Chapter 2

Review of Literature

2.1 About Probiotics

2.1.1 Introduction to probiotics

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular of milk products on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that these probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children. As there are no international consensus on the methodology to assess the efficacy and the safety of these products, at present, it was considered necessary to provide some useful studies to evaluate and suggest general guidelines for such assessments. The present thesis evaluates the latest information and scientific evidence available on the functional and safety aspects of probiotics, as well as the methodology to assess such aspects, by bringing together the work of worldwide scientific experts in the field.

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health (Salminen et al., 1998). The concept of probiotics evolved at the turn of the 20th century from a hypothesis first proposed by Nobel Prize winning Russian scientist Elie Metchnikoff (Bibel, 1988), who suggested that the long, healthy life of Bulgarian peasants resulted from their consumption of fermented milk products. He believed that when consumed, the fermenting bacillus (Lactobacillus) positively influenced the microflora of the colon, decreasing toxic microbial activities.

The present studies deals with the use of Lactic acid bacteria as probiotics.

Lactic acid bacteria (LAB) are present in the intestine of most animals. The beneficial role played by these microorganisms in the humans and other animals, including the effect on the immune system, has been extensively reported.
The probiotic approach is attractive because it is a reconstitution of the natural condition; it is a means of repairing a deficiency rather than the addition of foreign chemicals to the body which may have toxic consequences or, as in the case of antibiotics induce resistance and compromise subsequent therapy. Since to be used as probiotics the Lactic acid bacteria have to be cultivated in large amounts. Therefore for their economic production in large amounts some cheap source of nutrients is required. For this purpose use of waste materials is suggested in present studies. The organisms selected as probiotic strains are Enterococcus faecium and Lactobacillus acidophilus, whose useful properties are provided in the thesis.

2.1.2 What are Probiotics?

Probiotics are dietary supplements of live microorganisms thought to be healthy for the host organism. According to the currently adopted definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics; but also certain yeasts and bacilli are available.

At first, probiotics were thought to beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and toxin producing bacteria. Today specific health effects are being investigated and documented including alleviation of chronic intestinal inflammatory diseases, prevention and treatment of pathogen-induced diarrhea, urogenital infections, and atopic diseases.

Probiotics are live microorganisms (in most cases, bacteria) that are similar to beneficial microorganisms found in the human gut. They are also called "friendly bacteria" or "good bacteria." Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as complementary and alternative medicine (CAM) a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Complementary medicine is used together with conventional medicine, and alternative medicine is used in place of conventional medicine.

Experts have debated how to define probiotics. One widely used definition, developed by the World Health Organization and the Food and Agriculture Organization of the United Nations, is
that probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." (Microorganisms are tiny living organisms—such as bacteria, viruses, and yeasts—that can be seen only under a microscope.)

Probiotics are not the same thing as prebiotics—nondigestible food ingredients that selectively stimulate the growth and/or activity of beneficial microorganisms already in people's colons. When probiotics and prebiotics are mixed together, they form a synbiotic.

Probiotics are available in foods and dietary supplements (for example, capsules, tablets, and powders) and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and unfermented milk, miso, tempeh, and some juices and soy beverages. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation.

Most probiotics are bacteria similar to those naturally found in people's guts, especially in those of breastfed infants (who have natural protection against many diseases). Most often, the bacteria come from two groups, Lactobacillus or Bifidobacterium. Within each group, there are different species (for example, Lactobacillus acidophilus and Bifidobacterium bifidus), and within each species, different strains (or varieties). A few common probiotics, such as Saccharomyces boulardii, are yeasts, which are different from bacteria.

Some probiotic foods date back to ancient times, such as fermented foods and cultured milk products. Interest in probiotics in general has been growing; Americans' spending on probiotic supplements, for example, nearly tripled from 1994 to 2003.

2.1.3 Definition of Probiotic.

Probiotics are live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.

The key aspects of this definition are shown in Table 2.1, which include the fact that the microbe must be alive when administered (the fact that they may die during transit through the host does not exclude them) and must have undergone controlled evaluation to document health benefits in the target host (often, but not always, humans). Hamilton-Miller and colleagues provide a
historical context for use of this term, including noting that it once referred to microbial-produced substances and the microbes themselves.

Table 2.2 lists the many different definitions for probiotic that have been advanced through the years. Subcategories of the general term *probiotic* include probiotic drugs (intended to cure, treat, or prevent disease), probiotic foods (which include foods, food ingredients, and dietary supplements), direct-fed microbials (probiotics for animal use), and designer probiotics (genetically modified probiotics). Currently, there is no legal definition of the term *probiotic*. As the field of probiotics has advanced, the types of clinical indications tested for probiotic impact and the range of physiologic status of subjects being tested have greatly expanded. A given probiotic, tested in different clinical situations, might exert a beneficial effect, show no effect, or result in an adverse effect. However, a negative or adverse effect in certain situations does not negate probiotic status. Such results do, however, stress the need to be specific about the benefits that are documented for each probiotic and the situations in which use is considered to pose an undue risk. Use of “probiotic” to describe a strain refers to proven beneficial effects of the strain. Furthermore, it should not be presumed that a probiotic will be effective or safe under all conditions of use.

Table 2.1. What a Probiotic Is and Is Not

<table>
<thead>
<tr>
<th>Characteristics of a probiotic</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What a probiotic is...</strong></td>
<td>Bacteria and yeast have been studied as probiotics.</td>
</tr>
<tr>
<td><strong>Microbe</strong></td>
<td>The microbe must be alive when administered, although it may die after administration to the host. Viability at the site of action is presumed to be important, but some effects may be mediated by cell</td>
</tr>
</tbody>
</table>
components. Recovery of the fed strain in feces is suggestive that the microbe is alive at active sites within the alimentary canal.

**Defined and properly named**

The microbe must be identified at the genus, species, and strain levels, according to current nomenclature and using current best methods, generally DNA based. Phenotypic, morphologic, and biochemical characterization contribute to thorough strain characterization.

**Safe**

A probiotic must be safe for its intended use, as stipulated by regulatory authorities for the category of product. In the case of drugs but not foods, risks are balanced by benefits.

**Many possible regulatory categories**

“Probiotic” is an overarching category. Subcategories include:

- Probiotic food (food)
- Probiotic supplement (dietary supplement)
- Probiotic drug (drug)
- Designer probiotic (genetically modified microbe)
- Direct-fed microbial (animal uses)
What a probiotic isn't...

**Synonymous with native putatively beneficial microbes** Candidate probiotics are commonly isolated from the pool of native putatively beneficial bacteria found in humans, but it is not correct to equate probiotic and native commensal microbes.

**Synonymous with “live active cultures”** Live cultures are microbes associated with foods, often as food fermentation agents. Many of these have not been directly tested for health benefits. Probiotics are live microbes that have been shown to have a health effect. Also, some probiotics would not typically be associated with foods (such as *Escherichia coli*) and as such would not typically be referred to as a “live culture.”

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Table 2.2 Various Definitions of Probiotics that Have Been Published and Proposed for Use

<table>
<thead>
<tr>
<th>Definition</th>
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<tbody>
<tr>
<td>Substances secreted by one microorganism that stimulate another microorganism</td>
<td></td>
</tr>
<tr>
<td>Tissue extracts that stimulate microbial growth</td>
<td></td>
</tr>
<tr>
<td>Organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance</td>
<td></td>
</tr>
<tr>
<td>A live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance</td>
<td></td>
</tr>
<tr>
<td>A viable mono- or mixed culture of microorganisms that, applied to animals or humans, beneficially affects the host by improving the properties of the indigenous microflora</td>
<td></td>
</tr>
<tr>
<td>A live microbial culture of cultured dairy product that beneficially influences the health and nutrition of the host</td>
<td></td>
</tr>
<tr>
<td>Viable bacteria, in a single or mixed culture, that have a beneficial effect on the health of the host</td>
<td></td>
</tr>
</tbody>
</table>
• Living microorganisms that on ingestion in certain numbers exert health benefits beyond inherent basic nutrition
• A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract
• A preparation of or a product containing viable, defined microorganisms in sufficient numbers that alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host
• Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host
• Specific live or inactivated microbial cultures that have documented targets in reducing the risk of human disease or in their nutritional management
• Preparation of viable microorganisms that is consumed by humans or other animals with the aim of inducing beneficial effects by qualitatively or quantitatively influencing their gut microflora and/or modifying their immune status

2.1.4 Scientific Basis of Functionality

Hundreds of microbial species live in association with humans—on skin and in oral, intestinal and vaginal tracts. Bacterial populations have been estimated to reach 100,000,000,000,000 cells at all sites of the human body (Tannock, 1994), a number that is more amazing when considered in the context of exceeding by 10-fold the number of human cells associated with the human body. Studies with germ-free (gonotobiotic) animals prove that microbial colonization is not required for survival. In fact, microbial colonization can have negative effects as a result of the toxic, genotoxic, mutagenic, or carcinogenic potential of microbial metabolites (Hill et al., 1971). Germ-free animals, however, are more susceptible to infection than conventional counterparts (Hentges, 1992). The increased susceptibility to infection is attributed, at least in part, to poor immune function and the absence of “colonization resistance” (competition of normal microflora with invading microorganisms; Vollaard and Clasener, 1994). Differences between conventional and germ-free animals provide a basis for the belief that microbial colonization has important health implications for host organisms. It is a big
jump, however, from the assertion that colonizing microflora have a profound effect on normal human health to the probiotic hypothesis that the addition of certain exogenous microorganisms to the intestinal ecosystem will have a positive effect.

The intestinal tract is a fairly stable microbial ecosystem in the adult (Tannock, 1990). Acute perturbations as might result from antibiotic use, disease, or certain dietary changes seem to be self-correcting (Tannock, 1983). Probiotic bacteria consumed even in high numbers do not become permanent colonizers and are rarely detectable in fecal or intestinal samples beyond a couple of weeks after ingestion. This residence time likely coincides with washout kinetics, extended perhaps by some in situ replication of probiotics suited to the environment.

Therefore, it is necessary to consider that probiotic effects may, in fact, be mediated by associations and mechanisms less intimate and more transient than those of native microflora. Whether or not a specific probiotic bacterium will have beneficial, detrimental, or no effect on health cannot be presumed strictly through determination of its genus or species.

The tempting speculation that the members of one genus or species will consistently mediate specific effects is not supported by research. Strain-specific effects are frequently reported in a diversity of assays. Conversely, for targets including immune function, anti-cancer effects, and anti-diarrheal effects, similar positive effects have been demonstrated for different strains of different genera, e.g., Lactobacilli, Bifidobacteria and Enterococci.

Although direct comparisons of different strains are rarely done, it appears that generalizations about the probiotic performance of genera and species are difficult to make. Until mechanisms are better understood and controlled studies comparing isogenic strains differing in a well-defined manner are completed, it is prudent to assume that probiotic effects are strain specific. In addition, physiological conditions of the host are likely to be as important to probiotic efficacy as the microbial strain.

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The Benefits Of Probiotics

Fig 2.1 Diagramatic view of some beneficial effects of Probiotics
The term probiotic is a relatively new word meaning “for life” and it is currently used to name bacteria associated with beneficial effects for humans and animals. The original observation of the positive role played by some selected bacteria is attributed to Eli Metchnikoff, the Russian born Nobel Prize winner working at the Pasteur Institute at the beginning of the last century, who suggested that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Metchnikoff, 1907). At this time Henry Tissier, a French paediatrician, observed that children with diarrhea had in their stools a low number of bacteria characterized by a peculiar, Y-shaped morphology. These “bifid” bacteria were, on the contrary, abundant in healthy children (Tissier, 1906). He suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora. The works of Metchnikoff and Tissier were the first to make scientific suggestions concerning the probiotic use of bacteria, even if the word "probiotic" was not coined until 1960, to name substances produced by microorganisms which promoted the growth of other microorganisms (Lilly and Stillwell, 1965). Fuller (1989), in order to point out the microbial nature of probiotics, redefined the word as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". A similar definition was proposed by Havenaar and Huis in ’t Veld (1992), “a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora”. A more recent, but probably not the last definition is "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma, 1998).

It is clear that these definitions have:

1) restricted the use of the word probiotic to products which contain live microorganisms;
2) pointed out the need for providing an adequate dose of probiotic bacteria in order to exert the desirable effects.

The observations of Metchnikoff and Tissier were so appealing that commercial exploitation immediately followed their scientific works. Unfortunately, results were not always positive and most of these observations were suspicious. The probiotic concept was therefore regarded as scientifically unproven and it received minor interest for decades, with some research involving animal feeding, in order to find healthy substitutes for growth promoting agents. In the last 20 years however, research in the probiotic area has progressed considerably and significant advances have been made in the selection and characterization of specific probiotic cultures and substantiation of health claims relating to their consumption. Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used, but not exclusively, as probiotic microorganisms and a growing number of probiotic foods are available to the consumer. Some ecological considerations on the gut flora are necessary to understand the relevance, for human health, of the probiotic food concept. Bacteria are normal inhabitants of humans (as well as the bodies of upper animals and insects) including the gastrointestinal tract, where more than 400 bacterial species are found (reviewed by Tannock, 1999): half of the wet weight of colonic material is due to bacterial cells whose numbers exceed by 10-fold the number of tissue cells forming the human body. Normally the stomach contains few bacteria (10^3 colony forming units per ml of gastric juice) whereas the bacterial concentration increases throughout the gut resulting in a final concentration in the colon of 10^{12} bacteria/g. Bacterial colonization of the gut begins at birth, as new-borns are maintained in a sterile status until the delivery begins, and continues throughout life, with notable age-specific changes (Mitsuoka, 1992). Bacteria, forming the so-called resident intestinal microflora, do not normally have any acute adverse effects and some of them have been shown to be necessary for maintaining the well being of their host. As an example of the beneficial role of intestinal microflora, it is possible to cite what has been referred to as "colonization resistance" or "barrier effect" (van der Waaij et al., 1971; Vollaard and Clasener, 1994) meaning the mechanism used by bacteria already present in the gut to maintain their presence in this environment and to avoid colonization of the same intestinal sites by freshly ingested microorganisms, including pathogens. Therefore, it could be assumed that dietary manipulation of gut microflora, in order to increase the relative numbers of "beneficial
bacteria" could contribute to the well being of the host. This was also the original assumption of Metchnikoff who however, cautioned that:

"Systematic investigations should be made on the relation of gut microbes to precocious old age, and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body."

This prudent statement can still be regarded today as an invitation to scientists to investigate the probiotic bacteria in more depth and with care.

2.1.6 Probiotics as Foods or Drugs

The definition above specifically dealt with probiotics in food (including water). However, the definition did not include the term “food.” Although almost identical to a previously published definition by Guarner and Schaafsma—"live microorganisms, which when consumed in adequate amounts, confer a health effect on the host"—the word “administered” is substituted for “consumed,” presumably to expand the concept of probiotics to include administration in ways other than by mouth. Given that food must be consumed orally, the consultation apparently intended that the definition not be limited to food or by route of administration (eg, oral, vaginal, topical, rectal). Furthermore, the expert consultation report describes the functions of probiotics, including their role in “alleviation of infectious diseases.” This is consistent with much of the literature on probiotics that is focused on what are seen by regulatory bodies as therapeutic end points, such as reduction of duration of infectious diarrhea or extension of remission of pouchitis. In the United States, products that are intended to treat, cure, prevent, or mitigate disease are drugs, not foods. Taken together, it appears that this definition was intended to encompass health maintenance or improvement and therapeutic (ie, both food and drug) uses for probiotics. An important point must be made from a regulatory perspective. It is tempting to see the character of “food” or “drug” as an inherent property of a substance. However, in the United States, a substance is characterized as a “food” or a “drug” not based on its intrinsic characteristics as much as on its intended use. A product that by all appearances is a food and may even be available to the general public in grocery stores would be considered a drug by the US Food and Drug Administration (FDA) if it is sold to treat, cure, prevent, or mitigate disease [Food, Drug, and Cosmetic Act of 1938, §201(g)((1)]. Drug status by FDA interpretation is also extended to
any product administered along with a drug to either enhance effects or alleviate side effects if
the side effects themselves constitute diseases. There are both commercial and scientific
ramifications to this regulatory approach. For example, although controlled human studies on
“drug” uses for probiotics are numerous, such benefits cannot be claimed for a probiotic
marketed as a food or a dietary supplement (a subcategory of foods). This approach imposes
limitations on what publicly funded human studies can be conducted on probiotics in the United
States. For example, Hickson and colleagues conducted a study in England in which the diet of
hospitalized patients undergoing antibiotic treatment over the age of 50 years was supplemented
with a commercially available fermented milk. The results showed a significant reduction in
antibiotic associated side effects and Clostridium difficile–associated diarrhea. The FDA would
view such a study as a drug trial and, if consulted on such a study, would require the filing of an
Investigational New Drug application before the study is begun. A predicament arising from this
position is that given foods are not manufactured under drug Good Manufacturing Practices and
food companies generally do not have an interest in marketing drugs. Investigational New Drug
status may not be pursued. Therefore, a problematic disconnection exists between probiotic uses
and the regulatory framework in the United States, even as it pertains to research designed to
determine the validity of physician recommendations including, for example, the use of
probiotics when taking antibiotics.

Table 2.3 Characterized probiotic strains (partial list). Species identification is as reported
by the manufacturer and may not reflect the most current taxonomy (Yeung et al., 1999)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus acidophilus</em> NCFM</td>
<td>Rhodia, Inc. (Madison, Wis.)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> DDS-1</td>
<td>Nebraska Cultures, Inc. (Lincoln, Neb.)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> SBT-2062a</td>
<td>Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> LA-1</td>
<td>Chr. Hansen, Inc. (Milwaukee, Wis.)</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em> Shirota</td>
<td>Yakult (Tokyo, Japan)</td>
</tr>
<tr>
<td><em>L. casei</em> Immunitas</td>
<td>Danone (Paris, France)</td>
</tr>
<tr>
<td>Probiotic Strain</td>
<td>Source</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------</td>
</tr>
<tr>
<td><em>Lactobacillus fermentum</em> RC-14</td>
<td>Urex Biotech (London, Ontario, Canada)</td>
</tr>
<tr>
<td><em>Lactobacillus johnsonii</em> La1 (same as Lj1)</td>
<td>Nestlé (Lausanne, Switzerland)</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em> CRL 431</td>
<td>Chr. Hansen, Inc. (Milwaukee, Wis.)</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> 299V</td>
<td>Probi AB (Lund, Sweden)</td>
</tr>
<tr>
<td><em>Lactobacillus reuteri</em> SD2112 (same as MM2)</td>
<td>Biogaia (Raleigh, N.C.)</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> GGa</td>
<td>Valio Dairy (Helsinki, Finland)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GR-1</td>
<td>Urex Biotech (London, Ontario, Canada)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> 271</td>
<td>Probi AB (Lund, Sweden)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> LB21</td>
<td>Essum AB (Umeå, Sweden)</td>
</tr>
<tr>
<td><em>Lactobacillus salivarius</em></td>
<td>UCC118 University College (Cork, Ireland)</td>
</tr>
<tr>
<td><em>Lactobacillus lactis</em> L1A</td>
<td>Essum AB (Umeå, Sweden)</td>
</tr>
<tr>
<td><em>Bifidobacterium lactis</em> Bb-12</td>
<td>Chr. Hansen, Inc. (Milwaukee, Wis.)</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em> BB536a</td>
<td>Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)</td>
</tr>
<tr>
<td><em>B. longum</em> SBT-2928a</td>
<td>Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)</td>
</tr>
<tr>
<td><em>Bifidobacterium breve</em> strain</td>
<td>Yakult Yakult (Tokyo, Japan)</td>
</tr>
</tbody>
</table>

*Strains have been awarded Foods for Specified Health Use status in Japan*

### 2.1.7 Guidelines for the Assessment of Probiotic Microorganisms

In order to assess the properties of probiotics, it is suggested that the following guidelines be used. For use in foods, probiotic microorganisms should not only be capable of surviving passage through the digestive tract but also have the capability to proliferate in the gut. This means they must be resistant to gastric juices and be able to grow in the presence of bile under conditions in the intestines, or be consumed in a food vehicle that allows them to survive passage through the stomach and exposure to bile. They are Gram positive bacteria and are included...
primarily in two genera, *Lactobacillus* and *Bifidobacterium* (Holzapel et al., 1998; Klein et al., 1998).

### 2.1.7.1 Selection of probiotic strains for human use

Probiotics must be able to exert their benefits on the host through growth and/or activity in the human body (Collins et al., 1998; Morelli, 2000). However, it is the specificity of the action, not the source of the microorganism that is important. Indeed, it is very difficult to confirm the source of a microorganism. Infants are born with none of these bacteria in the intestine, and the origin of the intestinal microflora has not been fully elucidated. It is the ability to remain viable at the target site and to be effective that should be verified for each potentially probiotic strain. There is a need for refinement of *in vitro* tests to predict the ability of probiotics to function in humans. The currently available tests are not adequate to predict the functionality of probiotic microorganisms in the intestine. Many types of bacteria have been used as probiotics since time immemorial. They mainly consist of lactic acid producing bacteria (*Lactobacilli, Streptococci, Enterococci* and *Lactococci*). *Bifidobacteria, Bacillus* species, yeasts like *Saccharomyces* species too find a place in the long list of probiotics. The mode of ingestion is either through food or in a non food format. The probiotics generally subsist in the stomach or intestinal tract. They can potentially boost the immune system and can help in the treatment of a variety of conditions including lactose intolerance, diarrhea, colitis, hypertension, cancer, constipation, food allergies, irritable bowel syndrome and other intestinal disorders. How to select a probiotic and the ideal properties of probiotics

The ideal property of a probiotic is its ability to impart beneficial effect and not harm to the consumer. Hence, it is necessary that all the strains must be carefully and thoroughly studied prior to their utilization in humans or animals and should be having confirmed genetically regarded as safe (GRAS). The following are some of the properties, the probiotics are supposed to possess.

They are

1. Colonization or adhesion properties.

2. Human origin

3. Good in *vita* development
4. Ability of cells to produce metabolites and enzymes

5. Stability in gastric juices and bile

6. Production of antimicrobial substances

7. Safe for clinical use

8. Antagonistic action against noted pathogenic bacteria and / or viruses.

9. Factors that influence the strain survival during / after administration and

10. No adverse interactions with host especially, in terms of pathogenicity.

2.1.7.2 Classification and identification of individual strains: Classification is the arranging of organisms into taxonomic groups (taxa) on the basis of similarities or relationships. Nomenclature is the assignment of names to the taxonomic groups according to rules. Identification is the process of determining that a new isolate belongs to one of the established, named taxa. It is recommended that probiotics be named according to the International Code of Nomenclature to ensure understanding on an international basis. It is strongly urged that for the sake of full disclosure, probiotic strains be deposited in an internationally recognized culture collection. Since probiotic properties are strain related, it is suggested that strain identification (genetic typing) be performed, with methodology such as pulse field gel electrophoresis (PFGE). It is recommended that phenotypic tests be done first, followed by genetic identification, using such methods as DNA/DNA hybridization, 16S RNA sequencing or other internationally recognized methods. For the latter, the RDP (ribosomal data base project) should be used to confirm identity.

2.1.7.3 Defining and measuring the health benefits of probiotics

A number of health effects are associated with usage of probiotics. The use of probiotic microorganisms to confer health benefits on the host must indicate the dosage regimens and duration of use as recommended by the manufacturer of each individual strain or product based upon scientific evidence, and as approved in the country of sale. While this practice is not currently in place, it is strongly recommended that each product should indicate the minimum daily amount required for it to confer specific health benefit(s). Such evidence should, where
possible result from in vitro, animal (where appropriate) and human studies. Examples have been cited below to illustrate studies on specific strains and clinical outcomes. In doing so, the emphasis should not be on one particular strain being termed as superior to another, rather that the benefit conferred and the methods used to obtain and measure said benefits are of most importance.

2.1.8 Use of probiotics in otherwise healthy people

Many probiotic products are used by consumers who regard themselves as being otherwise healthy. They do so on the assumption that probiotics can retain their health and well being, and potentially reduce their long-term risk of diseases of the bowel, kidney, respiratory tract and heart. Several points need to be made on this assumption and its implications. The Consultation recognized that the use of probiotics should not replace a healthy lifestyle and balanced diet in otherwise healthy people. Firstly, there is no precise measure of “health” and subjects may actually have underlying and undetectable diseases at any given time. Secondly, no studies have yet been undertaken which analyse whether or not probiotic intake on a regular basis helps retain life-long “health” over and above dietary, exercise and other lifestyle measures. One study of day care centres in Finland showed that probiotic use reduced the incidence of respiratory infections and days absent due to ill health (Hatakka et al., 2001). The Consultation would like studies to be done to give credibility to the perception that probiotics should be taken on a regular basis by healthy men, women and children. Such studies should be multi-centred and require randomization on the basis of age, gender, race, nutritional intake, education, socio-economic status and other parameters. It is currently unclear as to the impact of regular probiotic intake on the intestinal microflora. For example, does it lead to the depletion or loss of commensal microorganisms which otherwise have beneficial effects on the host? While there is no indication of such effects, the issue needs to be considered. Furthermore, the concept of restoring a normal balance assumes that we know what the normal situation in any given intestinal tract comprises. It was deemed important by the Consultation to further study the various contributions of gut microorganisms on health and disease. Another point worthy of note is that, to date, the ingestion of probiotic strains has not led to measurable long-term colonization and survival in the host. Invariably, the microorganisms are retained for days or weeks, but no longer (Tannock et al., 2000). Thus, use of probiotics likely confers more transient than long-term effects, and so
continued intake appears to be required. In newborn children, where a commensal flora has not yet been established, it is feasible that probiotic microorganisms could become primary colonizers that remain long-term, perhaps even for life. While such probiotic usage can prevent death and serious morbidity in premature, low birth weight infants (Hoyos, 1997), the alteration of flora in healthy babies is a more complex situation. Just so, an implication of the Human Genome Project is that selected probiotics may be used at birth to create a flora that improves life-long health. These issues are very important for the future, and will require full discussion including human ethical considerations.

2.1.9 Potential benefits

Experiments into the benefits of probiotic therapies suggest a range of potentially beneficial medicinal uses for probiotics. For many of the potential benefits, research is limited and only preliminary results are available. It should be noted that the effects described are not general effects of probiotics. Recent research on the molecular biology and genomics of *Lactobacillus* has focused on the interaction with the immune system, anti-cancer potential, and potential as a biotherapeutic agent in cases of antibiotic-associated diarrhoea, travellers' diarrhoea, pediatric diarrhoea, inflammatory bowel disease and irritable bowel syndrome.

Table 2.4 Potential and established effects of probiotic bacteria (adapted from Sanders and Huis in’t Veld, 1999)

<table>
<thead>
<tr>
<th>Target Health Benefit</th>
<th>Postulated Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aid in lactose digestion</td>
<td>Bacterial lactase hydrolyses lactose</td>
</tr>
<tr>
<td>Resistance to enteric pathogens</td>
<td>Secretory immune effect</td>
</tr>
<tr>
<td></td>
<td>Colonization resistance</td>
</tr>
<tr>
<td></td>
<td>Alteration of intestinal conditions to be less favorable for pathogenicity</td>
</tr>
<tr>
<td></td>
<td>(pH, short chain fatty acids, bacteriocins)</td>
</tr>
</tbody>
</table>

30
<table>
<thead>
<tr>
<th><strong>Anti-colon cancer effect</strong></th>
<th>Alteration of toxin binding sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influence on gut flora populations</td>
</tr>
<tr>
<td></td>
<td>Adherence to intestinal mucosa, interfering with</td>
</tr>
<tr>
<td></td>
<td>pathogen adherence</td>
</tr>
<tr>
<td></td>
<td>Upregulation of intestinal mucin production, interfering with</td>
</tr>
<tr>
<td></td>
<td>Pathogen attachment to intestinal epithelial cells</td>
</tr>
<tr>
<td><strong>Small bowel bacterial overgrowth</strong></td>
<td>Mutagen binding</td>
</tr>
<tr>
<td></td>
<td>Carcinogen deactivation</td>
</tr>
<tr>
<td></td>
<td>Inhibition of carcinogen-producing enzymes of</td>
</tr>
<tr>
<td></td>
<td>colonic microbes</td>
</tr>
<tr>
<td></td>
<td>Immune response</td>
</tr>
<tr>
<td></td>
<td>Influence on secondary bile salt concentration</td>
</tr>
<tr>
<td><strong>Immune system modulation</strong></td>
<td>Influence on activity of overgrowth flora,</td>
</tr>
<tr>
<td></td>
<td>decreasing toxic metabolite production,</td>
</tr>
<tr>
<td></td>
<td>Alteration of intestinal conditions to be less favorable to</td>
</tr>
<tr>
<td></td>
<td>overgrowth flora activities or populations</td>
</tr>
<tr>
<td><strong>Allergy</strong></td>
<td>Strengthening of non-specific defense against</td>
</tr>
<tr>
<td></td>
<td>Adjuvant effect in antigen-specific immune responses</td>
</tr>
<tr>
<td><strong>Blood lipids, heart disease</strong></td>
<td>Enhancement of secretory IgA production</td>
</tr>
<tr>
<td></td>
<td>Prevention of antigen translocation into blood stream</td>
</tr>
<tr>
<td><strong>Antioxidative effect</strong></td>
<td>Assimilation of cholesterol within bacterial cell</td>
</tr>
<tr>
<td></td>
<td>Increased excretion of bile salts due to deconjugation by</td>
</tr>
<tr>
<td></td>
<td>bile salt hydrolase</td>
</tr>
<tr>
<td><strong>Antihypertensive effect</strong></td>
<td>Peptidase action on milk protein yields tripeptides which</td>
</tr>
</tbody>
</table>
inhibit angiotensin 1 converting enzyme
Cell wall components act as angiotensin converting enzyme inhibitors

**Urogenital infections**
- Adhesion to urinary and vaginal tract cells
- Colonization resistance
- Inhibitor production (H₂O₂, biosurfactants)

**Infection caused by Helicobacter pylori**
- Production of inhibitors of *H. pylori* (lactic acid and others)

**Hepatic encephalopathy**
- Inhibition of urease-producing gut flora

All effects can only be attributed to the individual strain(s) tested. Testing of a supplement does not indicate benefit from any other strain of the same species, and testing does not indicate benefit from the whole group of LAB (or other probiotics).

### 2.1.9.1 Managing lactose intolerance

As lactic acid bacteria actively convert lactose into lactic acid, ingestion of certain active strains may help lactose intolerant individuals tolerate more lactose than what they would have otherwise. In practice probiotics are not specifically targeted for this purpose, as most are relatively low in lactase activity as compared to the normal yogurt bacteria.

### 2.1.9.2 Prevention of colon cancer

In laboratory investigations, some strains of LAB (*Lactobacillus bulgaricus*) have demonstrated anti-mutagenic effects thought to be due to their ability to bind with heterocyclic amines, which are carcinogenic substances formed in cooked meat. Animal studies have demonstrated that some LAB can protect against colon cancer in rodents, though human data is limited and conflicting. Most human trials have found that the strains tested may exert anti-carcinogenic effects by decreasing the activity of an enzyme called β-glucuronidase (which can generate carcinogens in
the digestive system). Lower rates of colon cancer among higher consumers of fermented dairy products have been observed in some population studies.

2.1.9.3 Lowering cholesterol

Animal studies have demonstrated the efficacy of a range of LAB to be able to lower serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its reabsorption (which enters the blood as cholesterol). Some, but not all human trials have shown that dairy foods fermented with specific LAB can produce modest reductions in total and LDL cholesterol levels in those with normal levels to begin with, however trials in hyperlipidemic subjects are needed.

2.1.9.4 Lowering blood pressure

Several small clinical trials have shown that consumption of milk fermented with various strains of LAB can result in modest reductions in blood pressure. It is thought that this is due to the ACE inhibitor-like peptides produced during fermentation.

Improving immune function and preventing infections

LAB are thought to have several presumably beneficial effects on immune function. They may protect against pathogens by means of competitive inhibition (i.e., by competing for growth) and there is evidence to suggest that they may improve immune function by increasing the number of IgA-producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T lymphocytes and Natural Killer cells.

Clinical trials have demonstrated that probiotics may decrease the incidence of respiratory tract infections and dental caries in children. LAB foods and supplements have been shown to be aid in the treatment and prevention of acute diarrhea, and in decreasing the severity and duration of rotavirus infections in children and travelers' diarrhea in adults.

2.1.9.5 Helicobacter pylori. LAB are also thought to aid in the treatment of Helicobacter pylori infections (which cause peptic ulcers) in adults when used in combination with standard medical treatments. However more studies are required into this area.
2.1.9.6 Antibiotic-associated diarrhea

Antibiotic-associated diarrhea (AAD) results from an imbalance in the colonic microbiota caused by antibiotic therapy. Microbiota alteration changes carbohydrate metabolism with decreased short-chain fatty acid absorption and an osmotic diarrhea as a result. Another consequence of antibiotic therapy leading to diarrhea is overgrowth of potentially pathogenic organisms such as *Clostridium difficile.* Probiotic treatment can reduce the incidence and severity of AAD as indicated in several meta-analyses. However, further documentation of these findings through randomized, double blind, placebo-controlled trials are warranted. Efficacy of probiotic AAD prevention is dependent on the probiotic strain(s) used and on the dosage. Up to a 50% reduction of AAD occurrence has been found. No side-effects have been reported in any of these studies. Caution should, however, be exercised when administering probiotics to immunocompromised individuals or patients who have a compromised intestinal barrier.

2.1.9.7 Reducing inflammation

LAB foods and supplements have been found to modulate inflammatory and hypersensitivity responses, an observation thought to be at least in part due to the regulation of cytokine function. Clinical studies suggest that they can prevent recurrences of inflammatory bowel disease in adults, as well as improve milk allergies. They are not effective for treating eczema, a persistent skin inflammation. How probiotics counteract immune system overactivity remains unclear, but a potential mechanism is desensitization of so-called T lymphocytes, an important component of the immune system, towards pro-inflammatory stimuli.

2.1.9.8 Improving mineral absorption

It is hypothesized that probiotic lactobacilli may help correct malabsorption of trace minerals, found particularly in those with diets high in phytate content from whole grains, nuts, and legumes.

2.1.9.9 Preventing harmful bacterial growth under stress

In a study done to see the effects of stress on intestinal flora, rats that were fed probiotics had little occurrence of harmful bacteria latched onto their intestines compared to rats that were fed sterile water.

2.1.9.10 Irritable bowel syndrome and colitis

*B. infantis* 35624, sold as Align, was found to improve some symptoms of irritable bowel syndrome in women in a recent study. Another probiotic bacterium, *Lactobacillus plantarum*
299v, was also found to be effective in reducing IBS symptoms. Additionally, a probiotic formulation, VSL\#3, was found to be safe in treating ulcerative colitis, though efficacy in the study was uncertain. *Bifidobacterium animalis* DN-173 010 may help.

### 2.1.9.11 Managing urogenital health

Several in vitro studies have revealed probiotics' potential in relieving urinary tract infections and bacterial vaginosis. Results have been varied on these studies, and in vivo studies are still required in this area to determine efficacy.

### 2.1.10 Probiotics as mediators of health effects

Effects on Human Health Research suggests that probiotic bacteria may mediate a variety of health effects through numerous proposed mechanisms (Table 2.5). Some randomized, controlled studies conducted with a meaningful number of human subjects have yielded sufficiently positive results (Aso and Akazan, 1992; Belloma et al., 1980; Gade and Thorn, 1989; McFarland et al., 1994; Saavedra et al., 1994) to justify further investigation of the probiotic hypothesis. Alleviation of lactose intolerance symptoms and anti-diarrheal effects are the best substantiated effects. Anticancer and immune modulation effects are encouraging, but need more thorough substantiation in humans. Modulation of the gut microflora (populations and activities) and influence on mucosal immunity are mechanisms of probiotic function with potential to broadly influence human physiology. Probiotics have ameliorated acute toxic effects of gut flora metabolism in clinical systems, such as small bowel bacterial overgrowth (Simenhoff et al., 1996) and liver disease (Nanji et al., 1994; Read et al., 1966). A brief assessment of probiotic effects targeted toward several endpoints, with emphasis on results from human studies, where possible, follows.

- **Cancer.** Research has demonstrated that dietary components may increase or decrease cancer incidence (Williams and Wynder, 1996). Evidence that probiotic bacteria may be a dietary constituent that reduces cancer risk (Table 2.5) is derived from several lines of evidence (Hirayama and Rafter, 1999; Mital and Garg, 1995; Rafter, 1995). One study demonstrated that a powder preparation of *L. casei* (10^{10} CFU three times/day for one year) increased the recurrence-free period among human subjects with superficial bladder cancer (Aso and Akazan, 1992). Additional population studies and human intervention trials are needed to confirm this type of
effect. Such studies can be justified by the positive results seen to date from studies using a variety of biomarkers and the plausibility of proposed mechanisms. Results suggest that probiotic bacteria appear able to counteract mutagenic and genotoxic effects in the colon and other organ sites. Additionally, mechanistic studies suggest that probiotic bacteria or their byproducts influence epithelial cell kinetics in the colon, decreasing cancer cell proliferation. What cannot be determined from scientific evidence to date is to what extent regular probiotic consumption might influence cancer in humans. In addition, the cumulative evidence involves many different probiotic strains, feeding levels, exposure times, target cancer sites, and study methods. Therefore, precise translation of these results into specific recommendations is difficult.

Table 2.5 Activities of probiotics or probiotic-containing products that may play a role in reducing risk of cancer

<table>
<thead>
<tr>
<th>Activity Suppressed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell growth and differentiation of tissue culture cells</td>
<td>Baricault et al., 1995</td>
</tr>
<tr>
<td>Aberrant crypt foci in colonic tissue of animals</td>
<td>Rowland et al., 1998; Rao et al., 1999.</td>
</tr>
<tr>
<td>Recurrence of superficial bladder cancer in humans</td>
<td>Aso and Akazan, 1992</td>
</tr>
<tr>
<td>Enzyme activities (nitroreductase, b-glucuronidase, azoreductase, 7-a-dehydroxylase, glycocholic acid hydrolase) involved in conversion of procarcinogens into carcinogens in feces of laboratory animals and humans; not all parameters positively influenced in all studies.</td>
<td>McConnell and Tannock, 1993; Goldin et al., 1980</td>
</tr>
<tr>
<td>Mutagenic activity</td>
<td>Renner and Munzner, 1991</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td>Venturi et al., 1997</td>
</tr>
</tbody>
</table>
• **Intestinal Tract Function.** Gastrointestinal disturbances can range from annoying to life-threatening. Diarrheal illnesses have a host of microbiological, immunological and physiological causes, some related to the disruption of normal microecology. Improper function of the immune system that is related to decreased tolerance to indigenous microflora can lead to immunopathogenesis in chronic inflammatory bowel disease (Merger and Croitoru, 1998), which can be refractory to conventional treatment. Underlying pathology (e.g., chronic kidney failure) and achlorhydria (brought on by aging) can result in bacterial overgrowth of the small bowel and lead to abnormal and harmful microbial activities (Nanji et al., 1994; Simenhoff et al., 1996). Disruptions in intestinal permeability barriers can lead to translocation of bacteria into the bloodstream (Wells et al., 1988). Consumption of non-digestible foodstuffs (e.g., lactose for lactose intolerant people, soluble fibers) can provide fermentable substrates for growth of intestinal microbes, sometimes with deleterious effects. Probiotic bacteria have been shown to improve the clinical outcome in many intestinal disease targets (Table 2.6; Elmer et al., 1996; Salminen et al., 1998a). Most convincing are the blinded, placebo-controlled trials with human subjects (Bello et al., 1980; Gade and Thorn, 1989; McFarland et al., 1994; Saavedra et al., 1994). Although the clinical protocols frequently fall short of the double-blinded, placebo controlled ideal, the scientific evidence suggests that probiotic bacteria, consumed at high levels ($10^9$-$10^{11}$/day), can decrease the incidence, duration, and severity of some intestinal illnesses.

**Table 2.6** Targets and postulated mechanisms of probiotic influence on abnormal gastrointestinal conditions. Many studies were designed as pilot studies (small numbers of subjects, not blinded) and results have not been confirmed in large trials.

<table>
<thead>
<tr>
<th>Intestinal Conditions Mediated by Probiotic Bacteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic-associated diarrhea</td>
<td>Orrhage et al., 1994</td>
</tr>
<tr>
<td>Toxic amines in bloodstream of chronic kidney and liver disease patients with small bowel bacterial overgrowth</td>
<td>Muting et al., 1968; Simenhoff et al., 1996</td>
</tr>
<tr>
<td>Inflammatory bowel diseases</td>
<td>Kruis et al., 1997</td>
</tr>
<tr>
<td>Lactose intolerance symptoms</td>
<td>Suarez et al., 1995</td>
</tr>
</tbody>
</table>
Immune Function. Exposure to foreign antigens elicits a complex cascade of responses from the human body, including launching protective reactions against pathogens and suppressing activity against food antigens and colonizing microflora. Improperly directed or balanced immunologic activity can lead to serious health problems; the suffering brought on by allergic and inflammatory reactions is a testimony to these occurrences. Immune function can be compromised in the elderly and in those on immunosuppressing medications and suffering from certain diseases. The role of functional foods, directed for the most part toward a healthy population with competent immune function, is not clear. However, it is an area of much active research, consumer interest, and product positioning. The oro-gastro-intestinal system is a prime means of interface between foreign antigens, (either pathogens, harmless bacteria, or food antigens) and mucosal surfaces. The total mucosal surface area of the adult human gastrointestinal tract is up to 300 m², the largest body area interacting with the environment. The gut-associated lymphoid tissue (GALT) transforms the gastrointestinal tract into the largest immune organ in the human body. Approximately $10^{10}$ immunoglobulin-producing cells per meter of small bowel are estimated to be present, accounting for approximately 80% of all immunoglobulin-producing cells in the body (Targan and Shanahan, 1994). Subsequently, the intestinal tract can evoke a variety of immunological responses from many different local immune cells (Mccracken and Gaskins, 1999). It is through this mechanism that probiotic...
bacteria are postulated to influence the immune response, although genera in addition to *Lactobacillus* and *Bifidobacterium* have been evaluated as immune stimulators or "biological response modifiers" with varying degrees of success. The influence of probiotic bacteria on the immune response has been thoroughly reviewed by Marteau and Rambaud (1993), Mc-Cracken and Gaskins (1999), Perdigón and Alvarez (1992), and Perdigón et al. (1995). Many studies using *in vitro* assessments of immune response or non-oral routes of probiotic introduction have questionable relevance. Animal models and human studies provide a baseline understanding of the degree and type of probiotic-induced immune response. From these studies, it appears that probiotic bacteria are able to enhance both non-specific and specific immune responses by activating macrophages; increasing levels of cytokines; increasing natural killer cell activity; and increasing levels of immunoglobulins, especially secretory IgA. Viable probiotic cells, dead cells, or fermentation products have been shown to mediate immune activity. Important in this capability is the positive influence of probiotics on immune activity without eliciting a harmful inflammatory response. Biological effects correlated with enhanced immune function, such as protection against viral and bacterial pathogens and tumor inhibition, have also been measured (Marteau and Rambaud, 1993). This effort is important because it provides a physiologically relevant measure of functionality. It is difficult to interpret statistically significant results in immune function studies in terms of what degree of influence these changes would be expected to have on human health. Importantly, long-term studies have not established whether the immune response to exogenous probiotic strains is a temporary response upon exposure to a foreign strain or one that would continue to be expressed after extended feeding (Tannock, 1999b).

- **Allergy.** Preliminary research on probiotic-mediated modulation of certain allergic reactions has been reported. A breakdown of the intestine’s mucosal barrier function, allowing extensive antigen challenge, may be a factor in some allergic reactions. Since probiotic bacteria have been shown to improve mucosal barrier function, the hypothesis that they may play a role in moderating allergic response was tested. In one study, changes in receptors mediating phagocytosis was measured in eight milk-hypersensitive (not lactose intolerant) adults consuming milk and milk with *Lactobacillus GG* (Pelto et al., 1996). Consumption of the probiotic containing milk did not result in a significant increase in receptor expression whereas consumption of regular milk did, suggesting that this strain may suppress a milk-induced
immune inflammatory response. Unfortunately, symptoms were not evaluated. Symptoms were evaluated, however, in a partially blinded 12-month study in which yogurt and pasteurized yogurt were fed to college-age and elderly individuals (Trapp et al., 1993). A significant decrease in allergy symptoms was observed in individuals consuming yogurt containing live, active bacteria compared to individuals consuming pasteurized yogurt and those who did not consume yogurt. No microbiological characterization of the yogurt was reported. Another report, however, did not show clinical improvement in 15 asthmatic subjects fed yogurt and yogurt with *L. acidophilus* in a crossover design study (Wheeler et al., 1997). Further study of the role of probiotics in allergic response is necessary.

• **Stomach Health.** The ability of probiotic bacteria to influence colonization and activity of *Helicobacter pylori*, which is associated with chronic gastritis, peptic ulcers, and risk for gastric cancer (Marshall, 1994), has been evaluated. Conventionally colonized (stomach microflora is dominated by lactobacilli) mice resisted infection by *H. pylori*, whereas germ-free mice did not (Kabir et al., 1997). Results from animal (Aiba et al., 1998; Kabir et al., 1997) and human (Michetti et al., 1999) studies suggest that some probiotic bacteria or their end-products may inhibit *H. pylori* infection. The ability of *Lactobacillus salivarius* WB1004 to inhibit *H. pylori* colonization of human or murine gastric epithelial cells *in vitro* was extended *in vivo* in gnotobiotic mice (Kabir et al., 1997). Interestingly, complementary studies indicated that neither *S. aureus* nor *Enterococcus faecalis* afforded the same protection from *H. pylori* infection. Colonization by *H. pylori* was also reduced by feeding *L. salivarius* after infection with *H. pylori*. Aiba et al. (1998) found that *L. salivarius*, but not *L. casei* or *L. acidophilus*, inhibited *H. pylori* colonization in a mouse model. Lactic acid production was thought to correlate with inhibition. Lactic acid was shown to be more inhibitory to *H. pylori* *in vitro* than acetic or hydrochloric acids (Midolo et al., 1995). Michetti et al. (1999) administered to humans in a double-blind, controlled clinical study a whey protein-based fermentation product supernatant fluid prepared by growth of *L. johnsonii* Lai. They assessed effects using a carbon-13-urea breath test, endoscopy, and stomach biopsy. Reductions in 13CO2 in the 13C urea breath test were observed in subjects consuming the probiotic product, suggesting an inhibition of infection; the inhibition extended four weeks after cessation of treatment. Gastric biopsies, however, indicated that *H. pylori* colonization persisted. *In vitro* studies suggested that inhibition resulted from more than lactic acid, and was strain-specific. These results suggest that probiotic
bacteria and their metabolic end-products can inhibit *H. pylori* and its physiological effects. Results regarding the influence of probiotics on *H. pylori* infection must be extended and confirmed.

- **Urogenital Health.** Similar to the intestinal tract, the urogenital tract in women is highly colonized and susceptible to infection upon colonization disruption (Reid et al., 1998). Both urinary and genital tract infections have been linked to bacteria originating from the colon. This has led to the hypothesis that modulation of gut microflora through consumption of bacteria can have an effect on urogenital ecosystems (Sobel, 1999). Populating the colon with lactobacilli enables the colon to serve as a source of beneficial, not just harmful, bacteria. Several studies have correlated vaginal health (absence of infections) with the presence of lactobacilli, and specifically hydrogen peroxide-producing lactobacilli (Hillier et al., 1992). Some clinical substantiation of the ability of probiotics to decrease recurrence of urogenital infections in women exits (Bruce and Reid, 1988; Hallen et al., 1992; Hilton et al., 1992; Reid et al., 1995; Shalev et al., 1996). Most of these studies were conducted with intravaginal instillations of probiotic bacteria. Oral consumption of certain probiotic-containing products, however, was found to mediate decreased recurrence of *Candida* infections and bacterial vaginosis (Hilton et al., 1992; Shalev et al., 1996). To confirm a role of dietary sources of probiotic bacteria in the prevention of urogenital infections, placebo-controlled, blinded studies using oral routes of introduction with sufficiently large study groups are needed.

- **Cholesterol.** Elevated levels of certain blood lipids are a risk factor for cardiovascular disease. The observation that conventional animals excrete higher levels of cholesterol in feces than germ-free animals suggests that colonizing microbes may influence cholesterol excretion, with potential implications for serum cholesterol levels (Eyssen, 1973). Studies of the effects of culture-containing dairy products or probiotic bacteria on cholesterol levels has yielded equivocal results (Taylor and Williams, 1998). Since 1974, 15 published studies have evaluated blood lipids in human subjects consuming fermented milk products, with a total of 534 subjects. Statistically significant lowering of cholesterol ranged from 2.4 – 23.2% for total cholesterol and 9 – 9.8% for low-density lipoprotein cholesterol. The studies have been criticized for failure to stabilize baselines prior to onset of the feeding protocol, small sample size, short study duration, unreasonably large fermented milk intake requirements, small differences, and failure to control...
for diet and physical activity of subjects. In the studies showing statistically significant results on
the lowering of either total cholesterol or low-density lipids, the treatment duration was six
weeks or less. Furthermore, one report showed increases in both total cholesterol and low-
density lipoprotein (Rossouw et al., 1981) and another showed decreases in high-density
lipoprotein (Anderson and Gilliland, 1999). The effect of probiotic bacteria on reduction of serum
cholesterol and mechanisms of any effects are unknown. One hypothesis suggests that some
strains of *L. acidophilus* can assimilate the cholesterol molecule (Gilliland et al., 1985); this
activity has been reported in *in vitro* assays (Gilliland et al., 1985; Rasic et al., 1992). However,
some have questioned the physiological relevance of binding or assimilation observed in an *in
vitro*, aqueous assay conducted at pH 6.0 or lower. It has been suggested that pH dependent,
transient cholesterol precipitation in laboratory media were misinterpreted as assimilation
(Klaver and Meer, 1993; Tahri et al., 1996).

The ability of certain probiotic lactobacilli and bifidobacteria to enzymatically deconjugate bile
acids has also been suggested to have a role in regulating cholesterol levels in humans. Deconjugated bile acids are more efficiently excreted (De Smet et al., 1994). Because cholesterol is a precursor of bile acids, this could lead to reduction of serum cholesterol as cholesterol molecules are converted to bile acids to replace those lost through excretion. The desirability of bile salt hydrolysis as a probiotic attribute, however, is questionable. Deconjugated bile acids escaping re-entry into the hepatic circulation enter the colon where they can be converted into secondary bile acids by colonic microbes. Secondary bile acids are known cancer promoters; hence, this activity could result in a health risk. A potential increased risk of colon cancer may outweigh any benefit of reduction of serum cholesterol levels. These hypotheses have not been confirmed in animal or human studies, although Gilliland et al. (1985) established a cholesterol-lowering effect of a cholesterol-assimilating (but not a nonassimilating) strain in boars. Further research on any mechanisms should be preceded by evidence for clinical effect in at least one thorough, long-term human study.

**Hypertension.** Dietary recommendations accompany more aggressive strategies to control
hypertension, and some preliminary evidence suggests that food products derived from probiotic
bacteria could possibly contribute to blood pressure control (Takano, 1998). This antihypertensive effect has been documented with studies in spontaneous hypertensive rats.
(Nakamura et al., 1995; 1996) and one human clinical study (Hata et al., 1996). Two tripeptides, valine-proline-proline and isoleucine-proline-proline, isolated from fermentation of a milk-based medium by *Saccharomyces cerevisiae* and *Lactobacillus helveticus* have been identified as the active components. These tripeptides function as angiotensin-I-converting enzyme inhibitors and reduce blood pressure. Based on this technology, Calpis (Kanagawa, Japan) developed a pasteurized product (Ameal-S) having Foods for Specified Health Use (FOSHU) status. Unlike many other probiotic-induced effects, it is important to note that this effect is mediated by a fermentation end-product, not viable probiotic cells. Another antihypertensive activity was associated with cell wall fragments of *Lactobacillus casei* YIT9018 (Sawada et al., 1990). Tested in a placebo-controlled trial with 28 human hypertensive subjects, powdered cell extracts (not viable cells) were administered orally and effects on systolic pressure, diastolic pressure, and heart rate were determined. Small, but statistically significant, decreases in all three measurements were noted. These results suggest that probiotic bacteria or their fermentation endproducts may be effective in mediating a mild antihypertensive effect. Confirmation in long-term, placebo-controlled human subjects is needed.

- **Other Health Effects.** Probiotic influence on a variety of other clinical targets has been evaluated. Probiotic-mediated reduction in the severity of reaction to exposure to radioactive isotopes has been shown in mice (Dong et al., 1987) and humans (Henriksson et al., 1995; Korschunov et al., 1996; Salminen et al., 1988). The effects of endotoxemia associated with alcoholic liver disease were reduced by probiotics (Nanji et al., 1994). Probiotics have been used in enteral feeding to provide nutritional support of surgery patients (Bengmark and Gianotti, 1996). In addition to proposed direct effects on humans, probiotics may also have implications for human health through their use in animal agriculture. Probiotics have been tested for prevention of food animal colonization with pathogens.

Probiotics may also benefit animal agriculture through greater resistance of farm animals to infectious diseases, increased growth rate, improved feed conversion, and increased yield of milk and eggs (Fuller, 1998). Commercial probiotic products for use in animal agriculture are available.

### 2.1.11 Testing Methods for Establishing Health Benefits Conferred by Probiotic Microorganisms

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Proper *in vitro* studies should establish the potential health benefits of probiotics prior to undertaking *in vivo* trials. Tests such as acid and bile tolerance, antimicrobial production and adherence ability to human intestinal cells should be performed depending on the proposed health benefit (Collins et al., 1998; Havenaar and Huis in’t Veld, 1992). In order to ascertain that a given probiotic can prevent or treat a specific pathogen infection, a clinical study must be designed to verify exposure to the said pathogen (preventive study), or that the infecting microorganism is that specific pathogen (treatment study). If the goal is to apply probiotics in general to prevent or treat a number of infectious gastroenteritis or urogenital conditions, the study design must define the clinical presentation, symptoms and signs of infection, and include appropriate controls. For *in vivo* testing, randomized double blind, placebo controlled human trials should be undertaken to establish the efficacy of the probiotic product. The Consultation recognized that there is a need for human studies in which adequate numbers of subjects are enrolled to achieve statistical significance (Andersson et al., 2001). It would be preferable to have such findings collaborated by more than one independent center. For some foods, it may be difficult to separate a probiotic effect from an effect related to the general product characteristics of the food. Therefore, it is essential that proper controls be included in these human trials. Furthermore, data obtained with one specific probiotic food cannot be extrapolated to other foods containing that particular probiotic strain or to other probiotic microorganisms. With respect to measuring the health benefits in human studies, consideration should be given to clinically relevant outcomes in the population being studied. For diarrheal studies, this might be preventing death in some countries, while in others it might be prevention of a defined and statistically significant weight loss, decreased duration of watery/liquid stools, and faster recovery to normal health, as measured by restoration of normal bowel function and stool consistency. Although it is known that certain probiotics can elicit beneficial effects, little is known about the molecular mechanisms of the benefits reported (Andersson et al., 2001). The mechanisms may vary from one probiotic to another (for the same benefit via different means) and the mechanism may be a combination of events, thus making this a very difficult and complex area. It could involve the production of a specific enzyme(s) or metabolite(s) that act directly on the microorganism(s), or the probiotic could also cause the body to produce the beneficial action.

Examples of possible probiotic mechanisms of action, in the control of intestinal pathogens include:
- Antimicrobial substance production
- Competitive exclusion of pathogen binding
- Competition for nutrients
- Modulation of the immune system

The Consultation proposes that clear experiments (in vitro and/or in vivo) should be designed at the molecular level to elucidate the mechanisms of probiotic beneficial effects. Appropriate experiments including genetic analysis to elucidate the mechanism of actions should be performed. Probiotic bacteria containing beta-galactosidase can be added to food to improve lactose maldigestion (Kim and Gilliland, 1983). However, a similar health effect is also observed for lactose fermenting starter bacteria such as L. delbrueckii ssp. Bulgaricus and S. thermophilus in fermented milk products like yogurt (Kim and Gilliland, 1984; Kolars et al., 1984). These traditional starters are not considered probiotics since they lack the ability to proliferate in the intestine (Klein et al., 1998).

2.1.12 Safety Considerations

- Antimicrobial resistance profiles of probiotics

As with any bacteria, antibiotic resistance exists among some lactic acid bacteria, including probiotic microorganisms (Salminen et al., 1998). This resistance may be related to chromosomal, transposon or plasmid located genes. However, insufficient information is available on situations in which these genetic elements could be mobilized and it is not known if situations could arise where this would become a clinical problem. There is concern over the use in foods of probiotic bacteria that contain specific drug resistance genes. Bacteria, which contain transmissible drug resistance genes, should not be used in foods. Currently, no standardized phenotypic methods are available which are internationally recognized for lactobacilli and bifidobacteria (non-pathogens). The Consultation recognizes the need for the development of standardized assays for the determination of drug insensitivity or resistance profiles in lactobacilli and bifidobacteria. The Consultation is aware that plasmids exist in lactobacilli and bifidobacteria, especially in strains isolated from the intestine, which have genes encoding antibiotic resistance. Due to the relevance of this problem, it is suggested that further research be done relating to the antibiotic resistance of lactobacilli and bifidobacteria. When dealing with selection of probiotic strains, it is recommended that probiotic bacteria should not harbour
transmissible drug resistance genes encoding resistance to clinically used drugs. Research is required relating to the antibiotic resistance of lactobacilli and bifidobacteria and the potential for transmission of genetic elements to other intestinal and/or foodborne microorganisms.

-Safety of probiotics in humans

In terms of safety of probiotics, the Consultation believes that a set of general principles and practical criteria need to be generated to provide guidelines as to how any given potential probiotic microorganism can be tested and proven to have a low risk of inducing or being associated with the etiology of disease, versus conferring a significant health benefit when administered to humans. These guidelines should recognize that some species may require more vigorous assessment than others. In this respect, the evaluation of safety will require at least some studies to be performed in humans, and should address aspects of the proposed end use of the probiotic strain. Information acquired to date shows that lactobacilli have a long history of use as probiotics without established risk to humans, and this remains the best proof of their safety (Naidu et al., 1999; Saxelin et al., 1996). Also, no pathogenic or virulence properties have been found for lactobacilli, bifidobacteria or lactococci (Aguirre and Collins, 1993). Having stated that, the Consultation acknowledges that under certain conditions, some lactobacilli strains have been associated with adverse effects, such as rare cases of bacteremia (Saxelin et al., 1996). However, a recent epidemiological study of systematically collected lactobacilli bacteremia case reports in one country has shown that there is no increased incidence or frequency of bacteremia with increased usage of probiotic lactobacilli (Salminen et al., 2001). It is also acknowledged that some members of lactic acid bacteria, such as enterococci may possess virulence characteristics. For this and other reasons, the Consultation recommends that Enterococcus not be referred to as a probiotic for human use. The rationale is based upon: A. Strains can display a high level of resistance to vancomycin (Shlaes et al., 1989; Eaton and Gasson, 2001; Lund and Edlund, 2001), or can acquire such resistance. If this resistance is present, transfer to other microorganisms may occur and this could enhance the pathogenesis of such recipients (Noble et al., 1992; Leclercq and Courvalin, 1997). B. Certain strains of vancomycin resistant enterococci are commonly associated with nosocomial infections in hospitals (Leclercq and Courvalin, 1997; Woodford et al., 1995).
The Consultation recognizes that some strains of *Enterococcus* display probiotic properties, and may not at the point of inclusion in a product display vancomycin resistance. However, the onus is on the producer to prove that any given strain cannot acquire or transfer vancomycin resistance or be virulent and induce infection.

2.1.13. Probiotic Product Specifications, Quality Assurance and Regulatory Issues

**REGULATIONS AND SAFETY OF PROBIOTICS IN MANUFACTURE:**

Since the early of last century beneficial effects of probiotics have been proved and well used in the dairy industry, which suggested of daily intake to get the beneficial effect on the host. That directs the research and industry organizations to suggest special regulations for the use of probiotics in food industry to obtain the desired therapeutic effects. Probiotics are sensitive because they die after exposure to low pH in the human stomach. Therefore, the high-count number of probiotics recommended in the products at a minimum count of $10^6$ CFU/g at the expiry date (Kurmann and Rasic, 1991; Gomes and Malcata, 1999). However, it has been suggested that the daily intake should be at least $10^8$ CFU (Salminen et al., 1998b; Lourens-Hattingh and Viljoen, 2001; Playne, 2002).

The more use of probiotics in the worldwide as dairy products the more suggested the need of principles and regulations as standard for the minimum count of viable probiotics bacteria in the dairy and fermented milk products to get the beneficial effects. The National Yogurt Association (NYA) of the United States suggested that any yogurt with live culture and use for health benefit recognize as containing significant amounts of live and active cultures. The seal is a voluntary identification available to all manufacturers of refrigerated yogurt products contain at least $10^6$ cfu per gram at the time of manufacture, and at least $10^7$ CFU per gram in frozen yogurt contains at the time of manufacture.

Exactly in the same way, the International Standard of IDF requires 107 CFU of L. acidophilus in products containing Lactobacillus bacteria and $10^6$ CFU /g of bifidobacteria in fermented milks containing bifidobacteria at the time of sale (IDF, 1992). In Japan, the Fermented Milks and Lactic Acid Beverages Association have already established a standard that requires $\geq10^7$ CFU mL$^{-1}$ to be present in dairy products that claims to contain bifidobacteria (Ishibashi and Shimamura, 1993; Martinez-Villaluenga et al., 2006). Likewise, The Swiss Food Regulation requires a minimum of $10^6$ CFU of viable bifidobacteria in those
25 products (Ishibashi and Shimamura, 1993; Bibiloni et al., 2001). Some countries like Australia and New Zealand are still not introduce regulations for probiotics use. However, the Australian and New Zealand Food Standards Code (ANZFA) doesn’t put any minimum count of probiotics products, but it’s mentioned the important of viable organisms in the manufactured of fermented milk products and that should be at least $10^6$ CFU/g and pH of 4.5 at the end of manufactured of yogurt (ANZFA, 2003).

Bifidobacteria and lactobacillus strain have a long history as safe organisms in the dairy manufactured and they recognized as —GRAS (Donohue and Salminen, 1996). There are no guidelines for the safety of probiotics exists so far. However there is responsibility of probiotic for four types of side effects; Systemic infections, deleterious metabolic activities, Disproportionate immune stimulation and gene transfer (Marteau et al., 2002). Some potential infections of different species of probiotics has reported such as; Streptococcus likely to cause opportunistic infections (Salminen et al., 1998b). The recent studies have shown that this probiotic effect is link to a strain specific. Most the case of safety considerations were related to products because of certain species of Streptococcus, Enterococcus and bacillus, which are opportunistic pathogens (Donohue and Salminen, 1996; Spinosa et al., 2000).

The guidelines for the Evaluation of probiotics in Food by FAO/WHO working group in 2002 suggested the use of valid methodology such as phenotypic and genetic tests, which will help to establish the species currently used and evaluation of probiotics. Therefore, the introduction of regulations and safety for probiotic productions by food authorities are essential in the manufacturers to guarantee a specific count and species of probiotic bacteria in their products.

**Regulatory issues**

Government regulations differ among countries, however the status of probiotics as a component in food is currently not established on an international basis. For the most part, probiotics come under food and dietary supplements because most are delivered by mouth as foods. These are differentiated from drugs in a number of ways, especially with respect to claims. Drugs are allowed to claim effectiveness in the treatment, mitigation or cure of a disease, whereas foods, feed additives and dietary supplements can only make general health claims. In order to understand where probiotic products currently fall in terms of regulatory agencies, and the claims that can be made with their use, the following US example is provided (www.fda.gov).
Consumers are permitted access to products ingested as pills, capsules, tablets and liquids, or in capsules sold in health food stores or via the internet.

- A ‘health claim’ is defined as “a statement, which characterizes the relationship of any substance to a disease or health-related condition, and these should be based upon well-established, generally accepted knowledge from evidence in the scientific literature and/or recommendations from national or international public health bodies. Examples include ‘protects against cancer’.

- A structure/function claim is defined as “a statement of nutritional support that describes the role of a nutrient or dietary ingredient to affect the structure or functioning of the human body, or characterizes the documented mechanism by which a nutrient or dietary ingredient acts to maintain such structure or function. Examples include ‘supports the immune system’. Claims that substances can treat, diagnose, cure or prevent a disease are not structure/function claims. The US-Consultation recommends that disease reduction claims be permitted for specific probiotics if these have been demonstrated using guidelines outlined in this report.

The new paradigm of risk analysis is making its way into regulatory food safety and focuses on a functional separation of the science-based risk assessment and risk management. However, the issue of communication is now also considered an important integrated part of risk analysis. Communication includes exchange between assessors and managers and two-way interaction with other interested parties.

Within this concept, the transparency of the decision making process for food safety regulatory action is emphasized, as well as the importance of providing a vehicle for consumers and others to participate in the development process. Therefore communication efforts relative to the use of probiotics should be considered as an integrated part of the development of regulatory initiatives.

**Appropriate labeling**

To clarify the identity of a probiotic present in the food, the US-Consultation recommends that the microbial species be stated on the label. If a selection process has been undertaken at the strain level, the identity of the strain should also be included, since the probiotic effect seems to be strain specific. There is a need to accurately enumerate the probiotic bacteria in food products.
in order to include them on the label. The label should state the viable concentration of each probiotic present at the end of shelf life (Reid et al., 2001c).

-**Manufacturing and handling procedures**

To ensure that any given culture maintains the beneficial properties, the stock culture should be maintained under appropriate conditions and be checked periodically for strain identity and probiotic properties. Furthermore, viability and probiotic activity must be maintained throughout processing, handling and storage of the food product containing the probiotic, and verified at the end of shelf-life.

Adequate quality assurance programmes should be in place. Good manufacturing practices should be followed in the manufacture of probiotic foods. The Consultation recommends that the Codex General Principles of Food Hygiene and Guidelines for Application of HACCP (CAC, 1997) be followed.

-**Prebiotics**

Prebiotics as an area is distinct from probiotics and therefore, will not be covered in detail in this report. The Consultation recognizes both the potential benefits of prebiotics with respect to probiotics, in addition to their ability to stimulate indigenous beneficial bacteria in the host. Prebiotics are generally defined as ‘nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health’ (Gibson and Roberfroid, 1995).

The concept of prebiotics essentially has the same aim as probiotics, which is to improve host health via modulation of the intestinal flora, although by a different mechanism. However, there are some cases in which prebiotics may be beneficial for the probiotic, especially with regard to bifidobacteria, that is the synbiotic concept.

Synbiotics are defined as ‘mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host’ (Andersson et al., 2001). If a synbiotic relationship is intended, then it should be verified scientifically, following the guidelines.
2.1.14 Post Market Surveillance

The Consultation recommends that probiotic producers, medical professionals and public health officers consider some form of system to monitor the health outcomes of long-term probiotic administration. This is suggested as a means to gain insight into potential side effects as well as assess long-term benefits. A necessary prerequisite for surveillance is a proper trace-back system.

**Adverse effects**

There is no published evidence that probiotic supplements are able to completely replace the body’s natural flora when these have been killed off; indeed bacterial levels in faeces disappear within days when supplementation ceases. While the oral use of probiotics is considered safe and even recommended by the World Health Organization (WHO) under specific guidelines, in some specific situations (such as critically ill patients) they could be potentially harmful. In a therapeutic clinical trial conducted by M. Besselink and colleagues in The Netherlands, the consumption of a cocktail containing genetically modified strains of probiotic bacteria, increased the death rate of patients with acute pancreatitis. Probiotics have been shown to be beneficial for other types of patients. In a clinical trial conducted at the University of Western Australia, aimed at showing the effectiveness of probiotics in reducing childhood allergies, Dr Susan Prescott and her colleagues gave 178 children either a probiotic or a placebo for the first six months of their life, those given the good bacteria were more, not less, likely to develop a sensitivity to allergens.

Some hospitals have reported treating lactobacillus septicaemia which is a potentially fatal disease caused by the consumption of probiotics by people with lowered immune systems or who are already very ill.

2.1.15 Side Effects and Risks

Some live microorganisms have a long history of use as probiotics without causing illness in people. Probiotics' safety has not been thoroughly studied scientifically, however. More information is especially needed on how safe they are for young children, elderly people, and people with compromised immune systems.
Probiotics' side effects, if they occur, tend to be mild and digestive (such as gas or bloating). More serious effects have been seen in some people. Probiotics might theoretically cause infections that need to be treated with antibiotics, especially in people with underlying health conditions. They could also cause unhealthy metabolic activities, too much stimulation of the immune system, or gene transfer (insertion of genetic material into a cell).

Probiotic products taken by mouth as a dietary supplement as a product that contains vitamins, minerals, herbs or other botanicals, amino acids, enzymes, and/or other ingredients intended to supplement the diet. The U.S. Food and Drug Administration has special labeling requirements for dietary supplements. are manufactured and regulated as foods, not drugs.

2.1.16 Complementary and alternative medicine

Probiotics are live microorganisms (in most cases, bacteria) that are similar to beneficial microorganisms found in the human gut. They are also called "friendly bacteria" or "good bacteria." Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as complementary and alternative medicine (CAM).

People use probiotic products as CAM to prevent and treat certain illnesses and support general wellness. Effects found from one species or strain of probiotics do not necessarily hold true for others, or even for different preparations of the same species or strain.

2.1.17 Composition of Probiotics

Probiotics in theory can be composed of any live microbe. A large number of probiotics are from the Lactobacillus or Bifidobacterium genera. Also popular is Saccharomyces Boulardii (a yeast). Less commonly used are strains of Escherichia coli or Bacillus coagulans. One category of microbe that is typically not considered to be a probiotic is a virus. Live viruses have been administered as vaccines, but such use is generally considered to be outside the realm of probiotics. A distinction should be made between a probiotic and a live, active culture.

Fermented foods, especially fermented dairy products, frequently contain live, active cultures. As it is essential that probiotics be documented to have a health
benefit, and given that live, active cultures are generally tested only for food fermentation properties and not health benefits, equating live, active cultures and probiotics is not correct. Until the live cultures are shown to confer a health benefit, they should not be called probiotic. Therefore, not all fermented foods, even those retaining live cultures, should be considered to be probiotic foods. Another misuse of the term *probiotic* comes from equating probiotic with native beneficial bacteria. Given that probiotics must be isolated, characterized, demonstrated to have a health benefit, and then administered, it is not correct to talk about “native probiotic bacteria.” A final consideration with regard to probiotic composition is that probiotics must be defined. The FAO published guidelines for characterizing a probiotic that specifically refer to microbes defined at the strain level. Therefore, this eliminates use of the term *probiotic* to describe undefined combinations of microbes such as fecal enemas, even though a therapeutic benefit has been suggested.

The probiotic strain must be properly identified at the genus and species level according to current scientific practices, generally deoxyribonucleic acid (DNA)-based, and properly named according to current nomenclature. Furthermore, strain-specific identification is also necessary. Defined probiotic strains should also be deposited into an international culture collection, which provides the scientific community with the resource to repeat published studies. An international strain deposit also provides a standard for comparison of the probiotic entity over time. The ready availability and affordability of genomic sequencing technology have made a requirement for total genomic sequencing of probiotic strains tenable.

### 2.1.18 Mechanism and Sites of Probiotic Action

Many definitions of probiotics proposed through the years are restrictive by stipulating either a mechanism or a site of action (eg, intestinal or extraintestinal). The mechanism of action of probiotics (eg, benefits result from colonizing the intestine or enhancing immune function) has been dropped from the definition, in part, to allow for a product capable of imparting a clear health benefit to be called a probiotic even if the mechanism of action is not fully understood. This is currently the case for many observed effects. Furthermore, this approach does not restrict use of the term to one specific mechanism of action. Requiring that probiotics only impart benefits, for example, by influencing intestinal microbiota, would preclude use of the term for
products able to interact directly with immune cells. Some mechanisms of action associated with probiotics are listed in Table 2.7.

**Table 2.7. Proposed Mechanisms of Action of Probiotics**

- Produce antimicrobial substances, such as organic acids or bacteriocins
- Upregulate immune response (e.g., secretory IgA) to possible pathogens or to vaccines
- Downregulate inflammatory response
- Assist in early programming of the immune system to result in a better balanced immune response and reducing risk of development of allergy
- Improve gut mucosal barrier function
- Enhance stability or promote recovery of commensal microbiota when perturbed
- Modulate host gene expression
- Deliver functional proteins (e.g., lactase) or enzymes (natural and cloned)
- Decrease pathogen adhesion

It is common for probiotic products to be marketed on the premise that they have an important effect on the intestinal microbiota. However, evidence is mixed on the type and extent of impact different probiotics have on the intestinal microbiota. One difficulty is that many such studies were conducted using methodologies that relied on the ability to cultivate the microbial components of the colonizing bacterial communities. Given that modern surveys of intestinal microbiota indicate that a majority of resident microbes are not cultivated using conventional culture techniques, studies using culture methods likely have not assayed the impact of probiotics on some dominant members of the intestinal microbial community. A need remains to assess probiotic impact on bacterial communities using culture-independent DNA-based methods. Review of numerous studies on this topic makes a general conclusion on the role of probiotics on gut microbiota difficult at present because studies differ so widely with regard to probiotic strain(s), dose, subject age (from premature infants to elderly), subject health status, and methods used. One generalization that does emerge from these studies is that strains selected for the ability to resist the challenges of stomach acid and pancreaticobiliary secretions in the small intestine are usually recovered alive in feces and often lead to a transient increase in the levels of
genus of the fed probiotic. This recovery is typically, although not always, dose dependent. Recovery of the probiotic, with few exceptions, is short-lived once feeding is stopped. Exceptions include Collins and colleagues who noted recovery of *Lactobacillus salivarius* UC118 from one adult test subject 100 days after feeding had stopped, whereas the strain could not be isolated from any of the other 19 subjects 3 weeks postfeeding. Schultz and colleagues documented *Lactobacillus rhamnosus* GG in the feces of two infants 24 months after exposure to the probiotic from their mothers during vaginal delivery. Changes in other microbial groups are generally modest and are reversed once feeding has stopped.

There is some evidence that certain probiotic preparations can temper the impact of antibiotics on intestinal bacterial communities. Taken together, probiotics may or may not impact the populations of gut microbiota. However, an influence of a probiotic strain on intestinal microbiota cannot be presumed, and a causal link between such changes and health benefits needs to be established. Furthermore, microbiologic changes can be less important than functional changes. For instance, biochemical parameters, such as lactate, acetate, ammonia, amines, pH, phenols, p-cresol, and enzymatic activities, may be more indicative of health status than alterations in microbial populations. With regard to site of action, recent studies lead one to appreciate that probiotic function is being assessed in sites outside the intestine. Functional benefits in the oral cavity, stomach, vaginal tract, skin, and systemic immune responses have all been evaluated. Recently, pilot studies have looked at the influence of probiotics on symptoms of vernal keratoconjunctivitis, ultraviolet-exposed skin, and sleep patterns in the elderly.

2.1.19 Not All “Probiotics” Are the Same

Products contain different genera, different species, or even different strains of the same species, and not all products should be expected to work the same. Therefore, claims of efficacy should be target specific and should be made only for products that have been found efficacious in carefully designed studies. The marketplace has many examples of different strains of the same species: *Lactobacillus acidophilus* NCFM and La-1; *L. rhamnosus* GR-1 and GG; *Lactobacillus casei* Shirota and DN-114 001; *Lactobacillus reuteri* RC-14 and ATCC 55730; and *Bifidobacterium lactis* HN019 and BB-12. Each of these strains has a unique dossier to document individual health benefits. It is noteworthy, however, that among dozens of European
commercial products, the same biotype (based on pulsed-field gel electrophoresis of chromosomal DNA) was predominant among Bifidobacterium-containing products, suggesting that Bifidobacterium strains used commercially may not be so diverse. Head-to-head comparisons of different strains of the same species in clinical settings have not been conducted. However, several studies have compared different commercial probiotic products and documented differences in efficacy. For instance, Weizman and colleagues compared the effects of B. lactis BB-12 with those of L. reuteri ATCC 55730 on the incidence, duration, and severity of infections among infants in day care. In this randomized, doubleblinded, placebo-controlled trial of 201 infants between 4 and 10 months of age, formula was fed containing either no probiotic or BB-12 or ATCC 55730 at levels of $10^7/g$ dried formula. Infants consuming the BB-12-containing formula showed reduced duration and episodes of fever and reduced episodes and duration of diarrhea. However, only the infants consuming ATCC 55730 also showed fewer clinic visits, reduced absences from the child care center, and lower antibiotic prescriptions. Another comparative study was conducted on five different commercial probiotic preparations assessing the duration of acute diarrhea in children. Products were composed of L. rhamnosus strain GG; Saccharomyces boulardii; Bacillus clausii; a mixture of L delbrueckii var bulgaricus, Streptococcus thermophilus, L. acidophilus, and Bifidobacterium bifidum; or Enterococcus faecium SF68. The study randomized 571 children (3–36 months of age) who presented with acute diarrhea to receive one of these products or unsupplemented oral rehydration solution for 5 days. The results showed that only the L. rhamnosus GG and the product containing a mixture of lactic acid–producing bacterial probiotics were effective at reducing the duration of diarrhea. These studies illustrate that one cannot presume that different commercial probiotic products will perform in the same manner and that an evidence-based approach to product selection is the best approach.

2.1.20 Probiotics Must Be Safe

Probiotics must be safe under the intended conditions of use. Factors to consider include the inherent properties of the microbe, the physiologic status of the consumer, the dose administered, and the possibility that the probiotic could be a potential source of genes in the gastrointestinal tract environment that could be transferred to less innocuous members of the colonizing
microbiota by horizontal gene transfer. For foods, although 100% safety can never be ensured, probiotics must offer a reasonable certainly for no harm when consumed by the generally healthy population, and an assessment of risk versus benefit is not applicable. By contrast, for drugs, safety also includes balancing possible deleterious side effects with potential benefits. Owing to this clear distinction of safety standards and susceptibility of target populations between foods and drugs, it is advisable to use the term probiotic drug when a probiotic is being used on an unhealthy population to cure, treat, or prevent disease. When considering the safety of commercially available products, probiotic products in the United States are either foods or dietary supplements and as such are targeted for use by the generally healthy population. *Lactobacillus, Bifidobacterium, Saccharomyces,* and *S. thermophilus* all have excellent safety records for such uses. *Lactobacillus* and *Bifidobacterium* species have been recovered from patients with bacteremia, but infection from probiotic consumption has not been reported in healthy consumers. Some dietary supplement forms of probiotics include species of *Bacillus, Clostridium, Enterococcus,* and *Escherichia,* genera associated with safety concerns such as toxicity, infectivity, or potential sources of genes that may be transferred to less innocuous members of the commensal microbial community. These genera of bacteria should only be used when the manufacturers adequately demonstrate safety of the specific strain they intend to market for the target populations. Use of probiotics in either diseased or immunocompromised individuals must be done mindfully. Frequently, controlled studies reporting no product-related adverse incidents have been conducted in unhealthy or at-risk subjects, such as very low birth weight infants, patients with chronic inflammatory bowel diseases, intensive care unit patients, and patients with acute infectious diarrhea. Successful outcomes to such studies suggest that the identical product could be used with similar subjects under medical supervision. However, a report of increased mortality in the probiotic-consuming group of a randomized, clinical trial in subjects with acute pancreatitis highlights the importance of care when designing and launching studies with compromised individuals. As a general rule, caution should be used when considering probiotics in newborns, immunocompromised patients, patients with severe underlying illness, or short bowel patients (who are at increased risk of bacterial translocation). Another risk was reported when catheter line contamination leading to fungemia was observed in hospitalized patients given *Saccharomyces.* The manufacturer should be able to provide guidance as to the type and extent of safety assessments that have been conducted on its product.
Choosing probiotic products can be difficult. Several documents have been prepared by professional societies to assist health care professionals and consumers in this process. As mentioned previously, probiotic products in the marketplace in the United States are either foods or supplements. These products make different types of relevant claims on their labels and in advertising: content claims and health benefit claims. In the United States, foods and dietary supplements are allowed to make what are called structure or function health benefit claims, which relate the product to a physiologic effect on the normal (not diseased) structure or function of the human body. Such claims are required by statute to be “truthful and not misleading.” Therefore, companies making such claims are required to have substantiating scientific documentation. However, the claims are not subjected to premarket regulatory approval. In practice, the FDA does not police the accuracy or degree of substantiation for such claims; therefore, it is likely that some number of commercial products assert unsubstantiated communications on labels, Web sites, or advertising.

With regard to content claims, numerous published articles report independent analysis documenting probiotic products that do not have either the number or the type of microbes claimed on the label. Although Good Manufacturing Practices exist for both foods and dietary supplements, products still may not be accurately labeled. Accordingly, it is difficult for the consumer and the health care professional to distinguish the substantiated from the unsubstantiated claim.

- Probiotic Claims

Currently, there is no third-party objective rating system for product claims on probiotic products. Most probiotics are sold as either dietary supplements or ingredients in foods and cannot legally declare that they cure, treat, or prevent disease. Therefore, it is common to see claims such as “supports a healthy immune system” or “helps keep your microflora in balance.” What comprises a legitimate dossier to substantiate such claims is beyond the scope of this article. However, manufacturers should provide citations to published references that support health benefit claims. The quality of the studies and the extent of the effects observed provide quality of support for the claim. Given that different strains of the same species may have
different health effects, it is logical to make certain that claims of health benefits are based on research done on the particular probiotic. The product should contain the specific strain(s) of bacteria at the same levels used in the published supporting research.

-Dose

Product effects are also dose specific. It is not possible to provide one “minimum dose” that applies to all probiotics because different probiotics are effective at different levels. Some products are effective at 50 million colony-forming units (CFUs)/day to more than 1 trillion CFU/day. This huge range in effective doses likely reflects differences in strains, clinical end points, and perhaps the best guess of the researcher of what level would be sufficient. Dose-response studies are not common in the probiotic literature database. Therefore, it is essential that product doses should be based on levels that were tested in human studies and shown to be effective.

-Label

The label should disclose the genus, species, and strain designation of each probiotic strain contained in the product. This approach provides a level of confidence that the product manufacturer is formulating the product with specific strains consistently over time. Furthermore, strain designations tie the product content back to the scientific publications that document claimed health effects. The product label should also indicate the number of live microorganisms that are delivered in each serving or dose, as well as an expiration date. Levels are typically communicated as CFUs. The suggested serving size or dose should be indicated. Labels should describe health benefits that have been substantiated for the product. Finally, proper storage conditions and corporate contact information (including a Web site or consumer hotline number where additional information can be obtained) should be indicated.

-Medical Recommendations

Efforts to provide clinical recommendations for probiotics were published recently. Meta-analyses of available high-quality studies are also helpful in this regard. However, these efforts are hampered by limited data for many specific probiotic strains. Only 2 of over 25 meta-analyses and systematic reviews published to date focused on specific strains: Szajewska and
Mrukowicz’s article on *Saccharomyces cerevisiae* var *boulardii* strain Lyo and Szajewska and colleagues’ article on *L. rhamnosus* GG. The remaining publications have grouped together studies conducted on different probiotic preparations. This approach is not without merit as it indicates which preparations have positive results. However, compelling data suitable for meta-analysis for any one preparation are not available for most probiotics. Sometimes medical recommendations are based on a limited number of positive studies.

### 2.1.22 Availability of Probiotics/Sources of Probiotics

Probiotics can be found in capsule, liquid, powder, or tablet form. Acidophilus drinks can be found in health food stores and some grocery stores and Asian grocers.

Probiotics can also be found in cultured dairy products such as yogurt or kefir, however, the number of live organisms varies greatly from product to product due to differences in processing methods. Fermented foods such as sauerkraut also contain probiotics.

Once ingested, probiotics colonize the intestines and other parts of the body and can sustain themselves unless they are destroyed by antibiotics or other factors.

Although they are thought to be essential for health, because they can sustain themselves in the body under normal circumstances, there is no recommended daily intake of probiotics. Probiotics are available in foods and dietary supplements (for example, capsules, tablets, and powders) and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and unfermented milk, miso, tempeh, and some juices and soy beverages. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation.

### 2.1.23 Organisms used as Probiotics

Most probiotics are bacteria similar to those naturally found in people's guts, especially in those of breastfed infants (who have natural protection against many diseases). Most often, the bacteria come from two groups, *Lactobacillus* or *Bifidobacterium*. 
Within each group, there are different species (for example, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*), and within each species, different strains (or varieties). A few common probiotics, such as *Saccharomyces boulardii*, are yeasts, which are different from bacteria. Some probiotic foods date back to ancient times, such as fermented foods and cultured milk products. Interest in probiotics in general has been growing; Americans' spending on probiotic supplements, for example, nearly tripled from 1994 to 2003.

### 2.1.24 Prebiotics in the Diet:

Prebiotics that feed the beneficial bacteria in your gut mostly come from carbohydrate fibers called oligosaccharides. You don't digest them, so the oligosaccharides remain in the digestive tract and stimulate the growth of beneficial bacteria.

Sources of oligosaccharides include fruits, legumes, and whole grains. Fructo-oligosaccharides (compare prices) may be taken as a supplement or added to foods. Yogurt made with bifidobacteria contain oligosaccharides.

### 2.1.25 Probiotics in the Diet:

Probiotic bacteria like lactobacilli are naturally found in fermented foods like sauerkraut and yogurt. Some foods will have added probiotics as healthy nutritional ingredients, which will be evident on the label.

### 2.1.26 Prebiotic and Probiotic Supplements:

Probiotics are widely available as supplements. However, not all probiotic supplements are created equal. To make sure your probiotics get a good start, add some oligosaccharides to keep the probiotic bacteria healthy in your digestive system. The prebiotics may be taken as a supplement or may be added to your health foods. Be sure to check your local health food stores and look on labels when you buy probiotic supplements.

### 2.1.27 Probiotics and Children

In fact, the use of probiotics can be very beneficial for your children. A healthy digestive system is very important for a strong immune system in kids as well as in adults. The human intestinal tract is home to millions of friendly bacteria. These friendly bacteria make substances that help
keep the cells in the intestinal tract healthy, plus they keep unfriendly bacteria, yeasts and molds in check. Sometimes the good bacteria become out of balance with the unfriendly bacteria, and the bad bacteria, yeast and mold grow unchecked.

This can happen with use of antibiotics or with a bad diet. Probiotics are friendly bacteria found in foods and dietary supplements that can help bring back the balance of good bacteria. They can be taken as a daily supplement at any age as they heavily influence the entire digestive and immune system. Probiotics can help with any specific childhood disorders. If a child has been on an antibiotic, it is extremely important to replenish the beneficial bacteria lost by taking a quality probiotics supplement over the following two weeks.

Probiotics can help boost the immune system whenever a child might be coming down with symptoms of a cold or flu. Allergies are another indication of one's immune system underfunctioning and probiotics are ideal in this situation. Acne and any digestive issues like irritable bowel syndrome or Crohn's disease would also be an indication.

2.1.28 Researchs on probiotics

2.1.28.1 Some other areas of interest to researchers on probiotics are

- What is going on at the molecular level with the bacteria themselves and how they may interact with the body (such as the gut and its bacteria) to prevent and treat diseases. Advances in technology and medicine are making it possible to study these areas much better than in the past.
- Issues of quality. For example, what happens when probiotic bacteria are treated or are added to foods—is their ability to survive, grow, and have a therapeutic effect altered?
- The best ways to administer probiotics for therapeutic purposes, as well as the best doses and schedules.
- Probiotics' potential to help with the problem of antibiotic-resistant bacteria in the gut.
- Whether they can prevent unfriendly bacteria from getting through the skin or mucous membranes and traveling through the body (e.g., which can happen with burns, shock, trauma, or suppressed immunity).
2.1.28.2 Considerations for the Future

The increased worldwide interest in probiotics has set the stage for expanded marketing of these products, even though much research remains to be done. Some research goals are shown in Table 2.8. Broadened clinical evaluation in both healthy and diseased human populations will do much to increase understanding of important aspects of probiotic bioactivity, including strain specificity, dose requirements and extent of clinical efficacy. One exciting area of current research is chromosome sequencing of probiotic *Lactobacillus* species, including *L. johnsonii* Lal (Zink, 1999) and *L. acidophilus* (Cano and Willoughby, 1999; Klaenhammer, 1998). The information gleaned from sequence data will provide opportunity to improve probiotic functionality and expand understanding of mechanisms. The application of gene-based technologies to track the influences of probiotic consumption on gut microecology is another exciting area of research (Kitts, 1999). Dynamic models of the stomach, small intestine, and colon to simulate the *in-vivo* gastrointestinal environment have been developed (Marteau et al., 1997; Minekus et al., 1995) and may provide an important link between conventional *in-vitro* methods and *in-vivo* clinical studies. Advances in the understanding of genetic transfer systems and gene regulation of the lactobacilli (Klaenhammer, 1995) have enabled the construction of isogenic strains of lactobacilli that can be applied to animal or human feeding studies to determine which strain attributes are essential for probiotic effects. The use of a *Lactobacillus*-free mouse colony could be quite useful for such controlled studies (Tannock et al., 1988). Increased clinical evaluation is paramount, and will be most likely to succeed if well-defined probiotic bacteria are used in established clinical systems.

The benefit of probiotics as healthful ingredients could be enhanced if used in combination with other health-promoting dietary strategies. In the United States, the growth of the probiotics market is occurring mostly in the dietary supplement arena. Whole foods, however, represent a very attractive vehicle for delivery of probiotics, although delivery through a traditional dietary supplement is convenient for some (especially clinical) applications. Probiotic bacteria have always had a natural association with dairy foods. Dairy products containing probiotics provide not only viable bacteria, but also high-quality macronutrients and unique micronutrients (such as calcium, fermentation end-products, bioactive peptides, sphingolipids, and conjugated linoleic acids) found in fermented milk products (McBean, 1998; 1999; Van der Meer et al., 1998). At
the same time, the natural buffering of stomach acid by the food carrier would enhance stability of the probiotic after consumption. Formulation of food products with additional vitamins, non-digestible carbohydrates, soluble fiber, phytochemicals, or other bioactive ingredients could further enhance the dietary value of the product.

**Table 2.8 Future research in the probiotics area**

- Determine the physiological role, mechanisms of action, and extent of influence of probiotics in human health using human feeding studies. Studies on high-risk human populations for colon cancer or cancer recurrence would be a possible target for some studies.
- Validate biomarkers used for assessing probiotic function. Testing of predictions based on biomarker studies with actual results in human clinical evaluations is needed. Biomarker validation in the areas of immune system, cancer, and gut microecology is especially important. Once validated, biomarkers will be useful tools to assess dose-dependence and strain-specific responses. Biomarkers commonly used to select strains for probiotic use (adherence in tissue cultures, cholesterol assimilation, competitive exclusion of pathogens in tissue culture, inhibitor [bacteriocins, organic acids] production, lactase activity) have never been tested in controlled studies to determine if mutants without the characteristic perform any differently in clinical evaluations.
- Assess effects of probiotics on populations and activity of gut microbes. The application of genebased methods holds much promise in this field.
- Determine the role of probiotics as part of a whole food compared to isolated component.
- Improve reliability and ease of taxonomic classification of probiotic bacteria. Improve strain performance and activity.
- Conduct studies with consumers to understand how best to communicate the concept of probiotics and to determine favorable probiotic formats.
- Conduct research to improve product formats, consumer acceptance, stability, and efficacy of probiotic-containing products.
2.1.28.3 Recent research on Probiotics

- Probiotics prevent asthma

Sunday, October 18, 2009 by: Mike Adams, the Health Ranger, Natural News Editor showed that probiotics help prevent asthma. Asthma is often the result of systemic inflammation caused by food allergies. Probiotics can help deal with food allergies, preventing the inflammation and helping to eliminate many health problems (not just asthma, but also some skin conditions and intestinal disorders as well).

A short collection of research quotes about probiotics and asthma, gathered from some of the top health books in the world are provided below.

-Asthma is one of the most common chronic diseases in childhood and one of the most common causes of school absenteeism. Asthma is inflammation of the bronchial tubes. It causes obstruction of the airway, chest tightness, coughing and wheezing. If probiotics are primarily in the intestinal tract, they would probiotics aid the lungs and help to fight asthma since probiotics have a great influence on the immune system.

-Probiotic Rescue: How You can use Probiotics to Fight Cholesterol, Cancer, Superbugs, Digestive Complaints and More by Allison Tamis

The intestinal microflora plays a role in immune system regulation. Probiotics promote healthy intestinal barriers and immune reactions. Lack of probiotics in infants may prevent proper immune maturation leading to allergies. Leaky gut allows allergens through the intestines and promotes allergies. Probiotics appear to prevent and treat certain atopic diseases (asthma, eczema). Eczema can be effectively treated with probiotics. Asthma may be prevented or treated with probiotics.

-Research shows that both asthma and eczema are linked to abnormal immune responses in newborns. Finnish researchers have found that children who were exposed to probiotics around the time of birth were 40% less likely to develop atopic eczema than children in a placebo group; however, exposure to probiotics did not have any protective effect against asthma. Lactobacillus casei can reduce the rate of eczema in infants, but there has been no word yet on its ability to prevent asthma.

-If steroids/antibiotics have been taken for some time, probiotics friendly flora is needed for
actions to boost your own auto-immune system. Probiotics are essential in preventing disease and maintaining health.

The double-blind, controlled study was conducted with 326 children between the ages of three and five separated into three groups that received one of three milk solutions twice a day for six months. The first group received milk containing the probiotic strain *Lactobacillus acidophilus*, the second group received milk containing *L acidophilus* as well as *Bifidobacterium animalis*, and the third group received plain milk.

The results of the single and combination probiotic groups, respectively, were reductions in fever incidence by 53% and 72.7%, coughing incidence by 41.4% and 62.1%, and rhinorrhea incidence by 28.2% and 58.8%, relative to placebo. Duration of fever, coughing, and rhinorrhea were also reduced by 32% and 48%, respectively. Consequently, there were crucial reductions in both the use of antibiotics in the single and combination probiotic groups equaling 68.4% and 84.2% as well as in truancy days from group child care equaling 31.8% and 27.7%, respectively.

Overall, the study results definitively prove the efficacy of probiotic supplementation in maintaining health and preventing disease. Since about 70% of a person's immune system resides in the glands, mucosa, and mucosa-associated lymphoid system of the gastrointestinal tract, it is vital that the intestinal flora residing in the tract maintain optimal levels and function. The colonic bacteria that interact with the gastrointestinal lymphatic and immune tissue are what regulate the systemic immune system and inhibit the bacterial enzymes responsible for synthesizing colonic carcinogens. In other words, probiotics populate the gut and maintain a vibrant immune system by fending off foreign invaders from taking over while simultaneously assimilating vital nutrients from food and supplements.

While the body is naturally inhabited by healthy probiotics, inadequate diet, environmental toxins, antibiotics, and other foreign invaders can disrupt and even destroy intestinal flora leading to stomach illness, digestive problems, and other serious maladies such as Crohn's disease or colon cancer. Thus, probiotic supplementation is a necessary component to any healthy lifestyle, whether it be through eating cultured and fermented foods like raw milk, kefir, yogurt, miso or kombucha, or through taking probiotic powder, liquid or capsule supplements.

Some of the popular probiotic strains commonly present in cultured foods and supplements include *L rhamnosus, L casei, B Bifidum, B longum, B lactis, L paracasei, L plantarum, L hexosa, L acidophilus, B animalis, B bifidum, B lactis, L casei, and L paracasei*.
salivarius, L bulgarious, L sporogenes, as well as L acidophilus and B animalis which were the other bacterial strains used in the study.

While there is still much to learn concerning probiotics, it is certain that they are gaining popularity in the mainstream for their essential health-maintaining properties.

- **New Study: Probiotic Strain Boosts Immune Response to Flu Virus**

Thursday, May 14, 2009 by: S. L. Baker, features writer (Natural News) A new study published in the journal *Postgraduate Medicine* has good news about a way to help fight a potential flu pandemic, naturally. Researchers found that a specific strain of probiotics, which are beneficial microorganisms similar to the "friendly" bacteria found naturally in the body's digestive system, increases the body's immune response to the flu virus -- specifically, to influenza A. And the currently much hyped and much feared so-called swine flu, also known as H1N1, is a variant of influenza A.

Although many mainstream medical doctors as well as natural health practitioners have long recognized that probiotics can often help people with digestive disorders such as irritable bowel syndrome (IBS), diarrhea, gas, and bloating, the idea that taking probiotics could help healthy people stay that way has been controversial. But the new study could change that notion. It shows that taking probiotics regularly can boost the immune system in a specific way which helps the body give influenza A the boot. The probiotics strain, which has the scientific name *Bacillus coagulans* GBI-30, PTA-6086, was found to cause significant increases in T-cell production of TNF-alpha, a key immune system activity marker, when adults were exposed to influenza.

Researcher Mira Baron, MD, measured changes in blood TNF-alpha levels in 10 healthy adult volunteers before and after they took doses of the probiotic strain Bacillus coagulans (which is marketed under the trade name GanedenBC30 and found in various dietary supplements) daily and then were exposed to an influenza A virus. Results showed a huge increase in TNF-alpha levels upon viral challenge after the research participants had taken the probiotic for about a month. Dr. Baron noted in her study that the initial, dramatic increase in the body's production of TNF-alpha in response to viral exposure shows a heightened immunological response aimed at protecting against infection.

The study did not evaluate an immune response to the specific swine flu virus, H1N1, currently
causing much worry. However, there's certainly reason to think that Bacillus coagulans could boost the body's natural defenses to fight a variety of flu viruses, including swine flu. "The study helps support the long-suspected belief about the beneficial effects of GanedenBC30 on the immune system and adds to the emerging body of evidence that probiotics can benefit healthy people as well as those with specific health issues."

Dr. Gary Huffnagle, a professor of microbiology and immunology at the University of Michigan, and author of the book *The Probiotics Revolution* reviewed Dr. Baron's findings and concluded the research adds to the growing body of scientific data that show probiotics boost the immune function of healthy adults to defend against infection and lessen the symptoms of disease. "I think it is a wise move to include the consumption of probiotics, such as Sustenex (a supplement that contains Bacillus coagulans), along with good diet, frequent hand washing and other recommendations by the CDC in the battle against flu. While more research is needed to demonstrate whether this translates into reduced hospitalization and/or deaths, it's a healthy, low-cost, proactive thing that people can do that has no risks associated with it."

**Probiotics Found to Block Pneumonia in Critically Ill Patients**

Wednesday, March 11, 2009 by: David Gutierrez, staff writer (Natural News) Probiotics are just as effective as conventional antiseptics at suppressing pneumonia-causing bacteria in the mouths of critically ill patients, according to a study conducted by researchers from University Hospital in Lund, Sweden, and published in the journal *Critical Care.* Probiotics are live microorganisms that provide a health benefit inside the body. Pneumonia is a well-known risk of being placed on a ventilator for assisted breathing, and often occurs when bacteria from the mouth are inhaled into the lungs from the mouth through the breathing tube. To prevent this from occurring, hospitals regularly swab the mouths of breathing tube patients with the antiseptic chlorhexidine. Some patients, however, may have allergic reactions to this drug, while a small number of bacteria are resistant the drug. Probiotics, in contrast, improve the body's own immune system rather than acting on the infectious bacteria directly. In contrast to antiseptics, which must be reapplied every few hours, a single probiotic swab can remain effective for 24 hours. In the current study, researchers treated 25 critically ill patients using breathing tubes with the standard care of tooth brushing, suction to remove mouth secretions and a twice-a-day swab with
clorhexidine. They treated 25 others with the same care, but replaced the clorhexidine swab with one of carbonated water and then Lactobacillus plantarum 299 (Lp299). Lp299 naturally occurs in the human mouth, as well as in fermented food products such as pickles, sauerkraut.

Prior to using the antiseptic or probiotic swab on days 1, 2, 3, 5, 7, 10, 14 and 21 of the study (which began for each patient at the same time as ventilation), the researchers swabbed each participant's mouth for bacterial culturing. They found no significant difference in the bacterial content of the mouths of patients who had been treated with probiotics and those who had received an antiseptic swab. Patients receiving probiotics also spent the same amount of time on ventilation and in the hospital, and had the same risk of death as those receiving the antiseptic.

- **Probiotics Improve Infant Immune Function**

Monday, December 01, 2008 by: David Gutierrez, staff writer (NaturalNews) A probiotic treatment for pregnant women and their infants was successful in improving the immune function of the newborns, in a study conducted by researchers from Helsinki University Central Hospital in Finland and published in the journal *Pediatrics*. The researchers treated pregnant woman with a mix of four probiotic bacteria (Lactobacillus rhamnosus GG and LC705, Bifidobacterium breve Bb99 and Propionibacterium freudenreichii ssp shermanii) for the last four weeks of pregnancy, then treated their newborns with the same mixture plus prebiotics called galactooligosaccharides (GOSs) for the first six months of life. GOSs are also found in breast milk.

The infants were examined after 3, 6 and 24 months, and the mothers filled out questionnaires about the children's health at 3, 6, 12 and 24 months. The researchers found that while 28 percent of children in the placebo group had been prescribed antibiotics, only 23 percent of children in the probiotic group had. Likewise, the average number of respiratory infections per child in the placebo group was 4.2, compared with only 3.7 in the probiotic group. The researchers did not find any difference in growth, infant health, morbidity or other adverse health effects between the two groups.

- **Probiotics Linked to 70 Percent Reduction in Kidney Stones**
People who naturally carry a probiotic bacteria called Oxalobacter formigenes are 70 percent less likely to develop kidney stones than people whose dietary tracts lack the bacteria, according to a study conducted by researchers from Boston University and published in the *Journal of the American Society-of-Nephrology*.

Researchers compared 247 people who suffered from recurring calcium oxalate kidney stones with 259 people with no history of kidney stones. They found that while 38 percent of the people in the healthy group had O. formigenes in their intestines, only 17 percent of people in the kidney stone group did.

Approximately 80 percent of all kidney stones are made of the compound calcium oxalate, which builds up in the kidneys in small, hard lumps. Kidney stones can also move into other parts of the urinary tract, causing intense pain, infection and even kidney failure. Kidney stones have a tendency to recur, meaning that a single person can suffer from them many times. O. formigenes is believed to prevent kidney stone formation by breaking down calcium oxalate in the intestinal tract before it can move into the kidneys.

The exact reasons for kidney stone formation are not known, but scientists believe that the problem is related to dehydration and a high rate of calcium excretion. Most patients are treated through the use of shock waves to break up the stones, a treatment that is only sometimes effective.

According to Derek Machin, clinical director of urology at University Hospital, Aintree, any more effective treatment would be a major advance.

**Taking Probiotics Greatly Reduces Infections in Athletes**

Athletes who took probiotic supplements suffered fewer infections and recovered more quickly than those who did not, in a study conducted by the Australian Institute of Sport in Canberra and published in the *British Journal of Sports Medicine*.

Researchers studied 20 top-level, long-distance endurance runners for two months, assigning them to take either a placebo or a supplement of the probiotic bacterium *Lactobacillus fermentum*. During that time, the athletes recorded any day on which they experienced symptoms of winter illnesses, including coughs and runny noses.
The researchers added together the total symptom days of both groups, and found that while the normal group experience symptoms for a total of 72 days, the probiotic group experience only 30 days.

In addition, blood tests revealed that the athletes who were taking probiotics had twice the levels of an immune chemical known as interferon gamma as the athletes in the placebo group. While researchers do not understand how probiotics function, particularly since they appear to be effective even in small concentrations, an increasing body of evidence shows that these beneficial bacteria can boost the body's immune system, among other beneficial health effects. Recent research has also found that probiotic bacteria help regulate metabolism.

The research was carried out on athletes, because the strenuous training undergone by marathon runners is known to compromise the immune system, and even minor cold symptoms can seriously setback training regimen.

- **Probiotics Ease Gut Problems Caused by Long Term Stress (press release)**

Monday, July 24, 2006 by: NaturalNews, citizen journalist

Probiotics may help to reduce gut symptoms caused by long term stress, indicates research published ahead of print in the journal Gut.

The researchers base their findings on analysis of gut tissue taken from rats subjected to either water avoidance stress, which involves placing the rat on a small platform surrounded by water, or sham stress for one hour a day for 10 consecutive days. The stress sessions were designed to mimic psychological stress to produce the type of effects that would be seen in the human gut.

Half the rats were fed drinking water containing probiotic bacteria in the form of *Lactobacillus helveticus* and *Lactobacillus rhamnosus* for a period of seven days before and during the stress sessions. Bacteria were also detected in the mesenteric lymph nodes, which drain fluid coming from the intestine, indicating that bacteria had entered the body and activated the immune system.

However, probiotic treatment minimised the changes in chemical signalling and prevented bacterial “stickiness” and movement to the mesenteric lymph nodes.
Chronic stress is known to be implicated in the development of irritable bowel syndrome and in the worsening of symptoms of inflammatory bowel disease, such as Crohn's disease and ulcerative colitis. It also sensitises the gut, producing allergies to certain foodstuffs.

The authors say that probiotics literally compete for space with harmful bacteria and dampen down inflammatory responses, and as such, offer a potentially promising approach to the management of intestinal problems caused by stress.

2.2 Gut Microflora

The human colon is the body's most metabolically active organ. This is because of the resident microbiota, which comprises $10^{12}$ bacterial cells for every gram of gut contents. There are numerous publications purporting that probiotic are active in the gut after ingestion, others have questioned such claims and the beneficial effects that probiotics are said to confer their hosts.

In terms of the microbiology of different digestive tract areas, there is variability both in terms of composition and activity. The lumen of the human stomach is essentially sterile due to a low gastric pH. However, micro-organisms are known to reside in the mucosal layer that overlies the gastric epithelium. This includes Helicobacter pylori, which has attracted a great deal of research interest. This organism uses its flagellae to invade the gastric mucus layer and thereafter adhere to epithelial cells. In conjunction with a production of ammonia, this allows effective colonization of the stomach (Rathbone and Heatley, 1992).

In the small intestine, the transit time of gut contents tends to maintain bacterial numbers at below $10^6$ / ml of contents. Intestinal secretions like pancreatic enzymes and physiochemical variables such as pH also contribute towards the type of microflora that develop. Facultatively anaerobic and aerotolerant bacteria such as streptococci, staphylococci and lactobacilli dominate the upper small gut with bacterial numbers showing a progressive increase.

In comparison to other regions of the gastrointestinal tract, the human large intestine is an extremely complex microbial ecosystem, with at least several hundred different bacterial species being present. The environment is favourable for bacterial growth with a slow transit time, ready availability of nutrients and favourable pH. The vast majority (>90%) of the total cells in the body are present as bacteria in the colon. It is thought that over 60% of the faecal mass exists as prokaryotic cells. Generally, the various components of the large intestinal microbiota may be considered as exerting pathogenic effects or they may have potential health promoting values.
Given that the microbiota has components that are positive for human health, there is currently much interest in the use of diet to specifically increase groups perceived as health promoting. As such, the gastrointestinal flora and its activities are a major focus for functional food developments.

2.2.1 Microbiological aspects of large intestine

The large intestine harbours the largest and most complex microbial ecosystem associated with the human body, consisting of several hundred different strains of anaerobic bacteria, with numbers exceeding $10^{11}$/g of intestinal contents (Fooks et al., 1999; McBain and Macfarlane, 1998). This is because of the resident microbiota, which comprises $10^{12}$ bacterial cells for every gram of gut contents. As the large intestine usually contains about 200 g of contents, there is enormous biological activity. The fact that these activities can be modulated or perhaps even controlled through diet is of high relevance (Gibson et al., 2000). The microbiota is involved in the catabolism of a vast range of dietary and endogenously secreted compounds. The products of these biotransformations are often toxicological significance to the host. For example, the occurrence of colon cancer is greatly influenced by diet, while metabolism of dietary components by intestinal bacteria has been demonstrated to be an important factor in tumour initiation. The colonic microflora may be involved in the aetiology of large bowel cancer by chemical modification or activation of a wide variety of chemical agents with carcinogenic or co-carcinogenic potential. Exposure of the intestinal microbiota to potential toxicants may occur due to their presence in the diet by biliary excretion of endogenously metabolised substances into the intestine, enzymic activation of procarcinogens by the gut microflora or by direct producing mutagenic substances by intestinal microorganisms (McBain and Macfarlane, 1998). In terms of functionality, the human colon is the body's most metabolically active organ.

Besides vertical transmission of microorganisms, the body surfaces mentioned are contaminated during and directly after birth with a variety of microbial strains from the immediate environment (horizontal transmission). A number of these microorganisms will colonise these body surfaces permanently or temporarily, while others disappear. The temporarily colonising strains act as pioneers, which initiate the successive domiciliation of other microorganisms. During time, under normal conditions, the microflora will mature to a balanced composition of many different microorganisms.
The occupation of special niches depends on the local environmental circumstances in these specific habitats. These local conditions are determined by multifactorial interactive processes between the host and the microorganisms. Therefore, the mature composition of the natural microflora is specific to the animal species and even specific to an individual. In general, the natural microflora is called the indigenous microflora of a given species (including autochthonous and allochthonous microorganisms), and the indigenous microflora of a given individual (mainly autochthonous microorganisms), respectively. However, the terminology around symbiosis of microorganisms on body surfaces is often confusing.

Species specificity of the microflora has been shown in several studies. Characterisation of Lactobacillus and Bifidobacterium from humans and different animal species shows various biotypes. The exchange of Lactobacillus from one species to another shows that these bacteria do not colonise mutually and that normalisation of the intestinal microflora do not occur by inoculating germ-free animals with a complex microflora from other animal species. Colonisation in specific habitats within the intestinal tract is demonstrated by the fact that some strains are found in crypts while others are found on epithelial surfaces of the villi and that some microorganisms (filamentous bacteria) are found on specific sites (columnar epithelium) in the small intestine.

During one's lifetime further adaptation of the indigenous microflora occurs, due to the changing local circumstances on the condition that these changes take place gradually, such as due to ageing, and not abruptly (e.g. by a sudden change in food composition or drug use).

A schematic review of the predominant microflora of human body surfaces has been published by Tannock (1988). In mature microbial ecosystems, most microorganisms are obligately anaerobic, even on surfaces which are in direct contact with the air. The density of the microbial population varies from site to site, but can reach $10^8$ bacteria per ml of saliva and $10^{10}$ per gram contents of the large intestine. The numbers of eukaryotic cells are very low in comparison to prokaryotic cells. The diversity of microbial species or strains is thrilling. Progress in isolation and identification techniques, such as the use of anaerobic chambers, cell-wall analysis and DNA-DNA hybridisation, offer an increasing possibility to discriminate between the different microbial strains, for example, on the gene level.
2.2.2 Importance of an indigenous microflora

A well-established and matured indigenous microflora on external and internal body surfaces of animals and man is very stable. The penetration and colonisation of non-indigenous microorganisms from the environment and/or from other animal species (xenochthonous microorganisms) onto these body surfaces is hindered.

The importance of an indigenous microflora in the gut as a natural resistance factor against potential pathogenic microorganisms was already recognised in the 19th century by Metchnikoff during his research on cholera. Many decades later the role of the indigenous microflora received renewed interest after findings in laboratory animals orally treated with antibiotics. The antibiotics caused intestinal disturbances owing to infectious agents. It was suggested that this effect was induced by suppressing the normal gut microflora. Later on, the protective effect of the normal intestinal microflora in chickens against Salmonella infantis infection was shown by Nurmi and Rantala (1973). Colonisation resistance of the gut microflora was further confirmed for Salmonella and for other pathogenic bacteria such as Escherichia coli, Clostridium and Yersinia enterocolitica. Although Impey et al. (1982) demonstrated a protective effect of 48 selected bacterial strains, the colonisation resistance is most effective when a complete species-species microflora has settled down. This has been demonstrated in ‘normalisation’ studies with germ-free animals inoculated with several dilutions of the total intestinal microflora from normal animals of the same species.

The colonisation resistance induced by an indigenous microflora is partly based on occupation of available niches (competitive inhibition of binding sites) and autogenic regulation factors (e.g. synthesis of fatty acids, hydrogen peroxide, bacteriocins). Another important factor might be the non-specific activation of the immune system. The gut, the mucosa, as well as the skin, have humoral and cellular immune systems which can influence the composition of the microflora (gut microflora). It has been shown that the activity of the immune system of germ-free animals is very low since less γ-globulin, smaller lymph nodes and fewer lymphocytes and phagocytes were found. Activation of macrophages was noticed following the introduction of indigenous microorganisms. In addition to this stimulating effect on non-specific resistance factors, the gut microflora has also an important complimentary function in the digestion of dietary components, such as plant polymers and the synthesis of vitamins (Wood, 1996).
2.2.3 Establishment of bifidobacteria in infants

The composition of human fecal flora changes with advancing age (Naidu et al., 1999). Initially, the foetus exists in a sterile environment until birth (Mackie et al., 1999). After birth it rapidly becomes colonised by bacteria, especially bifidobacteria (Wolin et al., 1998) from the maternal vagina and other environmental sources (Mutai and Tanaka, 1987). In both breast-fed and bottle-fed infants, the large intestine is first colonised by Enterobacteria, Streptococci, including Enterococci, Clostridia on the 1st to 2nd day of life. On the 3rd day, bacteroides, bifidobacteria and clostridia have been isolated from over 40% of infants. Between days 4 and 7, bifidobacteria becomes more predominant accounting for 1010-1011 organisms per gram of faeces of breast-fed infants, exceeding enterobacteria by 100-1000 times. Clostridia, bacteroides, streptococci and staphylococci decrease, whereas enterobacteria are the predominant organisms in the bottle-fed infants, exceeding bifidobacteria by about 10 times. Thus, nearly 100% of all bacteria cultured from the stools of breast-fed infants were bifidobacteria. At 1 month of age, bifidobacteria were the most prevalent organisms in both groups but the number of these organisms in the stool of bottle-fed infants was approximately one-tenth that of breast-fed infants.

Benno and Mitsuoka (1986) also identified all isolates from the stools of healthy infants during the first week of life down to the species level. A total of 37 different species or biovars were obtained. No Bifidobacterium or Eubacterium sp. was recovered from any of the neonates on the first and second days of life. Clostridium paraputrificum and Bacteroides fragilis group were detected from the faeces on the first day of life. From the third to fourth day of age, however, Bifidobacterium sp. were recovered from the stools of healthy neonates. The incidence of the Bacteroides fragilis group, Clostridium tertium, C. paraputri ficum and Klebsiella pneumoniae also increased. Escherichia coli was the most common facultative species isolated from the stools of all healthy neonates. The next most common species, Enterococcus faecalis, Streptococcus and Staphylococcus epidermis, appeared on the first day after birth. At the end of the study period, E. coli, E. faecalis, S. epidermis and Streptococcus sp. had frequently been isolated from 90% of neonates.

2.2.3.1 Differences of the faecal flora between breast-fed and bottle-fed infants

Tissier's turn of the century microscope observations of the faeces of breast milk-fed infants are still valid, but the situation regarding formula-fed babies appears to have altered with
improvements to formula feeds which now resemble, but still do not exactly match, the composition of human milk. Well documented modern studies show that bifidobacteria are just as common and likely to be numerically dominant in the faeces of formula-fed as in breast-fed infants. There is considerable infant-to-infant variation in the population size of particular bacterial genera during the first week of life in both infant groups which may have contributed to the somewhat variable interpretations of the status of the infant microflora reported in the literature. More consistent values are obtained in babies older than one week, however, and realistic comparisons between infant groups are possible.

Colonization of the gastrointestinal tract of newborn infants occurs within a few days of birth (Simon and Gorbach, 1984). The inoculum may be derived either from the mother's vaginal or faecal flora (in a conventional birth) or from the environment (in a caesarian delivery). Initially, facultative bacterial species such as Escherichia coli or streptococci, are transferred. These are relatively nutritionally undemanding bacteria. Subsequently, their activities create a highly reduced environment which allows the development of the strictly anaerobic bacteria that will later dominate the colon. Dependent on the type of feeding regime given in early life, there appears to be variability in microflora development. The breast-fed infant has a preponderance of bifidobacteria, which easily outcompete other genera. In contrast, the formula-fed infant has a more complex flora which resembles the adult gut in that bacteroides, clostridia, bifidobacteria, lactobacilli, Gram positive cocci, coliforms and other groups are all represented in fairly equal proportions. The type of delivery, dietary constituents and gestational age influence the colonization pattern. The initial period of bacterial colonization in the colon takes place over approximately a two-week period. During this period the bacterial colonization is similar for formula- and breast-fed infants. Escherichia coli and Streptococcus are always the first organism to be detected, at concentrations between \(10^8\) and \(10^{10}\) organisms per gram of faeces. Several anaerobic organisms, namely Bifidobacterium, Clostridium and Bacteroides, often then take up residence in the gastrointestinal tract. In breast-fed infants a major decrease in the bacterial populations of E. coli and Streptococcus then occurs, as well as partial or complete disappearance of Clostridium and Bacteroides resulting in predominance of Bifidobacterium. In formula-fed infants, these reductions or disappearances do not take place, resulting in a more complex flora. The relatively simple flora of the breast-fed baby remains until dietary supplementation occurs. Upon introduction of other foods to the diet of the breast-fed infant
there is a return of E. coli, Streptococcus and Clostridium to the faeces. The differences between breast-fed and formula-fed infants disappear. There is then a transition period, which continues into the second year of life in which the intestinal flora evolves to resemble that of the adult.

The classical studies concerning the acquisition of the infant intestinal microflora are generally considered to be those of Tissier (1990). He divided the colonization of the intestinal tract of suckled infants into three phases. In the first phase, which consisted of the first few hours of life, the faeces were devoid of microbes. The second phase began between the 10th and 20th hour of life with the detection of a heterogenous collection of microbial types in the faeces. After three days, by which time milk had passed through the length of the intestinal tract, the third colonization phase began. A Gram-positive Bacillus became the numerically dominant microbe in the faeces at this stage judged by microscope examination of faecal smears. The other microbial types disappeared in a fairly consistent manner, and from the start of the third and fourth day of life until weaning the collection of microbes in the faeces remained the same.

The faecal flora of breast-fed infants is relatively simple. Bifidobacteria are the dominant organisms, accounting for about 99% (range 85% to more than 99%) of cultivable flora. Coliforms, enterococci and lactobacilli comprise about 1% (range 1-15%) of the faecal flora, while bacteroides, clostridia, and other organisms may be absent or insignificant. The stools have an acid pH (4.5-5.5). Since the work of Tissier, it has been believed that bifidobacteria are found exclusively in faeces of breast-fed infants, whereas in bottle-fed infants Lactobacillus acidophilus is the most commonly found organism. Twenty-one genera and 103 species or biovars of microorganisms were isolated from the faeces of the breast-fed infants and 20 genera and 97 species or biovars from the bottle-fed infants. The organism that showed the highest number and the highest frequency of occurrence in both groups was Bifidobacterium breve. Bifidobacterium infantis, which was formerly the most prevalent Bifidobacterium species in baby faeces, was never isolated at this time.

2.2.4 The faecal flora of children and adults
The compositions of the bacterial flora in the large intestine and faeces of different age groups may differ. The most prevalent bacteria in the faeces of infants and adults are obligate anaerobes, while facultative anaerobes are generally expected to account for less than 103 of anaerobe numbers. During weaning, bifidobacteria decrease by 1 log, the species and biovars alter from
infant-type to adult-type, and a remarkable proliferation of bacteroides, eubacteria, peptostreptococcaceae, and clostridia occur. The faecal flora of children closely resembles that of adults, where the numbers of bacteroidaceae, eubacteria, peptococcaceae, and usually clostridia outnumbered bifidobacteria, which constitute 5-10% of the total flora. The counts of Enterobacteriaceae and Streptococci decrease to less than $10^8$ per gram of faeces, but the counts are usually less than $10^7$ per gram of faeces. (Mitsuoka & Hayakawa, 1973)

In adults, the flora of the large intestine is more complex that that of children. The stools of adults have a low redox potential (Eh), a neutral or slightly alkaline pH (6.0-7.0 or above), a typical odour and colour, and they contain relatively large amounts of putrefactive products, such as ammonia, amines, phenols, and degraded bile acids.

2.2.4.1 Faecal flora of elderly persons

In adults, little is known about the influence of the ageing process on faecal microflora (Andrieux et al., 2002). Research conducted by Mitsouka & Hayakawa (1973) compared the faecal flora of healthy adults and elderly persons. In elderly persons, bifidobacteria decrease or diminish, clostridia including C. perfringens significantly increase, and lactobacilli, streptococci and Enterobacteriaceae also increase (Hopkins and Macfarlane, 2002).

2.2.4.2 Activity of bacteria in the gastrointestinal tract

In terms of the microbiology of different digestive tract areas, there is variability both in terms of composition and activity. The lumen of the human stomach is essentially sterile due to a low gastric pH. However, micro-organisms are known to reside in the mucosal layer that overlies the gastric epithelium. In the small intestine, the transit time of gut contents tends to maintain bacterial numbers at below $10^6$/ml contents. Intestinal secretions like pancreatic enzymes and physiochemical variables such as pH and Eh also contribute towards the type of microflora that develops. The upper small gut is dominated by facultatively anaerobic and aero-tolerant bacteria such as streptococci, staphylococci and lactobacilli, with bacterial numbers showing a progressive increase.

2.2.4.3 Gastrointestinal bacteria influenced by diet

Most work on the microbes of the large bowel of adults has concentrated on the analysis of fecal samples. The composition of the fecal microbiota has been the subject of many investigations and has been summarized in numerous reviews. Most studies focus on enumeration of the major groups of microbes with some studies characterised to the genus level.
Few workers have been as thorough as Moore and Holdeman (1974) who identified the species level wherever possible. Populations of faecal bacteria constitute a major proportion (approximately 50%) of feces (Tomomatsu, 1994). Findings by Moore and Holdeman (1974) noted that despite considerably variation in diet and health, numbers of the major bacterial groups in all subjects were remarkably similar. Bacteroides were found in highest numbers in all fecal numbers. The most frequently isolated anaerobic cocci were peptostreptococci, ruminococci, viellonella, and anaerobic streptococci, with Peptostreptococcus being the most commonly isolated species. Of the anaerobic Gram-positive nosporing rods, eubacteria were found in highest numbers.

2.2.5 Structure and function of the gastrointestinal tract (GI)

The gastrointestinal tract is a tube extending from the lips to the anus and is divided into various well-defined anatomical regions. The digestive and absorptive functions are well known but, in addition to being an organ in the body, the intestine acts as a container for the most intimate portion of the chemical environment. Assimilation of food is not the only physiological function of the alimentary tract. It is also concerned with the excretion of chemical waste, the control of body metabolism and immune response. Furthermore, the gut harbours a complex ecosystem.

2.2.6 Bacterial fermentations in the large intestine

It is clear that a complex, resident gut flora is present in humans. While the transit of residual foodstuffs through the stomach and small intestine is probably too rapid for the microflora to exert a significant impact, this slows markedly in the colon. The average transit time is around 70 h, but can be higher. As such, colonic micro-organisms have ample opportunity to degrade available substrates. These may be derived from either the diet or by endogenous secretions. Fermentations by gut bacteria consist of a series of energy yielding reactions that do not use oxygen in the respiratory chains. The electron acceptors may be organic (e.g. some products of the fermentations) or inorganic (e.g. sulphate, nitrate). As carbohydrates form the principal precursors for fermentation, ATP is usually formed through substrate level phosphorylation by saccharolytic micro-organisms. The fermentation process in the large gut is influenced by a variety of physical, chemical, biological and environmental factors. Factors affecting fermentation in the large intestine are presented in Table 2.9.
Table 2.9 Factors affecting fermentation in the human large intestine (Gibson and Fuller, 2000)

- Amount of substrate available for fermentation
- Colonic transit time
- Physical form of the substrate
- Chemical composition of the substrate
- pH of gut contents
- Composition of the gut microbiota with respect to species diversity and relative numbers of different types of bacteria
- Ecological factors including competitive and cooperative interactions between bacteria
- Rates of depolymerisation of substrates
- Substrate specificities and catabolite regulatory mechanisms of individual gut species
- Fermentation strategies of individual substrate utilising bacteria
- Antibiotic therapy
- Availability of inorganic electron acceptors

Major substrates available for the colonic fermentation are starches that, for various reasons, are resistant to the actions of pancreatic amylases and can be degraded by bacterial enzymes as well as dietary fibers like pectins and xylans. Other carbohydrate sources available for fermentation in lower concentrations include oligosaccharides and a variety of sugars and non-absorbable sugar alcohols.

2.2.6.1 Metabolites produced in the large intestine

In terms of end products, a variety of different metabolites arise. Predominant among these are the short chain fatty acids (SCFA), acetate, propionate and butyrate. The majority are absorbed into the bloodstream and can be further metabolised systemically. Transport to, and further metabolism of SCFA in the liver, muscle or other peripheral tissues is thought to contribute about 7 to 8% of host daily energy requirements. Other products include metabolites, such as ethanol, pyruvate and lactate, which are mostly further converted to SCFA and therefore not allowed to accumulate to any significant level in the large bowel.

The resulting end products due to carbohydrate fermentation with the major bacterial groups involved and metabolic fate are presented in Table 2.10.
Table 2.10 Predominant products of carbohydrate metabolism in the human colon (Gibson and Fuller, 2000)

<table>
<thead>
<tr>
<th>End product</th>
<th>Bacterial group involved</th>
<th>Metabolic fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>Bacteroides, bifidobacteria, eubacteria, lactobacilli, clostridia, ruminococci, peptococci, Veillonella, peptostreptococci, propionibacteria, fusobacteria, butyribrio</td>
<td>Metabolised in muscle, kidney, heart and urine</td>
</tr>
<tr>
<td>Propionate</td>
<td>Bacteroides, propionibacteria, veillonella</td>
<td>Cleared by the liver; possible glucoegenic precursor; suppresses cholesterol synthesis</td>
</tr>
<tr>
<td>Butyrate</td>
<td>Clostridia, fusobacteria, butyribrio, eubacteria, peptostreptococci</td>
<td>Metabolised by the colonic epithelium; regulator of cell growth and differentiation</td>
</tr>
<tr>
<td>Ethanol, succinate, lactate, pyruvate</td>
<td>Bacteroides, bifidobacteria, Lactobacilli, eubacteria, peptostreptococci, clostridia, ruminococci, actinomycetes, enterococci, fusobacteria</td>
<td>Absorbed; electron sink products further fermented to SCFA</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>Clostridia, ruminococci, fusobacteria</td>
<td>Partially excreted in breath; metabolised by hydrogenotrophic bacteria</td>
</tr>
</tbody>
</table>

2.2.7 Cancer of the large intestine

The large intestine is the second most common site for carcinoma in man and faeces from individuals living in Western societies frequently contain mutagenic substances as indicated by the Ames test. There is no general agreement regarding the aetiology of bowel cancer, although factors such as diet, environment and genetics have been implicated. It has been speculated that tumours occur 100 times more often in the hindgut than in the small intestine, indicating that the colonic microbiota plays an important role in carcinogenesis. It has been suggested that a mechanism whereby intestinal bacteria may be involved in these processes is by the production of carcinogenic metabolites from non-toxic precursor molecules, and a variety of hydrolytic and reductive enzymes responsible for carcinoegen production are produced by colonic microorganisms (McBain and Macarlane, 1998).

2.2.7.1 Conditions favourable for cancer

Colorectal cancer is the second largest cause of cancer deaths in western countries (Singh et al., 1997). Studies have suggested the involvement of intestinal microflora in the pathology of
colon cancer. Epidemiological and experimental studies provide evidence that nutritional factors play a role in the aetiology of colon cancer (Kulkarni and Reddy, 1994). Lactulose, a disaccharide and keto hydrolytic product of β-galactosidase, is not absorbed in the small intestine. These sugars are used as a substrate by bifidobacteria resulting in an increase in the number of bifidobacteria in faeces (Salminen et al., 1993; Gibson et al., 1994). These sugars enhance the selective proliferation and colonisation of bifidobacteria. Shifts in the colonic flora, i.e. an increase in anaerobes and a decrease in aerobes have been found in populations which are at increased risk of colon cancer. With respect to colon carcinogenesis, the fermentation of carbohydrate and dietary fibre by colonic bacteria to short chain fatty acids is of major interest. An acidic pH level in the colonic lumen caused by increased production of short chain fatty acids inhibits the bacterial degradation of primary to secondary bile acids, which have been shown to promote colon cancer in carcinogen-treated rats.

2.2.7.2 Bacterial prevention of cancer

Many reported studies have shown the beneficial effect of consuming specific lactic acid bacteria in the prevention of chronic conditions such as cardiovascular disease and cancer. These lactic cultures which are primarily used for fermentation of milk and other dairy products have shown to possess antimutagenic and anticarcinogenic properties and from epidemiological and experimental studies reduce certain types of cancer and inhibit tumour growth (Singh et al., 1997). Japanese research by Kubota (1990) found that colon cancer incidence was lowest when the colonic population of bifidobacteria was highest and that of Clostridium perfringens was lowest.

Results of the study carried out by Challa et al. (1997) indicate that Bifidobacterium and lactulose exert an additive antitumorigenic effect in rat colon. While the mechanism of inhibition of colon carcinogenesis by dietary B. longum has not been clarified, it is likely that the effect of lactic bacteria can proceed through diverse mechanisms. These may include the alteration of physiological conditions in the colon affecting the metabolic activity of intestinal microflora, the action of bile acids, and to quantitative and/or qualitative alterations in the bile acid-degrading bacteria. The species of lactobacilli and Bifidobacterium, most often suggested as beneficial dietary supplements, have all been reported to exert antagonistic actions toward several enteropathogenic organisms in the intestine such as
Escherichia coli and Clostridium perfringens. C. perfringens and other enteropathogenic anaerobic bacteria contain high levels of 7α-dehydroxylase, which is an important enzyme in the formation of the secondary bile acids from the primary bile acids in the colon. These secondary bile acids have been shown to play a role as tumour promoters in the colon. Evidence of correlations between the incidence of colon cancer and the number of bacteria per gram of faeces possessing 7α-dehydroxylase enzyme activity have been found in humans. It is then possible to assume that dietary lactic cultures modulate the metabolic activity of intestinal microflora and the activity of 7α-dehydroxylase thereby producing lower levels of secondary bile acids in the colon (Reddy et al., 1993).

2.2.8 Natural microflora in gastrointestinal tract

Many different types of bacteria representing most bacterial groups have at some time been isolated from the intestine. Those isolated most frequently can be considered as members of the resident flora or as contaminants from the environment. The number of bacterial groups that may be detected is related to the methods used for their detection. Very few investigators have attempted a systematic investigation of the intestinal bacteria and so any list of the species present in the gut must be provisional.

Numerically, the most important genus of intestinal bacteria in animals and man is Bacteroides. This along with Fusobacterium, which contains pathogenic species, and Leptotrichia, which is also found in the mouth, comprise the family Bacteroidaceae which also contains members of the former genus Sphaerophorus. These are all Gram-negative, strictly anaerobic, non-sporing rods, although some may show varying degrees of polymorphism. B. fragilis is ubiquitous in animals and man.

Amongst the Gram-positive, non-sporing rods several genera are numerically important in the gut. Obligately anaerobic types include Propionibacterium (mainly P. acnes), Eubacterium and Bifidobacterium, including B. bifidum and B. infantis from the faeces of breast-fed infants. Among the facultative anaerobes the genus Lactobacillus contains many species occurring in the gut of most warm-blooded animals. Although numerically important throughout the alimentary tract their ecological significance has not been conclusively elucidated.
Several types of spore-forming rods and cocci are normal inhabitants of the gut. The genus Clostridium is probably the most ubiquitous. Others such as *C. perfringens*, *C. bifermentans* and *C. tetani* are found regularly, albeit in relatively low numbers but are of significance in humans. Facultative and obligately anaerobic Gram-positive cocci are numerically important in the gut. The facultatively anaerobic streptococci are well represented by many species from Lancefield group D including *Streptococcus faecalis*, *S. bovis* and *S. equinus* and come from group K such as *S. salivarius* which is usually associated with the mouth. Gram-negative anaerobic cocci include the closely related genera Veillonella which utilises lactic acid and Acidaminococcus which can utilise amino acids as a sole energy source.

Although they are not numerically important, the Gram-negative facultatively anaerobic rods include a number of very important pathogens. Members of the related genera Vibrio and Campylobacter cause enteric disease in man and animals. The taxonomy of the Enterobacteriaceae is complex and while some genera such as Proteus and Klebsiella and many serotypes of *E. coli* and Salmonella are commensal in animals particular biotypes of the latter two genera are major animal pathogens, particularly affecting the young.

Although Shigella causes dysentery in man it may be found existing as a commensal in the gut of other warm-blooded animals. The significance of the presence of yeasts and moulds in the gut is uncertain. Some yeasts associate with the murine stomach wall but in general they are thought to be transient contaminants.

2.2.9 Bacteria influenced by diet

2.2.9.1 Influence of diet on faecal bifidobacterial flora

It is often reported that the compositions of intestinal flora are influenced by diet. The quality of diet can immensely affect human health preventing and reducing susceptibility to particular diseases (Kolida et al., 2000; Gibson, 1999).

The physical and physiological characteristics of the gastrointestinal tract and its epithelial layer are greatly affected by the presence of a complex microflora whose density varies according to the section of the intestine colonized. The sensitivity of intestinal microorganisms to gastric acid and oxygen largely determines the sites of colonization. Since the oxidation-reduction
potential (Eh) varies according to the microbial population level, the microflora itself controls certain aspects of its own environment.

Some facultatively anaerobic groups of bacteria, such as the lactobacilli, streptococci and coliform bacteria, are ubiquitous and are distributed throughout most of the tract. Obligately anaerobic bacteria such as Bacteroides and Bifidobacterium are confined to parts of the gut where Eh values are very low. Such sites include the colon, caecum and the rumen or rumen-like anatomical modifications of the stomach in those animals possessing a fore-gut microbial fermentation.

Although diet is important in determining the qualitative and quantitative composition of the intestinal microflora, it is difficult to demonstrate experimentally. While the adult flora is characteristic of the host species, that of the neonatal mammal is common to a wide range of species since the milk diet produces a common environment in the gut. Characteristic of the gut flora of neonates are low numbers of potentially pathogenic species such as E. coli. These low numbers are maintained by the inhibitory effects of specific antibody (mainly IgA) and several non-specific factors including the iron binding protein, lactoferrin. Because of the complex intestinal flora, adult animals are normally extremely difficult to infect with enteric pathogens.

Disturbance or removal of the flora (for example by antibiotics) thus increases susceptibility to colonisation by these organisms. An additional consequence of oral antibiotic administration is that commensal and pathogenic bacteria may become resistant to these drugs by mutation or by transferable drug resistance. Both these problems are of considerable significance to animal and public health. Because of this renewed attempts are being made to induce changes in the intestinal flora of animals and man, beneficial to host health, by feeding normal constituents of the gut flora or fermented milk products.

The faecal flora of nine rural healthy Japanese and eight urban healthy Canadians were compared (Benno et al., 1986). The two populations are typical Japanese and Western diets, respectively. The numbers of eubacteria, bifidobacteria, lactobacilli and veillonellae and the frequency of occurrence of bifidobacteria were higher in the Japanese than in the Canadians. Higher numbers of bacteroides and C. perfringens were found in the Canadians.
Table 2.11: The 25 most prevalent bacterial species in the faeces of human subjects consuming a Western diet (10^{9-10} bacteria per gram wet weight) (Gibson and Macfarlane, 1995)

<table>
<thead>
<tr>
<th>Bacteroides vulgatus</th>
<th>Ruminococcus albus</th>
<th>Bifidobacterium adolescentis A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides species, other</td>
<td>Bacteroides distasonis</td>
<td>Bifidobacterium adolescentis C</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>Peptostreptococcus intermedius</td>
<td>Bacteroides clostridiiformis ssp. clostridiiformis</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>Peptostreptococcus</td>
<td>Peptostreptococcus prevotii</td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td>Peptostreptococcus productus</td>
<td>Bifidobacterium infantis ssp. liberorum</td>
</tr>
<tr>
<td>Bacillus species (all)</td>
<td>Eubacterium lentum</td>
<td>Clostridium indolis</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis D</td>
<td>Facultative streptococci, other</td>
<td>Enterococcus faecium</td>
</tr>
<tr>
<td>Eubacterium aerofaciens</td>
<td>Fusobacterium russii</td>
<td>Bifidobacterium longum</td>
</tr>
</tbody>
</table>

In summary, it seems that no general agreement exists in regard to whether or not the bifidobacterial flora of individuals on high-meat diets differ from those of individuals on low-meat diets. However, these results, which were obtained using a comprehensive method for cultivating intestinal flora, indicated that a Japanese-style diet is superior to a western-style diet from the viewpoint of bifidobacteria in the intestinal flora.

The characteristics of particular genera commonly found in human faeces are presented in Table 2.12 including metabolic products and metabolic processes.

Table 2.12: Characteristics of bacterial genera commonly detected in human faeces (Tannock, 1978)

<table>
<thead>
<tr>
<th>Genera</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>Gram-negative, nonspore-forming bacilli. Obligate anaerobe. Metabolic products include combinations of acetic, succinic, lactic, formic or propionic acids. If N-butyric acid is produced, isobutyric and isovaleric acids are also present.</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>Gram-positive, nonspore-forming, nonmotile bacilli sometimes club-shaped or spatulated extremities. Obligate anaerobe. Acetic and lactic acids are produced primarily in the molar ratio of 3:2. Glucose is degraded exclusively and characteristically by the fructose-6-phosphate 'shunt' metabolic pathway.</td>
</tr>
<tr>
<td>Clostridium</td>
<td>Gram-positive bacilli that form endoscopes. Obligate anaerobe.</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Gram-positive cocci. Facultative anaerobe. Lancefield group D. Can grow in 6.5% NaCl broth and in broth at pH 9.6.</td>
</tr>
</tbody>
</table>
2.3 Organisms used as Probiotics

Living microorganisms are widely used for several therapeutic purposes and their beneficial effects as biotherapeutic agents are well known. While certain strains of lactic acid bacteria and bifidobacteria are used as probiotics in pharmaceutical preparations, feed additives and so-called functional foods yeasts also possess some medicinal efficiency.

One rapidly developing area in the food microbial sciences is the use of dietary intervention to modulate the gut flora, with the consequent aim of improving health. Although probiotics have been used in human and animal nutrition for centuries, many new food products have recently become available, including fermented milks, lyophilized preparations and drinks. The most popular delivery system for human use is yoghurt, whereby additional cultures to the traditional starter strains (Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus) are used in the fermentation process and/or added to the product afterwards. Both bacteria and yeasts are used for their probiotic effects. The literature indicates over 50 reported human trials with a so-called ‘positive’ result. These have largely centred around gastrointestinal disorders, such as protection from travellers diarrhoea and alleviation of symptoms of irritable bowel syndrome. However, some systemic effects are also said to occur through the metabolic end products of probiotic growth in the gut (e.g. acetic acid is transported to muscle tissues where it can act as a source of ATP) and probiotics have been used to treat conditions such as atopic eczema and vaginosis. The annual European market for probiotics is said to be in excess of several billion euros, with new product developments occurring rapidly. The Greek translation of probiotic is ‘for life’, and it is formally defined as a ‘live microbial feed supplement which beneficially..."
affects the host animal by improving its intestinal microbial balance”. This has since been modified by a European working party on gastrointestinal function foods to a ‘live microbial food ingredient that is beneficial to health’. This implies that health outcomes should be defined and proven, which is not the case for all of the purported benefits of probiotics. Most research has been directed towards the use of intestinal isolates of bacteria as probiotics. Over the years, many species of micro-organisms have been used. They consist not only of lactic acid bacteria (lactobacilli, streptococci, enterococci, lactococci, bifidobacteria) but also Bacillus spp., yeasts such as Saccharomyces spp. and fungi such as Aspergillus spp. Most probiotic bacteria are Gram-positive strains. This is largely because of their ability to persist within the gut ecosystem and produce organic acids such as lactate and acetate. One difficulty with many probiotics, however, is stability within the product. For example, the bifidobacteria are strictly anaerobic, leading to processing difficulties. Attention has therefore turned to less fastidious microorganisms and recent reports have cited the use of E. coli as a probiotic. Most of the work on probiotic E. coli centres around one particular strain, known as Nissle 1917. It was isolated in World War I from a soldier who survived a particularly severe outbreak of diarrhoea. Nissle proposed the use of E. coli as early as 1916 and showed in the 1930s that administration of this strain improved symptoms in patients with non-infectious bowel disorders.

Subsequent work with Nissle 1917 has shown that administration to infants results in colonization of the gastrointestinal tract and a serum antibody response.

Further, such colonized infants showed reduced colonization by bacterial pathogens and potentially pathogenic species. It has also been found that postnatal colonization with Nissle 1917 results in a significantly reduced incidence of allergies by the age of 10. The use of this E. coli strain to treat Crohn’s disease and ulcerative colitis has generated attention. In well controlled, doubly blind trials, Nissle 1917 was found to be as effective as the drug mesalazine in maintaining remission periods in patients with ulcerative colitis. In addition, it was found to inhibit adhesion of pathogenic E. coli strains isolated from patients with Crohn’s disease to intestinal epithelial cells. As such, the use of E. coli as a probiotic for inflammatory bowel diseases has renewed interest in the use of microbial intervention for these conditions (established therapies include broad spectrum antibiotics like metranidazole and anti-inflammatory agents such as sulphasalazine – often, neither are especially effective). The data on
Nissle 1917 suggests that it may have some use as a probiotic, although much more data from human trials are required before firm conclusions can be safely drawn. Further research to clarify how it works is also necessary. Use of a Gram-negative species as a probiotic is rare, but is likely to stimulate interest in other species. One aspect to consider is that within the normal gastrointestinal microflora, *E. coli* is normally present only in relatively trivial numbers, when compared to bacteroides, bifidobacteria, eubacteria, clostridia, lactobacilli, etc. In the context of colonization of the neonatal gastrointestinal tract, the 'Gold Standard' is generally held to be human milk. The result of breast feeding is a gastrointestinal microflora very much dominated by Gram-positive micro-organisms (usually bifidobacteria), more so than in formula feeding. In this light, the strategy of colonization of infants with *E. coli* should be pursued with caution.

### 2.3.1 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are present in the intestine of most animals. The beneficial role played by these microorganisms in the humans and other animals, including the effect on the immune system, has been extensively reported. They are present in many foods and are frequently used as probiotics to improve some biological functions in the host. The activation of the systemic and secretory immune response by LAB requires many complex interactions among the different constituents of the intestinal ecosystem (microflora, epithelial cells and immune cells). Through different mechanisms they send signals to activate immune cells. Thus the knowledge of the normal intestinal microflora, the contribution of LAB and their role in the numerous functions in the digestive tract as well as the functioning of the mucosal immune system form the basis for the study and selection of a probiotic strain with immunostimulatory properties. In the selection of LAB by their immunostimulatory capacity it helps to know not only the effect which they have on the mucosal immune system, but the specific use to which these oral vaccine vectors are being put. Although there are reports of the protection of animals and humans against diseases such as microbial infections and cancer, more work remains to be done on the factors affecting the design of oral vaccine vectors and the use of LAB for therapeutic purposes.

All warm-blooded vertebrates live in symbiotic association with a complex population of microorganisms which inhabits their gastrointestinal tract. One of the benefits which the host
animal derives from this relationship is an enhanced resistance to infectious diseases (Fuller, 1992, 1997). Thus conventional animals with a complete gut microflora are more resistant to infection than are germfree animals. The detailed basis for this difference is not known but it seems certain that changes in immunity are likely to be involved. The gut microflora stimulates mainly a local response at the gut wall. This mucosal immunity is an important element of the animal's immune status because it is responsible for the control of infections as well as inducing tolerance to environmental and dietary antigens. Under natural conditions the level of immunity is adequate, but under domesticated conditions, stress factors cause deficiencies to occur which render the animal vulnerable to infection. Under these circumstances, supplementation with live microorganisms to repair the deficiencies in the composition of the gut microflora can stimulate an immune response and restore the animal's resistance to infection. These supplements known as probiotics have been defined as: "live microbial food supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller 1989).

This definition includes not only preparations specifically designed to act as probiotics but also the traditional yogurts and other fermented products where the benefits conferred on the consumer may be incidental to their primary role as a tasty and nutritious food. By far the most commonly used microorganisms in probiotic products are the lactic acid bacteria (LAB) and it is important to know how these LAB affect the immune status of the consumer.

The probiotic approach is attractive because it is a reconstitution of the natural condition; it is a means of repairing a deficiency rather than the addition of foreign chemicals to the body which may have toxic consequences or, as in the case of antibiotics induce resistance and compromise subsequent therapy. The discovery that probiotics can stimulate an immune response (Fuller and Perdigón, 2000) provides a scientific basis for some of the observed probiotic effects. This is an important function of probiotic preparations and a rapidly developing area of research.

### 2.3.1.2 Lactic acid bacteria and their utilization in food fermentation

Lactic acid bacteria (LAB) form a phylogenetically diverse group of microorganisms and are defined as Gram-positive, non-sporing, catalase-negative, devoid of cytochromes, of anaerobic habit but aero-tolerant, fastidious, acid-tolerant and strictly fermentative bacteria that secrete lactic acid as the major end product of sugar fermentation.
They are chemoorganotropic and grow only on complex organic media. They produce lactic acid from various fermentable carbohydrates (Axelsson 1998).

\[ \text{C}_{6}\text{H}_{12}\text{O}_{6} \rightarrow 2 \text{CH}_3\text{CHOH.COOH} \]

(Carbohydrate) (Lactic acid)

There are two groups of LAB based on their fermentation patterns:

- Homofermentative: produce more than 85% lactic acid from glucose and,
- Heterofermentative: produce lactic acid along with other acids.

LAB have a long history in industrial use such as in the fermentation of milk, vegetables and meat and among industrial microbes, the importance of LAB is next only to that of yeast. Interest in LAB as health-promoting bacteria was raised early last century, when Elie Metchnikoff suggested that proteolytic bacteria of the intestinal normal flora are harmful to human health and that modification of the intestinal flora by the consumption of LAB may contribute to prolonging of life, as exemplified by the apparently long life span of yogurt-eating Bulgarian peasants (Bibel, 1988).

Since then, several probiotic products have been marketed for human or animal use. LAB are major members of the complex microbial flora in the mammalian intestine and are gaining growing interest in basic and applied research i.e. as probiotic as well as delivery vehicles for pharmaceutically important compounds like genetically engineered vaccines (Well et al. 1993).

2.3.1.3 LAB as a probiotics

Over 400 distinct species of microbes inhabit the various regions of the human digestive tract, making up nearly 4 pounds of the human total body weight. The term "Probiotics" refers to the live microorganisms that survive passage through the gastrointestinal tract and have beneficial effects on the host (Lee & Salminen 1995; Fuller 1989 and 1993). Probiotic LAB have been the focus of much scientific and commercial interest, due to a range of possible health effects. Some of the proposed health benefits are due to live bacteria contained in the foods. Suggested minimum no. probiotic bacteria for consumption is \(10^5-10^6\) cfu/g (Donnet et al. 1999).
The potential benefits of LAB as probiotics includes:

- Improvement of gut function by normalizing microflora balance, reducing constipation & intestinal mobility.
- Treatment of infantile diarrhoea, traveller's diarrhoea and some antibiotic induced diarrhoea.
- Nutraceutical effects include the reduction of serum cholesterol, management of diabetes & prevention of osteoporosis.
- Enhanced general digestion and assimilation.
- Improves peristalsis and colon health by removing toxins.
- Helps digest lactose and thus decrease symptoms of intolerance.
- Synthesize vitamin B₃, B₅, B₆, folic acid, biotin & vitamin K.
- Produce lactic acid and natural antibiotic like hydrogen peroxide that inhibits growth of *Candida albicans*, viruses, pathogenic bacteria and parasites (Tannock 1990).
- Helps in maintaining a healthy hormonal balance.
- Reduces food allergens and sensitivities.
- Reduces bad breath.
- Prevention of colon cancer.
- Stimulation of the immune system (Fleromonti *et al.* 2003).
- Competition for nutrients otherwise consumed by pathogen (Tagg & Dierksen, 2003)
- Antimutagenic and anticarcinogenic properties (Mary *et al.* 2000)
- Aids in digestion by producing enzymes e.g. galactosidase, bile-hydrolase, protease and lipase.
- For targeting inflammatory bowel disorders, pouchitis, vaginites and throat infection.

2.3.1.4 *Enterococcus*

*Enterococcus* is a genus of lactic acid bacteria of the phylum Firmicutes. Members of this genus were classified as *Group D Streptococcus* until 1984 when genomic DNA analysis indicated that a separate genus classification would be appropriate.

*Enterococci* are Gram-positive coccis that often occur in pairs (diplococci) or short chains and are difficult to distinguish from *Streptococci* on physical characteristics alone. Two species are common commensal organisms in the intestines of humans: *E. faecalis* (90-95%) and *E. faecium*.
Enterococci are facultative anaerobic organisms, i.e., they do not require oxygen for metabolism, but can survive in oxygen-rich environments. They typically exhibit gamma-hemolysis on sheep’s blood agar. There are rare clusters of infections with other species: \textit{E. casseliflavus}, \textit{E. raffinosus}.

Enterococcus is a genus of bacteria which lives in the human body, notably in the digestive and urinary tracts. It is generally a benign bacteria in people with normal health and without compromised immune systems. It has the characteristic of being very resistant to antibiotics and treatment of the bacteria can be problematic.

There are more than seventeen identified species in the enterococci genus.\cite{SwanHeeGoh99} Only a very few are known to be of significant interest from a health and clinical treatment aspect.\cite{SusanLetal08}

Vancomycin-Resistant Enterococcus (VRE) is a particularly resistant strain, believed to have originated in Europe and the UK and spread to the US. In the UK, approximately 5\% of the population is believed to be carrying this strain of Enterococcus, but do not have any symptoms manifest unless they are in a high-risk group. Groups at high risk are hospital personnel – including doctors, nurses and other medical staff – and hospitalized patients or visitors. Very old, very young, or very ill individuals are also susceptible. \cite{VREbyNewYorkDepartmentofHealth2006}

Exposure can be by contact with a contaminated person or an object touched by a carrier. It can be transmitted in food, by direct touch, or indirect contact with a contaminated surface.

In the US, the only reported cases were all traced back to initial contact in a hospital. The best methods of controlling this strain are meticulous personal hygiene – paying particular attention to washing hands at frequent intervals – avoiding hospitals and limiting use of antibiotics for treatment of routine illness. Control of existing Enterococcus colonies – even VRE – is simple. Stay healthy and maintain a well-balanced concentration of stomach flora. Course of treatment involves a number of pharmaceuticals, dependent on test results and particular strain. Johns Hopkins has conducted exhaustive studies of treatment and control of the
Enterococcus in the intestinal, urinary and reproductive systems. (Lisa A. Spacek, 2009)

There was an extensive cost-benefit study conducted in 1999 that concluded the Enterococcus could be controlled and effectively diminished by use of disposable gown and glove use. Cost of the care versus the cost of treatment led to increases in initial expenditures, however, the cost was more than offset by the cost of treatment. (Laura Puzniak, et al; 2006)

Enterococcal infection poses a significant mortality risk to older, male, ICU hospital patients with a history of recent antibiotic use and with a compromised immune system or wounds from surgery or accident. (Meera Varman, MD, et al; 2009)

There are probiotic uses of ingested Enterococcus, and several studies on swine and piglets have yielded positive results in controlled experiments as published by the American Society of Microbiology. Results indicate a 20%-25% decrease in chlamydia infection with a 100% correlation between four independent testing methods. (Pollmann, et al; 2005)

While testing in humans is limited due to the relative youth of the genus (established in 1986), there are promising results from animal testing. When considered with the relatively low incidence of complications in healthy adults who are not in contact with a high-risk group, this bacteria could be of significant use in the control of more dangerous and harmful infections as chlamydia.

2.3.1.5 Lactobacillus acidophilus

Acidophilus is an “unique” name for a category of probiotics. These probiotics, as defined by the Food and Agricultural Organization of the United Nations, are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.” (FAO/WHO) One of the main functions of probiotics in the acidophilus group is to provide aid in the digestion process. Some of these bacteria include: Lactobacillus (L.) acidophilus, L. bulgaricus and L. fermentum.

Lactobacillus acidophilus is found naturally in humans in the mouth, the intestines and the vagina. Classified as “healthy” bacteria, L. acidophilus helps guard against infections and disease. There are a number of ways in which L. acidophilus performs. It assists in the breakdown of foods and thereby produces hydrogen peroxide, lactic acid and other substances that create an acidic, unfriendly environment for harmful organisms. L. acidophilus also creates
lactase, which is an enzyme that converts lactose (milk sugar) into a simple sugar. Because of this latter capability, ingestion of L. acidophilus may be useful for people who suffer from lactose-intolerance.

Scientists began to use Lactobacillus acidophilus for its probiotic benefits as far back as the early 20th century. Russian scientist and Nobel laureate Elie Metchnikoff, studying the helpful properties of yogurt, speculated that the lactic acid produced during yogurt fermentation could conquer decaying gut microbes. Metchnikoff proposed that when the probiotics found their way into the intestines, they would prevent the formation of the harmful microbes. While investigating the diets of people living in the Balkans and the Near East, Metchnikoff came to believe in a connection between long-term consumption of yogurt and longevity. (Metchnikoff O 1921)

In the 1920s, acidophilus milk was administered to treat diarrhea as well as constipation. (Kopeloff N., 1926.) Dannon, a leading manufacturer of yogurt products, began producing yogurt for delivery to pharmacies in the early 1920s. In the 1970s, the company produced a television commercial (the first ever filmed in the then-Soviet Union) that featured a purportedly 89-year-old man from Soviet Georgia eating his yogurt with his mother, allegedly 114 years old, smiling in the background. The campaign sparked sales of Dannon yogurt, and helped create awareness of yogurt among a new generation of users.

In addition to being present in yogurt, lactobacillus acidophilus is available as a supplement, in the form of tablets, liquids and powders.

**Benefits**

Lactobacillus acidophilus helps in the prevention Yeast Infections mostly in urinogenital tract. Urogenital infections such as bacterial vaginosis, yeast vaginitis and urinary tract infections affect millions of women. In many instances, the affliction recurs, particularly due to a buildup in resistance to certain antimicrobial therapies. L. acidophilus may prove to be effective in inhibiting the growth of candida albicans, which is the fungus responsible for many of these infections. An abstract from a 2003 article in the Post Graduate Medical Journal of the British Medical Journal states: “daily oral intake of probiotic strains Lactobacillus rhamnosus GR-1 and Lactobacillus fermentum RC-14, resulted in some asymptomatic bacterial vaginosis patients reverting to a normal lactobacilli dominated vaginal microflora.” (Reid G(2001), Reid G(2003))
When lactobacilli are introduced vaginally ... there will be an impact on the subject's microflora. If this is dominated by yeast, Gram-negative coils and anaerobes, or gram-positive cocci, then the outcome might significantly benefit the patient.” (Burton JP.2003)

Assists in the Absorption of Nutrients

Microflora such as Lactobacillus acidophilus are also necessary for the body’s assimilation of nutrients.

They assist in the production of key enzymes, and increase the rate at which vitamins are absorbed. Some of the nutrients best absorbed with L. acidophilus are the vitamins K and B, calcium, lactase and fatty acids.

Helps Reduce Lactose Intolerance

A 1984 study demonstrated that lactose is absorbed more effectively in yogurt and products containing L. acidophilus than in milk, and the other items sampled, which include sweet acidophilus milk, pasteurized yogurt and cultured milk. (DA Savaiano, 1984) The study also showed that pasteurization greatly inhibited the body’s ability to digest lactose and significantly decreased yogurt’s natural lactase activity.

Decreases Antibiotic Side Effects

Antibiotics kill bacteria in the body, both the good and the bad. While antibiotics are a crucial therapy for many illnesses, they can cause the demise of “friendly” flora. They can also produce unpleasant side effects such as diarrhea. Ingestion of L. acidophilus can reduce the likelihood of these side effects. A study conducted among healthy volunteers taking 400 mg of erythromycin showed that those who ate yogurt containing Lactobacillus probiotics exhibited fewer instances of diarrhea than those who ingested pasteurized yogurt. (Annals of Medicine, 1990) Diarrhea is
sometimes reported as a side effect among infants and young children who have been administered antibiotics for respiratory infections. L. acidophilus, when used as a prophylactic, decreases the likelihood of diarrhea among these young subjects. (Arvola T., 1999)

2.3.2 Saccharomyces cerevisiae

Saccharomyces cerevisiae, which in Latin means “sugar fungus,” has been utilized by humans for thousands of years. Saccharomyces cerevisiae is a species of yeast. It is perhaps the most useful yeast, having been instrumental to baking and brewing since ancient times. It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums; it exists among the waxes of the cuticle). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like Escherichia coli as the model bacterium. It is the microorganism behind the most common type of fermentation. S. cerevisiae cells are round to avoid, 5–10 micrometres in diameter. It reproduces by a division process known as budding. Many proteins important in human biology were first discovered by studying their homologs in yeast: these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes. The petite mutation in S. cerevisiae is of particular interest. Antibodies against S. cerevisiae are found in 60–70% of patients with Crohn's disease and 10–15% of patients with ulcerative colitis.

It is believed that it was first discovered on the skins of grapes. S. cerevisiae is a budding or brewing yeast, and has been put to use since antiquity to make dough rise and to provide ethanol in alcoholic beverages. One of the most elemental purposes of brewers yeast was that it transformed the way bread was made in ancient times. Once, all bread was unleavened, and could often have a hard, dry texture. One can only imagine the reaction when yeast was accidentally added to the bread mixture an estimated 5,000 years ago in Egypt, yielding a chewy, flavorful diet staple. (British Broadcast Corporation, 2006.) Yeast remains a key component in baking to this day. The pioneering research of Louis Pasteur in the 1860s included a method of enabling yeast to be commercially produced as an ingredient for baking and in the processing of alcoholic-beverages.
Its deeply detailed cellular structure makes S. cerevisiae, also known as "brewers yeast," one of the most highly researched model organisms in the study of biology. It exists in single-cell form, or in pseudomycelial form. Cellular reproduction occurs by budding. The ability of S. cerevisiae to ferment specific sugars is a major factor that differentiates it from other yeasts. S. cerevisiae exists and grows in the haploid and diploid cellular forms. The haploid life cycle consists of mitosis, growth, and ultimately death, the latter more rapid under extremely stressful conditions. Diploid cells also undergo mitosis as well as growth, but in the same stressful circumstances can experience sporulation. Subsequent to sporulation, the cells undergo meiosis and produce a number of haploid spores. These haploid spores progress to mate.

2.3.2.1 Beneficial effects present in cells of Saccharomyces Cerevisiae:

A probiotic in terms of its beneficial effects, S. cerevisiae has many properties from the most basic to highly advanced. When ingested in a quantity of two tablespoons daily, the commercially prepared product known as “nutritional yeast” provides 52 percent of the recommended daily amount (RDA) of protein. Nutritional yeast is also high in fiber, B vitamins and folic acid. It is also gluten-free, which makes it an attractive supplement to people who are wheat-intolerant.

Nutritional yeast also has the presence of beta-1,3 glucans, which have been shown to stimulate the body’s immune system. Researchers at the University of Louisville established a receptor found on the surface of certain immune cells is known for binding itself to beta-glucans, which permits the immune cells to recognize the beta-glucans as being dissimilar. (Vetvicka, V, 1996) While some pharmaceutical drugs are capable of over-stimulating the body’s immune system during therapy and are therefore not suitable for people with autoimmune illnesses, beta-glucans appear to assist the immune system without causing overactivity (Chiara, G. (1992)).

Beta-glucans are also apparently capable of lowering LDL cholesterol levels, assisting in the healing of wounds and aid in the prevention of infections. Beta-glucans derived from shiitake mushrooms have been applied as immunoadjuvant treatment for cancers since 1980. This therapy is particularly popular in Japan. Several studies indicate that beta-glucans can prevent the formation of tumors and the development of cancers. (DiLuzio, N.R, 1980 Morikawa, K, Mansell, P.W, 1975) In an experiment conducted with mice, beta
1,3 glucans administered with interferon gamma slowed the progress of tumors and metastasis to the liver. (Sveinbjørnsson, B., 1998) It was also proved that among human colorectal cancer patients, ingestion of beta-1,3 glucans from shiitake mushrooms, along with chemotherapy, generated a longer rate of survival. (Wakui, A., 1986)

Prepared yeast is also easily stored. Often vacuum-packed or sealed in individual doses, this form of S. cerevisiae is shelf-stable for approximately one year.

2.3.2.5 Mechanism of controlling pathogenic organisms by probiotic yeast

S. cerevisiae and S. boulardii share a common mechanism of action against pathogenic bacteria.

- Pharmacokinetics

Pharmacokinetics is the study of the process by which a drug is absorbed, distributed, metabolized, and eliminated by the body. Pharmacokinetics is a branch of pharmacology dedicated to the study of the time course of substances and their relationship with an organism or system. In practice, this discipline is applied mainly to drug substances, though in principle it concerns itself with all manner of compounds residing within an organism or system, such as nutrients, metabolites, endogenous hormones, and toxins.

*S. cerevisiae* and *S. boulardii* can resist gastric acidity, proteolysis and are able to achieve and maintain high populations in the GI tract. They can permanently colonize the colon and do not easily translocate out of the intestinal tract (Boddy et al., 1991). They can also be detected alive throughout the digestive system, if they are given daily in freeze dried form (WHO., 1995). In gnotobiotic mice, a single dose of *S. boulardii* was found to result in colonization of the intestinal tract, the yeast being detectable at a constant, albeit low, level (10^7 c.f.u./g) for 60 days. In healthy human volunteers, that received a single oral dose of 1 g *S. boulardii*, it took 36 to 60 hours to reach maximum yeast numbers, 2 to 5 days to decline to no-detectable concentrations. *S. cerevisiae* and *S. boulardii* are sensitive to non-absorbable antimycotics such as nystatine but can safely be administered with re-absorbable antifungal agents such as fluconazole.
Pharmacodynamics

Pharmacodynamics is the study of the biochemical and physiological effects of drugs, the mechanisms of drug action and the relationship between drug concentration and effect. Pharmacodynamics is the study of what a drug does to the body, whereas pharmacokinetics is the study of what the body does to a drug. The pharmacodynamics of S. cerevisiae and S. boulardii involves 3 different aspects.

A. Direct antagonism

*S. boulardii* reduces the growth of *Clostridium albicans*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Salmonella enteritidis* (Czerucka and Rampal 2002), and *Clostridium difficile* (Izadnia et al., 1998). *S. cerevisiae* reduces the growth of *E. coli*, *Shigella flexnerii*, *Clostridium difficile* and *Vibrio cholerae*.

*S. cerevisiae* and *S. boulardii* have been shown to protect against various enteric pathogens and members of the family Enterobacteriaceae in animal studies (Czerucka and Rampal 2002).

B. An antisecretory effect by acting specifically on the binding of toxins to intestinal receptors

Pathogenic strains of *C. difficile* produce two well-characterized toxins, A and B, that cause mucosal damage and inflammation of the colon (Pothoulakis and Lamont 2001). *S. cerevisiae* and *S. boulardii* significantly reduce the liquid secretion and mannitol permeability caused by *C. difficile* toxin A in the rat ileum, compared to controls (Pothoulakis 1993). Chromatography of filtered supernatant from *S. boulardii* led to the identification of an active fraction that decreases toxin A-induced rat ileal secretion by 46%, intestinal permeability by 74% and prevented toxin A-mediated inflammation and villus damage. It was demonstrated that this fraction was enriched in a protease that acted on the toxin A molecule and inhibited toxin A binding to its receptor on the brush border membrane of rat intestinal cells (Pothoulakis et al., 1993). This protease was identified as a 54-kDa serine protease (Castagliuolo, 1996). The amount of cholera toxin-
stimulated cAMP (cyclic adenosine monophosphate) was also decreased by 50% in cells treated by S. boulardii and cholera toxin compared to cells exposed to the toxin alone.

C. Trophic effect on the enterocyte with stimulation of enzymatic expression and intestinal defense mechanism. Rats treated with Saccharomyces spp showed significant increases in sucrase-isomaltase, lactase and maltase activities. In their study on human volunteers (Jahn et al., 1996) used an in situ technique to measure brush border enzyme activities in snap-frozen biopsies. After treatment with S. cerevisiae and S. boulardii, an increase in lactase, glycosidase and alkaline phosphatase activity was detected both at the basal and apical parts of villi, with increases ranging from 22 to 55% compared to the basal activities measured before treatment. S. cerevisiae and S. boulardii, both in humans and in a rat model, were found to enhance the expression of disaccharidases and alkaline phosphatase enzymes. This effect may improve the absorption of carbohydrates, usually defective in acute and chronic diarrheal disorders. S. cerevisiae and S. boulardii contain polyamines (spermine and spermidine which have the same trophic effect on the intestinal mucosa, with an increase in diasaccharidase activity, as an equivalent amount of spermine and spermidine given to test animals (Buts, 1994; Balasundram et al., 1994). The overall mechanism of controlling pathogenic organisms by biotherapeutic yeasts is shown in figure 2.3.

(i) Pharmacokinetics: Resistance to gastric acidity, proteolysis and able to achieve high population density in the gastrointestinal tract.

(ii) Pharmacodynamics

- Direct antagonism
  - Competition for the adhesion site
  - Antisecretory effect
  - Acting directly on binding site of bacterial toxin
  - Trophic effect
  - Stimulate enzymatic expression and intestinal defense

Figure 1. Mechanisms used by biotherapeutic yeasts to control pathogens.
2.3.3 Bacillus subtilis

Bacillus subtilis, as with many in the Bacillus genus, is an extremely common bacterium. It is found in soil, water, air, and decomposing plant matter. Bacteria in the Bacillus genus are spore-forming, which means that they create a thick wall which surrounds their DNA and other internal cell structures.

In this way, they are very hardy and impervious to extreme temperatures, chemicals, environmental factors, even some types of radiation. This makes them excellent for use in industrial processes.

Bacillus subtilis is widely used for laboratory studies, but more for genetic research as opposed to health research. The bacteria is highly responsive to genetic mutation, giving it a many experimental uses in a laboratory setting. Though Bacillus subtilis presents some risk to humans, the instances of this are incredibly rare. Part of the problem with its sometimes shady reputation can actually be attributed to other members of its genus. The Bacillus genus encompasses a large number of species. At one time all aerobic, spore-forming bacilli were named part of the subtilis species. Many of the species are closely related, making it very difficult to tell them apart. However, the disease-causing Bacillus species are now easily distinguishable from the helpful strains such as Bacillus subtilis.

The subtilis species is not to be confused with Bacillus cereus, which is a common cause of food poisoning, and Bacillus anthracis, which is pathogenic to humans and other animals. Bacillus subtilis is beneficial in many ways, including industrial applications. It is used to produce a variety of enzymes, including amylase, which is helpful in the de-sizing of textiles and starch modification for the sizing of paper. Bacillus subtilis also produces the enzyme protease, including subtilisin, which is used in detergents and the leather industry.

Perhaps more notably, Bacillus subtilis is used to produce many antibiotics, such as difficidin, oxydifficidin, bacilli, bacillomyin B, and Bacitracin, which is helpful in treating bacterial skin infections and preventing infection in minor cuts and burns. Bacillus subtilis is also used as a fungicide. The bacteria colonize the root system, leaving no room for fungal disease organisms. It is used on agricultural seeds of vegetables, soybeans,
cotton, and peanuts and on flower and ornamental seeds. It is also being used to produce insect toxins, including one to kill malarial mosquito larvae.

According to a Toxic Substances Control Act report from the Environmental Protection Agency, Bacillus subtilis “is considered a benign organism as it does not possess traits that cause disease. It is not considered pathogenic or toxigenic to humans, animals, or plants. The potential risk associated with the use of this bacterium in fermentation facilities is low.”

A 2009 report published in the Journal of Hepatology referenced a report by Swiss researchers and showed a possible different aspect of Bacillus subtilis. Liver injury occurred to two patients after taking an Herbalife product “contaminated” with Bacillus subtilis. They concluded that because liver damage resulted after use of the product, Bacillus subtilis possesses “potential hepatoxicity.”

Though the incidence of distress related to Bacillus subtilis is quite low, perhaps the best advice for its use comes from Gary Huffnagle, a Ph.D. and author of The Probiotics Revolution. Because of certain probiotic species’ similarity to disease-causing strains, Huffnagle recommends consulting a healthcare professional before using supplements containing strains of E. coli, Enterococcus faecium, and Bacillus subtilis.

2.3.4 Bifidobacterium

Bifidobacteria are one variety of “good” bacteria that live in a healthy intestinal tract. Since bifidobacteria exist naturally in your gastrointestinal system, you might guess that nature intends bifidobacteria to serve a specific purpose there, and your guess would be correct. Along with many of the other gut flora, which is the collective term for the bacteria that occur naturally in your intestines, bifidobacteria aid in the food digestion process.

Unfortunately, not every person has a perfectly functioning intestinal tract. In a 1992 study, the National Center for Health Statistics (NCHS), a division of the Center for Disease Control, reported that 5.9 percent of respondents to an National Health
Interview Survey (NHIS) questionnaire on digestive disorders had experienced functional colon issues at one time or another. (Advance Data Number 212, 1992.)

Because bifidobacteria keep healthy digestive systems working properly, researchers have taken an interest in the possibility that supplemental bifidobacteria might help boost the digestive systems of those suffering functional colon issues.

As with all probiotics, the bifidobacterium microbe is only classified as a probiotic when it is: administered live; capable of surviving the administering process and subsequently growing; and administered in an amount proven to provide health benefits to the recipient. (FAO/WHO)

2.3.4.1 Benefits

Only in the last decade or so have researchers begun to actively pursue the probiotic benefits of bifidobacterium. Historically, most available information about bifidobacterium came from the study of feces, animals, and post-mortem subjects. (Gerhard Reuter, 2001) Finding successful ways to administer bifidobacterium such that it survives its shelf life and your gastric fluids has also been tricky.

Researchers know that bifidobacteria exist in healthy digestive systems, but the performance of individual sub-strains has not been thoroughly identified. In 2006, researchers from the University of Manchester School of Medicine conducted a study on the effects of B. infantis on female Irritable Bowel Syndrome (IBS) patients.

The researchers identified a probiotic dosage level for B. infantis that could be administered in a stable, convenient capsule, and which improved abdominal pain, bloating, bowel dysfunction, incomplete evacuation, straining during bowel movements, and the passage of gas. (PJ Whorwell, et. al., 2006) Another ongoing clinical trial is studying the effects of B. breve on IBS patients. (Shin Fukudo)
An additional ongoing clinical trial is evaluating the effects of B. infantis and B. animalis on premature infants. The researchers noted higher levels of bifidobacteria in healthy breast-fed term babies versus formula-fed babies. (Mark Underwood) While researchers have proven B. infantis an effective treatment for IBS in women, another University of Manchester study has shown that B. pseudocatenulatum is associated with atopic eczema in infants.

The study also demonstrated higher levels of B. bifidum in breast-fed infants, and higher levels of B. pseudocatenulatum in formula-fed infants. (C Gore, et. Al, 2008)

2.3.4.2 Probiotic properties of bifidobacteria

Bifidobacteria are major components of the indigenous bacterial population present in the human gut and are arguably most relevant to the health-promoting properties that have been attributed to elements of this microbiota.

They exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules. Health-promoting properties of members of the genus

Bifidobacterium have been reported but research is still necessary for an in depth understanding of the probiotic function. In fact, although experimental evidence of the probiotic effectiveness of bifidobacteria has a long history, little information is available on the molecular mechanisms underlying the health-promoting claims, especially on such complex phenomena as anticarcinogenic and anti-inflammatory-effects.
2.4 Waste utilization

2.4.1 Problems in waste disposal

More recently the problem of effluent from processing operation and their disposal has gained public recognition. In many areas of the world, especially the developing countries, the environmental issues are the same (Okonko et al., 2006; Shittu et al., 2007). Human beings produce large quantities of wastes as we go about our daily lives. From our homes come wastes from food preparation, washing machines, baths, toilets, newspapers, junk mail, packaging, hobbies, auto and home maintenance projects, and the landscape. In addition, wastes are generated in producing the goods and services we utilize.

Waste is defined as any material, which has not yet been fully utilized, i.e. the leftovers from production and consumption. However, waste is an expensive and sometimes unavoidable result of human activity. It includes plant materials; agricultural, industrial, and municipal wastes and residues. Waste also refers to liquid or solid discharged from residences, business premises, small scale industries, and institutions. In general, waste can be characterized based on its bulk or organic contents, physical characteristics, and specific contaminants (Okonko et al., 2006). According to Okonko et al. (2006), each waste contains its unique quality and characteristics, which then suggests the type of treatment required. The two divisions of waste—Domestic and Industrial effluent have different make-ups and often require various treatment processes. Though, waste treatment is generally classified into four levels; primary, secondary, tertiary, and quaternary treatment with each treatment level aimed at removing a more specific class of contaminants (Mc Langhlin, 1992; Aririatu et al., 1999; Okonko et al., 2006).

The environmental impacts of the food sector

While it is true that the principle of waste prevention is universally accepted, the practice has lagged far behind. Food industry will also have to concentrate on waste avoidance as well as utilization of process wastes. Application of clean technologies enhances the safety and quality of the product as well as reducing the energy requirements and environmental impact of the food industry. The main environmental impacts of the food sector are aquatic, atmospheric and solid waste emissions. By choosing proper separation technology, wastewater treatment is usually
carried out and is implemented in process installations. The atmospheric emissions are mainly caused by extensive energy use. The food industry consumes a great deal of energy for heating buildings, processes, and process water, for refrigeration and for the transportation of raw materials and products. The increased share of renewable energy sources could slowly reduce the amount of conventional fossil fuel utilization.

Solid by-products and wastes are also generated in high amounts in the food industry. The main treatment method of solid wastes is, at present, composting. Recovery and re-use of by-products and wastes as raw materials is another option. However, microbiological quality and safety is always of major concern.

**Waste minimization in the waste management process**

The primary aim of waste legislation is the prevention of waste generation. Waste prevention refers to three types of practical actions, i.e., strict avoidance, reduction at source, and product re-use. However, waste prevention does not only include the reduction of absolute waste amounts but also avoidance of hazards and risks because safety is also of major concern. Considering the waste management options, at the top of the hierarchy stands waste minimization that includes (Riemer & Kristoffersen 1999):

- waste prevention i.e. reduction of waste by application of more efficient production technologies;
- internal recycling of production waste;
- source-oriented improvement of waste quality, e.g. substitution of hazardous substances;
- re-use of products or parts of products, for the same or other purpose.

**2.4.2 Sustainable development**

Sustainable development is a process in which the exploitation of resources, the direction of investments, the orientation of technological development and the institutional changes are all made consistent with future as well as the present needs. Sustainable development helps achieve the necessary balance between the resolution of social and economic problems and the protection of the environment, the provision of desirable living conditions for the present generations and
measures taken to preserve these conditions for future generations. Sustainable development is also a key phrase used by politicians, economists and environmentalists. Sustainable advancement and development in relation to a nation is the process of making living, that area of land and/or water more useful or profitable for mankind. The life sickness affects over 30% of global socio-economic and sustainable development turnover by way of healthcare, food and energy, agriculture and forestry. This percentage impact will grow with biotechnological developments which are increasingly improving the efficiencies of production processes in all spheres of life. This therefore implies that biotechnology occupy a very strategic position in the socioeconomic advancement and sustainable development of the nation in particular and the world at large. Scientific advances through the years have relied on the development of new tools to improve socio-economy such as health care, agricultural production, and environmental protection (Okonko et al., 2006).

Sustainable development is a policy which aims to ensure that development meets the needs of our present society without compromising the ability of future generations to meet their own needs (Okonko et al., 2006). Sustainable development aims to ensure that the development needs of the present do not compromise the needs of future generations. A way of addressing this sensitive issue could be through the sustainable development of waste as an alternative and renewable energy resource.

2.4.3 Waste utilization

Wastes are produced by virtually all types of industries, although many cleanup and disposal options exist, no single process can be applied to all types of waste streams. The trend in the world today is to convert waste into useful products through the manipulation of microorganisms and to recycle waste product as much as possible and the role of microorganisms in waste utilization has been studied extensively by several authors. Some workers have thus explored ways of minimizing the environmental hazard posed by the industry effluent, not just by getting rid of it but by converting it to useful products. Waste utilization is another approach in waste management practice. Waste utilization is an ecologically safe and economically efficient method of waste management since; the waste is not treated spending money or disposed off in the landfill causing pollution. Waste utilization could be brought about by the following methods:
-Bioconversion

Biological processes for the conversion of wastes to fuels include ethanol fermentation by yeast or bacteria, and methane production by microbial consortia under anaerobic conditions. Bioconversion is referred to as the enzyme-mediated conversion of organic substrates, such as cellulose, to other more valuable substances, such as protein, by other organisms. The conversion of biomass to useable energy, as by burning solid fuel for heat, by fermenting plant matter to produce fuel, as ethanol, or by bacterial decomposition of organic waste to produce methanol is also referred to as bioconversion (Okonko et al., 2006).

-Bioremediation

One of the promising methods for toxic waste cleanup problems is bioremediation. Bioremediation is an environmental biotechnology process that use either naturally occurring or deliberately introduced microorganisms, to consume and breakdown environmental pollutants into harmless by-products such as water, CO₂ and salts, in order to cleanup a polluted site. Naturally occurring bacteria or fungi that degrade specific substances are isolated, cloned, and manufactured in large quantities and introduced as combinations of microorganisms into a hazardous waste site to eliminate specific contaminants. Under carefully controlled conditions, it is a practical and cost effective method to remove pollutants from contaminated surfaces and subsurfaces.

-Biotechnological Processes

In the industry the production processes are now being modified using biotechnology for reduction in pollution caused by the conventional methods. The biotechnological processes also prove to be very economical and also they provide products, which are better or at least equal in quality to the conventional methods. But in these processes the cost of pollution eradication is also saved as these processes generally give out very little or nil pollution and are more efficient than the conventional processes. Biotechnology serves as a solution to many problems in various fields ranging from fuels to many other cleaner and innovative clean up technologies. Some examples are:

1. Biotechnological production of biosurfactants
2. Biochemical conversion of lignocelluloses substrates to cellulose, liquid glucose, and value-added chemicals.

Biotechnological, bioremediation or bioconversion process is often successful and the most inexpensive method, it is only one of many techniques for dealing with hazardous wastes. This biological waste treatment or bioconversion is desirable because it is inexpensive, can be done at the site of pollution, and causes minimal physical disturbance to the surrounding area compared to other methods (Okonko et al., 2006).

-Biocatalysis

To develop biocatalytic methods for the conversion of crop derived carbohydrates to high value polysaccharides or oligosaccharides. The project composed of two major objectives. Develop biocatalytic methods for the conversion of starch, corn coproducts, beet sugar, or cane sugar to value-added oligosaccharides.

-Biofilm reactors

Nicolella et al. (2000) reported that biofilm reactors are in operation at industrial scale throughout the world. Use of biofilm reactors is anticipated to be economical for the production of these industrial chemicals. It has been reported that the best biofilms were obtained with Pseudomonas fragi, Streptomyces viridosporus, and Thermoactinomyces vulgaris when used in combination with polypropylene composite chips.

2.4.4 Fruit & vegetable waste utilisation

India is the second major producer of fruits and vegetables and ranks next to Brazil and China respectively, in the world. It contributes 10 percent of world fruit production and 14 per cent of world vegetable production. Fruits and vegetables are more prone to spoilage than cereals due to their nature and composition, and this spoilage occurs at the time of harvesting, handling, transportation, storage, marketing and processing resulting in waste. Efficient management of these wastes can help in preserving vital nutrients of our foods and feeds, and bringing down the cost of production of processed foods, besides minimizing pollution hazards. According to India Agricultural Research Data Book 2004, the losses in fruits and vegetables are upto 30 per cent. Taking estimated production of fruits and vegetables in India at 150 million tones, the total waste
generated comes to 50 million tones per annum. The post-harvest technologies for perishable horticultural produce serve as an effective tool for getting better return to the produce and also help in avoiding wastage both at production site and distribution centers, which will help in regulating the market infrastructure. Recycling of fruit and vegetable waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. Microbial technology is available for recycling and processing of fruit and vegetable waste and following products can be made out of the different processes.

2.4.4.1 Fermented Edible products

A number of beverages such as cider, beer, wine and brandy, and vinegar can be obtained from the fermentation of fruit wastes. Apple pomace has been utilized for the production of cider. Best quality of cider can be made by carbonating it. Good quality apple cider and brandy can also be produced by fermenting milled apple pulp. The possibility of making brandy from dried culled and surplus apples, grapes, oranges and other fruits have also been explored. Vinegar can also be prepared from fruit wastes. The fruit waste is initially subjected to alcoholic fermentation by acetic acid fermentation by Acedobacter bacteria, which produce acetic acid. Vinegar production by fermenting waste from pineapple juice has been reported. Vinegar production by fermenting orange peel juice has also been attempted successfully. Apple pomace extract can also be mixed with molasses in the ratio of 2:1 for producing vinegar.

2.4.4.2 Single Cell Proteins

Single cell proteins can be produced from dried and pectin extracted apple pomace by using Trichoderma viride and Aspergillus niger. The grape waste and pressed apple pulp have also been employed as a substrate for Aspergillus niger to generate crude protein and cellulose. Pineapple waste for single cell protein production has also been utilized. Using Fusarium has also used citrus peel juice to generate single cell protein. The waste from brewery and distilleries can also be used for the production of single cell proteins. Potato peels supplemented with ammonium chloride have also been used for the production of protein by using a non-toxic fungi.
pleurotus ostreatus. Similarly, waste from orange, sugarcane and grape processing industry have also been utilized for the production of single cell protein.

2.4.4.3 Animal Feed

The waste obtained from processing of fruits and vegetables is rich in fibre, which includes cellulose, hemi-cellulose, lignin and silica with poor quality of protein. Fermented potato waste has been successfully tried as animal feed. Apple pomace after fermentation with different species yeast, followed by drying, makes the feed enriched with proteins, vitamins, minerals and fats and which can be used for feeding animals. Waste from wineries, breweries and distilleries can be used for feeding livestock. Animal feed can also be obtained from grape pomace after fermentation. Dry brewer's grains after addition of molasses become a very good cattle feed.

2.4.4.4 Ethanol

The waste from fruits and vegetable processing industries being polyaaccharides (cellulose, hemi-cellulose and lignin) can be subjected to solid state fermentation for the production of ethanol, which has several uses. It can be used as a liquid fuel supplement and as a solvent in many industries. Process for production of ethanol from apple has been developed. Pear and cherry waste have also been utilized for production of ethanol. Orange peel after enzymatic hydrolysis was found suitable for the production of ethanol by use of Saccharomyces cerevisiae.

2.4.4.5 Biogas Production

Bio-mass consisting of agricultural, forest, crop residues, solid and liquid wastes from industries, sewage and sludge can be utilized for production of biogas through microbial technology. Similarly, the waste from fruit and vegetable processing industries has been used for production of biogas. Biogas is produced by anaerobic digestion of fruit and vegetable wastes. Methanotropic bacteria like Methanobacterium and Methanococcus spp. can utilize CO₂ from waste materials to produce methane. During this process, the complex polymers are first hydrolyzed into simple substances by acid forming bacteria and finally these are digested anaerobically by methanotropic bacteria and methane gas is liberated. Thus, the waste from fruit and vegetable processing in real sense is not a waste as every thing can be profitably recycled, bio converted and utilized in one or the other form as food, feed or fodder. However, most of the
technologies for the waste utilization are developed at the laboratory scale, so these technologies needed to be standardized for commercial exploitation by the industry. Since the waste is a source of pollution, it has to be treated before discharging into the environment. The regulatory agencies can act as catalyst in developing different processes for the utilization and management of waste arising out of processing industries and industries engaged in food processing should invest a part of their investment on research and development for waste utilization and standardization of the various processes which are commercially viable. Furthermore, the wastage in fruit and vegetable can be effectively managed by the use of bio-technology, by maintaining efficient food distribution system and by promoting domestic and international trade. Thus, proper waste utilization will add to the wealth of the nation and will benefit all involved in the process. During the controlled digestion process of organic matter, biogas is produced which can be used effectively as an energy carrier. In addition, less CO\textsubscript{2} is released during the anaerobic fermentation process than during composting (an aerobic process), and no methane escapes to the atmosphere as is the case with landfill applications. These are important aspects when "global warming" is considered. The anaerobic fermentation process also produces compost which can be used as a soil conditioner.

2.4.5 Agricultural waste

Numerous agricultural residues generated due to diverse agricultural practices and food processing such as rice straw, yam peels, cassava peels, banana peels among others represents one of the most important energy resources. The major components of these are cellulose and hemicellulose (75-80%) while lignin constitutes only 14% (Bowen & Harper, 1989). Yearly accumulation of these agricultural residues causes deterioration of the environment and huge loss of potentially valuable nutritional constituents which when processed could yield food, feed, fuel, chemicals and minerals (Bisaria, 1991). Agricultural residues when dumped in open environment constitute health hazard due to pollution and support for the growth of microorganisms such as actinomycetes, fungi and bacteria (Barton, 1979). If these residues are industrially developed, a vast bulk of them could be rendered economically useful and could help control pollution and elimination of waste disposal problem. Recycling of agricultural residue can be achieved naturally and artificially by microorganisms. Aerobic organisms such as fungi, bacteria, and some anaerobic organisms have been shown to be able to degrade some
constituents of these residues. Fungi play a significant role in the degradation of cellulose under aerobic conditions (Schlegel, 1999). Microorganisms as potential cell matter are rich in B-group vitamins and in protein that contain essential amino acids. They therefore constitute the potential enrichment for deficient diets.

Agricultural waste contains three primary constituents: cellulose, hemicellulose and lignin, and can contain other compounds (e.g. extractives). Cellulose and hemicellulose are carbohydrates that can be broken down by enzymes, acids, or other compounds to simple sugars, and then fermented to produce ethanol renewable electricity, fuels, and biomass based products (Puri, 1984; Wyman and Goodman, 1993; van Wyk, 2001). When the amount of organic agricultural waste, such as corn stalks, leaves and wheat straw from wheat-processing facilities, sawdust and other residues from wood mills, is also considered, this component of solid waste could be a principal resource for biodevelopment (Louwrier, 1998; van Wyk, 2001). Materials of organic origin are known as biomass (a term that describes energy materials that emanate from biological sources) and are of major importance to sustainable development because they are renewable as opposed to non-organic materials and fossil carbohydrates (van Wyk, 2001). Common farm organic wastes such as maize cobs, banana peels, pawpaw fruit peels, maize chaff, stumps of palm tree, palm tree inflorescence, maize stem, rice straw and spinach weeds were earthworm (Eudrilus eugeniae) garden snail (Limicolaria aurora) and palm grub (Oryctes rhinoceros). It was demonstrated by Omoyinmi et al. (2004) that animal protein production varied from 0.91g/kg to 1.41g/kg of waste in earthworm, from 1.15g/kg to 1.40g/kg of waste in garden snail and from 0.90g/kg to 1.60g/kg of waste in palm grub. It was also shown that the short life cycle and production of large number of offsprings could be harnessed for the raising of feed for fish/livestock and in some cases human consumption. This culture of invertebrates offered economic benefits to the farmer and it improved on the environmental quality by transforming wastes into beneficial products.

A surveys on the potential for biomass waste to alleviate energy problems in Tanzania through utilization of agro-industrial residues for anaerobic conversion into biogas and biodiesel.

In 2004, Kareem and Akpan reported that the use of agricultural by-products as substrate for enzyme production was cheap and could facilitate large scale production of industrial enzymes in the tropics. Eight isolates of Rhizopus sp. was obtained from the environment and were grown on solid media for the production of pectinase enzymes. Three media formulated from agricultural
materials were the following: medium A (Ricebran + Cassava Starch, 10:2 w/w); Medium B (Cassava Starch + Soyabean, 1:2 w/w); Medium C (Ricebran + Soyabean + Casein hydrolysate, 10:20.5 w/w). The result obtained by Kareem and Akpan (2004) showed that medium A gave the highest pectinase activity of 1533.33μ/ml followed by medium A and C with 1366.66 and 1066.00μ/ml respectively after 72hrs fermentation. The three solid media supported profuse mycelia growth of Rhizopus species and enhanced its pectinase producing potential (Kareem and Akpan, 2004). A comparative study of the performance of cow dung and poultry manure as alternative nutrient sources in a bioremediation process was described by Obire and Akinde (2005) and Chukwura et al. (2005). Obire and Akinde (2005) also reported that that amelioration of oil polluted soil with cow dung and poultry manure facilitates the disappearance of crude oil in the soil thereby increasing the rate of soil recovery. Poultry manure performed better than Cow dung which will greatly enhanced food productivity at such a time like this when the world at large is facing food crisis.

Coffee-husk and Pulp

Coffee husk and coffee pulp are coffee processing by-products. Some of the husk is used as organic fertilizer (Cabezas et al. 1987) while coffee pulp has its application and utilization in Swine feeding (Jarquín, 1987). The presence of tannins and caffeine diminishes acceptability and palatability of husk by animals.

Caffeine

Caffeine is also a component of several cola drinks. The addition of caffeine in cola drinks is responsible for almost 70% of the world’s pure caffeine trading (Mazzafera, 2002; Mazzafera et al., 2002). Asano et al (1993) reported a successful microbial production of theobromine from caffeine while Braham and Bressani (1987) and Bressani and Braham (1987a,b) have reported the potential uses of coffee berry byproducts and the composition, technology, and utilization of coffee pulp in other species as well as its antiphysiological factors. The popularity of coffee beverage is also based on the stimulant effect of caffeine, because of this pharmacological effect; caffeine has long been added to medical formulations to compensate the depressive effects of other drugs (James, 1991).
Citrus Pulp

According to Wing (1975) and Wing et al (2003), the Florida Citrus Exchange established a fellowship for research into uses of citrus waste in 1911 and thus launched an area of investigation which remains strongly productive. Involved primarily is citrus pulp, consisting mainly of the rag, peel, and seeds of oranges with minor amounts from other fruits (Hendrickson and Kesterson, 1965). This waste collects on concrete slabs or in open pits at canneries.

Cattle eat citrus pulp in the fresh state, but it accumulates too fast for current consumption, and it ferments and spoils too rapidly to save as it is produced. The feeding value and nutritive properties of citrus by-products proved that the digestible nutrients of dried grapefruit refuse were good for growing heifers (Neal et al., 1935).

Citrus Molasses

Citrus molasses also serves as a substrate for fermentation in the beverage-alcohol industry (Becker et al., 1946). The remaining distillery waste can be condensed to a very acceptable feedstuff high in pentose sugars and, because of yeast used for fermentation, high in good quality protein. Large and increasing amounts of citrus molasses are used for production of beverage alcohol. The remaining sugars, which are pentoses, cannot be used by the beverage industry, but they are an excellent source of energy for cattle.

Cassava Wastes

Disposal of agricultural byproducts such as cassava wastes from processing activities is becoming a concern in Nigeria due to its foul dour. Conversion of these low-value cassava wastes into biosorbent that can remove toxic and valuable metals from industrial wastewater would increase their market value.

Recently, Cassava starch powder produced locally from Nigerian Cassava Starch Paste was tested for its ability to serve as a solidifying agent in microbiological nutrient media by Dabai and Muhammed in 2004 using different aqueous percentage concentration in pour plate and slants. Ten percent (10%) concentration produced getting usually associated with solid nutrient media within 30 minutes, though cassava starch has not been
observed to support microbial growth at the concentration used. In view of the findings of Dabai and Muhammed, (2004), Cassava starch powder can therefore be suggested as a potential solidifying agent in microbiological nutrient media as an alternative to Agar-agar.

**Pineapple Peels**

According to Tran et al. (1998) selection of a strain of *Aspergillus* for the production of citric acid from pineapple waste in solid-state fermentation proved valuable.

**Corn Cobs/Local Plant Tubers**

Corn cobs is waste produced from an agricultural products, it was converted into fermentable sugars by the pretreatment processes of dilution with distilled water and the action of concentrated hydrochloric acid respectively (Ashiru, 2005). According to Ashiru (2005), the presence of these fermentable sugars i.e. hexose sugars and additional nutrients added to this substrate, were then utilized each by two yeasts. *Candida albicans*, *Saccharomyces cerevisiae* and a mould, *Neurospora crassa* and converted it into ethanol after incubation for 96 hours at a temperature of 37°C. There has been preparation of several useful substances from corn cobs as far back as 1918 (LaForge and Hudson (1918)).

Growth culture media has also been composed from corn cobs and local plant tubers for microbial isolation as reported in a study by Bankole *et al* (2006). All media prepared from local plant tubers supported the growth fungi (Bankole and Aina, 2005). Corn cob was also acid hydrolyzed by Ashiru (2005) to obtained glucose and pentose sugars.

**Sugarcane bagasse**

Sugarcane bagasse is also waste product generated in large quantities in Nigeria and is classified as lignocelluloses. It was acid hydrolyzed by Ashiru (2005) to obtained glucose and pentose sugars. At the end of acid hydrolysis, detoxification was carried out for all substrates using potassium hydroxide and this was followed by the addition of other nutrients to increase yield and facilitate better fermentation. The highest yield and productivity was recorded with the fungus *Neurospora crassa* using sugarcane bagasse with values being 77.88g l⁻¹ (Ashiru, 2005).
Sugar Cane Molasses

There has been continuous production of citric acid from sugar cane molasses using a combination of submerged immobilized and surface stabilized cultures of *Aspergillus niger*, KCU520 as reported by Gupta (1994). Molasses are wastes products produced from sugar refineries and as an agricultural waste respectively, it was converted into fermentable sugars by the pretreatment processes of dilution with distilled water and the action of concentrated hydrochloric acid respectively. The presence of these fermentable sugars i.e. hexose sugars and additional nutrients added to these substrates, were then utilized each by two yeasts, *Candida albicans*, *Saccharomyces cerevisiae* and a mould, *Neurospora crassa* and converted into ethanol after incubation for 96 hours at a temperature of 37°C. *Neurospora crassa* produced the highest percentage yield of ethanol from the substrates, with 33.65% yield. *Candida albicans* produced the lowest percentage yield with 14.46% from molasses. *Saccharomyces cerevisiae* produced a percentage 23.59% ethanol from molasses (Ashiru, 2005).

Soy Whey/Soybean Curd Residue

Agarose-entrapped *Aspergillus niger* cells has been used for the production of citric acid from soy whey (Khare, 1994). Soybean curd residue supplement has been found to be very significant for enhancement of methane production from pretreated woody waste (Take et al., 2005).

Mango Peels

The use of fermented and unfermented mango peels (*Mangifera indica-R*) as animal feeds was reported by Ojokoh (2005). Ripe mango peels (*Mangifera indica-R*) was naturally fermented for 96 hours at room temperature (30°C). The quality of the unfermented and fermented mango peels were accessed by determining the proximate composition, mineral contents, anti-nutritional content as well as the microbiological quality. The result of the proximate analysis revealed that there was an increase in the protein content of the ripe mango peels fermented with value of 8.64% contents. Ojokoh (2005) used the fermented and unfermented samples to feed albino rats and found that there was an increase in the daily weight of the albino rat feds with these mango peels.
Banana Agro-Waste

Banana is major cash crop of this region generating vast agricultural waste after harvest. The agro-waste including dried leaves and pseudostem after harvest was used as substrate for the release of sugars. Thus, under these conditions the agro-waste left behind for natural degradation can be utilized affectively to yield fermentable sugars which can be converted into other substances like alcohol (Baig et al., 2003).

Processed Food Waste

Food waste can be defined as any edible material or byproduct that is generated in the production, processing, transportation, distribution, or consumption of food. The primary waste products fed to swine are plate and kitchen waste, bakery waste, and food products from grocery stores and this has proved very valuable as reported by many authors (MWPS, 1993) and dehydrated restaurant food waste products are used as feedstuffs for finishing pigs (Myer et al., 1999).

2.4.6 Potential biobased products and applications

Wastes from biomass can also provide raw materials for a diversity of biobased products. For e.g. plastics from biomass are being produced using polylactic acid from corn. According to Block (1999) executive order and proposed bill will boost biobased products and bioenergy in nations that sees the need for it.

2.4.6.1 Production of Biofuel

The demand for ethanol has the most significant market where ethanol is either used as a chemical feedstock or as an octane enhancer or petrol additive Brazil produces ethanol from the fermentation of cane juice whereas in the USA corn is used. In the US, fuel ethanol has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10% ethanol by volume (Sun and Cheng, 2002). The production of ethanol from sugars or starch impacts negatively on the economics of the process, thus making ethanol more expensive compared with fossil fuels. However, the huge amounts of residual plant biomass considered as waste can potentially be converted into various different value-added products including
biofuels, chemicals, and cheap energy sources for fermentation, improved animal feeds and human nutrients. High energy liquid fuels are also derived from plants (Nemethy et al., 1980).

2.4.6.2 Production of Biogas

Biogas is a renewable fuel and electricity produced from it can be used to attract renewable energy subsidies in some parts of the world (Wikipedia, 2008). Depending on where it is produced, biogas can also be called swamp, marsh, landfill or digester gas. A biogas plant is the name often given to an anaerobic digester that treats farm wastes or energy crops. Biogas can be produced utilizing anaerobic digesters. These plants can be fed with energy crops such as maize silage or biodegradable wastes including sewage sludge and food waste. The prospects for biogas cannot be underestimated (Pankhurst, 1983). The composition of biogas varies depending upon the origin of the anaerobic digestion process. Landfill gas typically has methane concentrations around 50%. Advanced waste treatment technologies can produce biogas with 55-75% CH₄ (Adelaide, 2007; Kolumbus, 2007; Wikipedia, 2008). Biogas can be utilized for electricity production, space heating, water heating and process heating. If compressed, it can replace compressed natural gas for use in vehicles, where it can fuel an internal combustion engine or fuel cells. Methane within biogas can be concentrated to the same standards as natural gas, when it is, it is called biomethane. If concentrated and compressed it can also be used in vehicle transportation (Wikipedia, 2008).

2.4.6.3 Production of Biorefinery Products

The most profitable way to operate a biomass-to-ethanol plant is as a refinery producing a variety of products from processing all the chemical components (hemicelluloses, cellulose, lignin, and extractives) of cellulosic feedstock. The plant could make use of extractives by converting them to resin acids, or pharmaceuticals (taxols from specific conifers, for example). Cellulose derivatives can be processed into a variety of products including higher value animal feeds.

2.4.6.4 Production of Biodiesel

Biodiesel production is a completely renewable resource. Biodiesel product is made from soya and canola, which is a self-sustaining fuel. Best of all it provides a market for excess soya bean
oil production. Biodiesel is a substitute for fuels that produce a lot of soot and carbons. These poisonous elements, which are, associated with regular diesel fuel emissions (especially buses). However, biodiesel has been around for decades as a supplement that is added to conventional diesel fuel to improve the lubricity of diesel engines. A Biodiesel fuel consists of methyl esters of soybean oil. Many car manufacturers are seeing the wisdom of creating vehicles that can accommodate a biodiesel product by creating a diesel car that is friendly to the use of vegetable oil blended with diesel fuel. In addition to displacing North America's reliance on imported petroleum, the use of biodiesel product has been shown to reduce air pollution and greenhouse gases.

2.4.6.5 Production of enzymes

- Production of Cellulases and Hemicellulases

Cellulases and hemicellulases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture (Farooq et al., 1994; Bhat, 2000; Sun and Cheng, 2002; Wong and Saddler, 1992a, b; Beauchemin et al., 2001, 2003). Hemicellulases are used for pulping and bleaching in the pulp and paper industry where they are used to modify the structure of xylan and glucomannan in pulp fibres to enhance chemical delignification (Suurnäkki et al., 1997; van Wyk, 1999a). Goyal et al (1991) expounded more on the characteristics of fungal cellulases while Kim et al (1998) studied the factorial optimization of a six cellulase mixture.

- Production of Xylanases

In the baking industry xylanases are used for improving desirable texture, loaf volume and shelf life of bread. A xylanase has shown excellent performance in the wheat separation process (Christopherson et al., 1997).

- Production of New enzyme systems

Valuable substances produced by organisms can be growing on food processing wastes. Diacetyl reductase, for example, has been isolated, purified, and characterized by some researchers. This enzyme is of industrial interest for the production of specialty chemicals from fats and oils. B-
glucosidase was produced by *Aspergillus niger* grown on fruit pomace. This enzyme is now under investigation for enzymatic release of natural flavors from food processing residues.

### 2.4.6.6 Production of other high-value products.

**Production of Xanthan**

Xanthan are produced by fermentation using glucose as the base substrate but theoretically these same products could be manufactured from “lignocellulose waste”.

**Production of Xylitol**

Xylitol used instead of sucrose in food as a sweetener, has odontological applications such as teeth hardening, remineralisation, and as an antimicrobial agent, it is used in chewing gum and toothpaste formulations (Roberto *et al.*, 2003; Parajó *et al.*, 1998). Various bioconversion methods, therefore, have been explored for the production of xylitol from hemicellulose using microorganisms or their enzymes (Nigam and Singh, 1995).

### 2.4.6.7 Production of industrial products

In recent times, many developed countries have returned to carbohydrate-based industries for industrial products (Louwrier, 1998). Renewable fuel production includes fuels such as ethanol, methanol, and hydrogen, biodiesel, and Fischer-Tropsch (FT) liquids (fuels derived using the Fischer-Tropsch conversion process). The FT process can produce diesel, naptha, and other fuels that can be used as substitutes for gasoline. Landfills produce a methane rich biogas that is most commonly used for power generation as previously discussed by many authors. Bioconversion of wastes could make a significant contribution to the production of organic chemicals (Coombs, 1987). Other examples of production of industrial chemicals produced in biofilm reactors include acetic acid or vinegar, lactic acid, succinic acid, and fumaric acid.

**Production of Ethanol**

There have been increased industrial uses of agricultural commodities to produce different products (Lee, 1994). The production of ethanol from biowaste can improve energy security and decrease pollution (Forward, 1994). Akpan *et al.* (1988) produced ethanol from gari effluents using *Aspergillus niger* in a one-step fermentation process. Rajagopal (1977) prepared a beer
using cassava. The same can be done using garri effluent to produce different types of alcoholic beverages. Ethanol has been produced continuously in an attached biofilm expanded bed bioreactor of *Zymomonas mobilis* and *Saccharomyces cerevisiae* in biofilm reactors (Bland *et al.*, 1982; Kunduru and Pometto, 1996) while Krug and Daugulis (1983) used *Zymomonas mobilis* immobilized on an ion exchange resin for ethanol production. Adsorbed cells of *Saccharomyces cerevisiae* have also been used in a packed bed continuous bioreactor to produce ethanol from molasses (Tyagi and Ghose, 1982). Lynd *et al* (1991) investigated ethanol yield and tolerance in continuous culture during thermophilic ethanol production. Ethanol is an excellent transportation fuel and blends of it have benefits, such as reduced gasoline use, thus lowering the need for fossil fuels. It also improves the performance of an ethanol–gasoline blend and ethanol provides oxygen for the fuel resulting in a more complete combustion with a low atmospheric photochemical reactivity. Even though CO₂ is released during the fermentation of sugars to form ethanol and the burning of ethanol as a fuel, the CO₂ is reutilized to grow new biomass replacing that harvested for ethanol production (Maddox, 1989).

**Production of Butanol/2,3-Butanediol**

Butanol is an important industrial chemical that can be produced from a number of carbohydrates using a number of microbial cultures. Butanol can be used as a fuel and has higher/greater energy content than ethanol (Maddox, 1989). Continuous production of 2, 3-butanediol from whey permeate using cells of *Klebsiella pneumoniae* immobilized on to bonechar was reported by Maddox *et al* (1988).

**Production of Acetic Acid/Vinegar**

Commercial production of acetic acid or vinegar using biofilm reactors as a bioconversion technology has been exercised for many years (Maddox, 1989). Production of these chemicals has been reported by Crueger and Crueger (1989). The percentage of starch (72%) in cocoyam was exploited in the production of ethanol and vinegar by Braide *et al.* (2005). Statistically, there exist a significant difference in the aroma and colour, but not in taste between cocoyam vinegar compared with commercial cider and white vinegar at 95% confidence limit. The acetic acid is produced by one of the bacteria grouped in the two genera, *Gluconobacter* and *Acetobacter*. The
species that are used commercially include *Acetobacter aceti*, *A. pasteurianus*, and *Gluconobacter oxydans*.

**-Production of Fumaric Acid**

Biofilm reactors as a bioconversion technology have also been used successfully for the production of fumaric acid from glucose (Cao et al., 1996) and mineral ore treatment (Crueger and Crueger, 1989).

**-Production of Citric Acid**

Citric acid (CA) is a carboxylic organic acid that is soluble in water with a pleasant taste. It is the most important acid used in the food industries. Until about 1920, all commercial CA was produced from lemon and lime juices. Rohr et al. (1983) reported that CA can be produced by fermentation process using species of microorganisms namely *Aspergillus niger*, a fungus which was used commercially for the first time in 1923 (Xu et al., 1989) and in solid-substrate fermentation (Lu, 1995). Sugar source has effect on the citric acid production by *Aspergillus niger* (Hossain et al., 1984; Murad et al., 2003).

**-Production of Lactic Acid**

Production of lactic acid in biofilm reactors is another example of industrial chemical production in such reactors as a bioconversion technology. Demirci et al. (1993a, b) evaluated a number of supports for biofilm formation using lactic acid producing cultures. Lactic acid was produced in repeated batch cultures in a biofilm reactor.

**-Production of Succinic Acid**

Succinic acid is a chemical that has been produced in biofilm reactors during bioconversion processes. The industrial potential for succinic acid fermentation was recognized as early as the late 1970s (Zeikus et al., 1999). Succinic acid (HOOCCH₂CH₂COOH) is a dicarboxylic acid, which can be used as a feedstock chemical for the production of high value products such as 1,4-butanediol, tetrahydrofuran, adipic acid, γ-butyrolactone, and n-methylpyrrolidone for applications in agriculture, food, medicine, plastics, cosmetics, and textiles.
Manures have been used as fertilizer for centuries, but crop fertilization with manure has received renewed attention in recent years as concern for water pollution potential from excess manure has increased. However, the content will vary depending on the type of animal, the feed that was consumed, the way the manure was handled, and other factors. Large, concentrated animal operations such as dairies and layer houses generally import more nutrients onto the farm in feed than goes out in milk, eggs, and animals (Johnson et al., 1991).

Forage producers can make good use of many materials considered wastes. Layer and broiler manure, dairy manure, septage and biosolids, composted urban plant debris, phosphogypsum, and waste lime from municipal water treatment plants are examples of materials that can be used for fertilizing and liming pastures and fields. Often these materials can be obtained at little or no cost to the farmer. In some circumstances the land owner may be paid for taking the waste, thus collecting a disposal fee while the soil and crops benefit from the materials added. Forage crops such as corn silage and hay are excellent candidates for such application since they remove nutrients in significant quantities (Kidder, 2002).

### 2.4.7 Potential applications of bioproducts

Food wastes will not pollute the soil if the materials are chosen carefully and applied properly. A waste in one situation can be a resource in another. Animal manures have been returned to the land as fertilizer and soil conditioner for centuries. A wide variety of biomass resources are available on our planet for conversion into bioproducts. These may include whole plants, plant parts (e.g. seeds, stalks), plant constituents (e.g. starch, lipids, protein and fibre), processing byproducts (distiller’s grains, corn solubles), materials of marine origin and animal byproducts, municipal and industrial wastes (Smith et al., 1987). These resources can be used to create new biomaterials and this will require an intimate understanding of the composition of the raw material whether it is whole plant or constituents, so that the desired functional elements can be obtained for bioproduct production (Howard et al., 2003). Application of biotechnology to the processing of food (including beverages) produced from agriculture has proved highly valuable (Cooper, 2003). Indeed, the combination of bio-based feedstock, bio-processes and new products offers the potential to revolutionize chemical industry structures. In less than 10 years, integrated
biorefineries will play a role comparable to today's oil and gas crackers. They will make use of row crops, energy crops, agricultural waste and food waste as inputs to extract oil and starch for food, protein for feed, lignin for combustion, cellulose for conversion into fermentable sugars, as well as other by-products. Sugar will be the key feedstock of the future, as it can be used to ferment ethanol for transportation fuel, but also for a whole set of new, basic building blocks. Molecules such as lactic acids, succinic acid, propylene glycol or 3-hydroxy propionic acid produced at 20 cents per pound can catalyze the innovation of new chemical product families, similar to the innovation boost based on the cracker chemicals in the middle of this 21st century. Indeed, the combination of bio-based feedstock, bio-processes and new products offers the potential to revolutionize chemical industry structures. Owing to simplicity in implementation and use of cheap raw materials in villages, it is one of the most environmentally sound energy sources for rural needs. Fossil oil and natural gas are being replaced by carbohydrates from renewable resources as low cost, renewable feedstock. In particular, the development of technology to convert cellulosic biomass from agricultural waste, food waste or energy crops into fermentable sugars offers the perspective of producing ethanol and other bulk organic chemicals at low cost. The cost of biomass-based ethanol produced on a commercial scale, for example, is expected to undercut the cost of gasoline with oil at $30 per barrel. Enzymes, for example, are fast-growing bio-products that make washing powder more effective, allow softer processing of textiles and pulp and paper, and reduce nitrogen emissions from animal farming.

2.4.8 Benefits of food waste utilization

Compared to burning fossil fuel, there may be benefits associated with greenhouse gas reduction with the creation of markets for agricultural waste where its disposal has a negative effect on the environment. For example, the use of biomass as feedstock reduces net carbon emissions to the atmosphere and provides reductions in methane emissions from natural decay processes. The ecologically responsible removal of slash from logged areas benefits the environment. The use of other agricultural residues also reduces emissions of volatile organic compounds, odors, dust, and nuisances associated with agricultural operations such as dairies and animal feeding operations. Improved management of animal manure and solid wastes also reduces ground water contamination (MWPS, 1993). There are economic benefits from the biomass based industrial activities such as electricity generation, erosion control, and for the production of fuels, animal
feed, and green or renewable chemicals (such as solvents and lubricants, polymers and plastics) and increased food productivity. Total economic benefits derived from these activities depend on the mixture of biomass based products generated from it, as well as the state of maturity of these industries.

2.4.9 Considerations for food waste utilization

1. The effect of utilization of food waste on the water budget should be considered, particularly where a shallow ground water table is present or in areas suitable to runoff.

2. Minimize the impact of odors of land-applied food wastes.

3. Food and agricultural wastes contain pathogens. Wastes should be utilized in a manner that minimizes the disease potential to humans or animals.

4. Flushing of animal wastes offers several advantages for the livestock producer including labor efficiency and a more pleasant if not actually healthier environment in animal housing areas (Nordstedt and Baldwin, 2002).

2.4.10 Challenges for waste utilization

2.5.10.1 Pollution of surface waters

Pollution of surface waters may result from runoff of applied food waste materials. To prevent this, one should not apply waste to frozen or saturated soils. Incorporating surface-applied food waste into soil will limit overland movement of nutrients and organic matter detrimental to water quality.

2.4.10.2 High Production Costs

The cost of processing cellulosic materials to produce ethanol is high. The development of new technologies has significantly decreased the cost of producing ethanol, particularly corn-derived ethanol. However, for some, technologies for cellulosic ethanol production are currently in the experimental state, while others believe that these technologies are already sufficiently mature but have not been widely applied due to lack of capital, which is difficult to attract for the implementation of new technologies (MWPS, 1993).
As the current trend in the world today is to utilize and convert waste into useful products and to recycle waste product as means of achieving sustainable development.

Bioproduct development would influence the activities of the food, pharmaceutical, cosmetic and petroleum sectors more in the future as the pressure on waste management and biodevelopment increases. As with any development, the sustainable re-use of food waste resources would not be without difficulties, but it would open up the opportunity for biotechnological developments. The economic environment would need to foster the type of conditions in which the emergent food industry can thrive. Developing countries are still grappling with socioeconomic issues including meeting the massive energy shortage demands, food security and developing biotechnological solutions in the agriculture, agro-processing and other related manufacturing sectors. Therefore to manage food wastes, the general pathways of industrial-food waste generation should be reduced, recycled or reused and what is left must be treated and disposed of in an environmentally acceptable way. If a process is not environmentally friendly, it should be redesigned such that it becomes so and where a process cannot be redesigned, then it is necessary to reconsider whether it should be undertaken at all.

2.4.11 Recent research in waste utilization for microbial growth

Studies on orange peels

-The growth behaviour of Memnoniella echinata and Fusarium roseum was examined in slurry fermentation systems using untreated orange peel as substrate (Clementi et.al 1985). The more concentrated the peel slurry, the greater the substrate inhibitory effect on microbial growth becomes. The optimal operating strategy for such SCP production process to be determined.

-Orange peels were hydrolyzed by two different methods to fermentable sugars, namely by dilute acid hydrolysis (0.5% (v/v) H$_2$SO$_4$) at 150 °C and by enzymatic hydrolysis by cellulase, pectinase and β- glucosidase. The fungus was able to produce ethanol with a maximum yield of about 0.36 g/g after 24 h when grown on acid hydrolyzed orange peels both by aerobic and anaerobic cultivation. A preliminary aerobic cultivation on enzymatic hydrolyzed orange peels gave a maximum ethanol yield of 0.33 g/g after 26 h. The major metabolite produced during the cultivations was ethanol. Apart from ethanol, glycerol was the only component produced in
significant amounts. In cultivations performed aerobically on acid and enzymatic hydrolyzed orange peels the glycerol yields were 0.048 g/g after 24 h. (Päivi Ylitervo, 2008)

**Studies on banana peels**

- The ability of *Aspergillus niger* to produce mycelia protein from pretreated banana peels as substrate was studied with yeast nitrogen base glucose broth as control. Banana peels gave the highest yield of mycelia protein with optical density of 0.28 (O.D) compared to the yeast nitrogen base glucose broth 0.08 (O.D). The residual glucose content in the banana peel medium decreased from 1800 µg/ml on the first day to 320 µg/ml on the 7th day. In the yeast nitrogen base glucose broth, it decreased from 2440 µg/ml on day one to 420 µg/ml on the 7th day. The mycelia protein from banana peels had a crude content of 20.4% and a lipid content of 33.3%. The results indicate the possibility of using banana peels as substrates for mycelia protein production with *A. niger*. (YABAYA, A. & ADO, S. A. 2008)

- Agricultural residues rich in carbohydrates can be utilized in fermentation process to produce microbial protein which in turn can be used to determine the factors influencing cell biomass production *Pseudomonas fluorescens* was cultivated using banana peel out, watermelon skin, and Cane molasses showed that the strain was capable of meeting its components required for growth. The organism was capable of growth at 37°C when supplemented with agricultural wastes in different concentration mixed with agar. The number of colony forming unit were more when compared with nutrient agar. (C. N. Murugalakshmi and S. S. Sudha, 2010)

- Alpha amylase is a hydrolytic enzyme and in recent years, interest in its microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries (Asghar et al., 2000). Alpha amylase was produced by *Bacillus subtilis* utilizing banana peel in a solid state fermentation (SSF).

  The effect of varying incubation period, substrate level, pH of the medium, incubation temperature, peptone (nitrogen source) and micronutrients on the production of α-amylase was investigated. The maximum activity of α-amylase (9.06 IU/mL/min) was recorded after 24 hours of SSF at pH 7 and 35°C temperature of the optimum banana peel medium containing, 50 g fresh chopped banana peel (substrate), 0.2% peptone, 0.02% MgSO₄·7H₂O, 0.04% CaCl₂·2H₂O and 0.1% KH₂PO₄. The enzyme produced by *Bacillus subtilis* can be used in industrial processes after characterization. (SHAISTA KOKAB, 2003)
Studies on Papaya peels

-Papaya (Carica papaya L.) is a sugar crop with soluble saccharides in the form of glucose, fructose and sucrose (Solvaraj and Pal, 1982), and it is widely cultivated in several countries (Samson, 1980). Sugars represent the part of the fruits which is used by microorganisms for single cell protein and alcohol production (Oura, 1983).

-Utilization of three food processing wastes (lemon pulp, papaya fruit waste and rice straw) for the production of fruit bodies mushroom with edible fungi Pleurotus ostreatus NRRL-0366 was done using solid state fermentation technique. Rice straw supplemented with different concentrations of the other two wastes forming eight substrates was used in this study. The highest mushroom harvested was with a substrate no. 2 (equal concentration of lemon pulp and rice straw) giving 957.80 g/Kg fresh fruit bodies with biological efficiency 26.98% followed by substrate no. 7 (double amounts of papaya fruit waste supplemented with one amount of rice straw) giving 431.1g/Kg fresh fruit bodies with biological efficiency 13.50%. The results revealed that the fruit bodies containing 26.0-31.5% digestible protein, 20.9 -33.0% total soluble carbohydrates and 2.0-5.9% fat (on dry basis). Calorific values for the eight fruit bodies ranged 189-253 Kcal per 100 g of dry mushrooms. GLC analysis of the eight fatty acids of the fruit bodies has revealed that the unsaturated fatty acids were at higher concentration (68.2-75.2%) than the saturated one. Studies were carried out on the activities of amylase, cellulase, invertase, polygalacturonase and pectinlyase enzymes of Pleurotus ostreatus fruit bodies cultivated in the previous eight substrates. The substrates affect the enzyme activities of Pleurotus ostreatus fruit bodies. A higher activity of amylase was observed in the unsupplemented lemon pulp and papaya waste. No significant change was obtained in cellulase and invertase activities within the eight substrates except substrate no. 7 using papaya waste. This fungus is able to produce high levels of pectinlyase in fruit bodies during solid state fermentation on lemon pulp.

-Extracts of papaya fruit were used as substrate for single cell protein (SCP) production using Saccharomyces cerevisiae. A 500 g of papaya fruit was extracted with different volumes of sterile distilled water. Extraction with 200 mL of sterile distilled water sustained highest cell growth. Biochemical analysis of dry biomass revealed the following composition: 35.5% protein, 40.7% saccharide, 4.09% lipids, 0.04% magnesium, 9.63% moisture and 7.90% total ash.
Nutrient found in papaya fruit extract were 9.8% saccharide, 0.1% crude protein and 7.3% total soluble sugars. (OJOKOH A.O.* and R.E. UZEH, 2005)

**Studies on Whey**

Cheese whey is a by-product of cheese manufacture that remains when CN and butter fat are separated as curd from milk. Whey permeate is further produced when proteins in whey are recovered as a whey protein concentrate by ultrafiltration (Haast et al., 1986; Lee et al., 2003). Whey permeate contains nutrients necessary for microbial growth and holds almost 100% of lactose in milk. However, because of its high lactose content (4 to 5%; Gonza’lez Siso, 1996), disposal of large volumes of whey permeate causes serious environmental problems because of its chemical oxygen demand (40 to 60 g/L; Martin, 1998). One possible solution to this problem is to use this potential pollutant as a growth substrate for economically valuable products.

The main purpose of this work was to isolate and characterize lactic acid bacteria (LAB) strains to be used for biomass production using a whey-based medium supplemented with an ammonium salt and with very low levels of yeast extract (0.25 g/L). Five strains of LAB were isolated from naturally soured milk after enrichment in whey-based medium. One bacterial isolate, designated MNM2, exhibited a remarkable capability to utilize whey lactose and give a high biomass yield on lactose. This strain was identified as *Lactobacillus casei* by its 16S rDNA sequence. A kinetic study of cell growth, lactose consumption, and titratable acidity production of this bacterial strain was performed in a bioreactor. The biomass yield on lactose, the percentage of lactose consumption, and the maximum increase in cell mass obtained in the bioreactor were 0.165 g of biomass/g of lactose, 100%, and 2.0 g/L, respectively, which were 1.44, 1.11, and 2.35 times higher than those found in flask cultures. The results suggest that it is possible to produce LAB biomass from a whey-based medium supplemented with minimal amounts of yeast extract. (Elene et al 2006)

-A novel approach to utilizing whey permeate, the cultivation of mycelia of the edible mushroom *Ganoderma lucidum*, is introduced. The major objective of this research was to use whey permeate as an alternative growth medium for the cultivation of mycelia of edible mushroom *G. lucidum* and to find an optimum condition for solid-state cultivation. (M. Song, 2007)