Chapter 4
Results and Discussion

4.1 Selection of different Probiotic organisms

The studies were done to select the best probiotic organism for economical production. According to already existing data, there are thousands of probiotic strains available and a lot of research have been done on their useful probiotic properties. The present study was aimed at the economical production of a particular probiotic without sacrificing its normal characters. Therefore the first step of the study was to select a probiotic strain. For this selection certain parameters were fixed and the different existing probiotic strains were screened for desired characters. The main criteria were that the strain having probiotic property should be easily available and a lot of research should have been done on its beneficial properties on host organisms. With respect to these primary criterions we have selected six probiotic strains namely *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Saccharomyces cerevisiae* and *Escherichia coli*. For further selection, secondary screening were performed. For secondary screening we have fixed sixteen characters and all the six organisms were screened on the basis of them. These characters were:

- Probiotic property
- Natural presence in fermented food products which are consumable
- Normal constituent of human digestive system
- Ease in isolation and identification
- Growth at wide range of temperature and pH
- Provision to exclude competing bacteria: The primary mechanism for probiotic action is known as competitive colonization or competitive suppression. It is best described as the proliferation of the probiotic bacteria in the human intestine, leaving little space for the growth of any pathogens (Balongue, 1992; Biavati et al., 2000).
- Growth on agricultural waste
- Ability to survive in human gastrointestinal tract
- Ability to produce antimicrobial substances
- Genome sequenced
• Sensitivity to common antibiotics
• Aerotolerancy
• Organotrophic nutrition
• Non-pathogenic
• Ability to produce lactic and acetic acid to control intestinal pH
• Important research going on the strain.

*Screening results for Lactobacillus acidophilus*: The table 4.1 shows the results for *Lactobacillus acidophilus*. On the basis of analysis, it has been inferred that the strain has many characters which make it suitable for the studies.

Table 4.1 Screening results for *Lactobacillus acidophilus*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characters</th>
<th>Result</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Probiotic property</td>
<td>Present</td>
<td>Can be taken as probiotics</td>
</tr>
<tr>
<td>2</td>
<td>Natural presence in fermented food products</td>
<td>Present</td>
<td>Not harmful to humans</td>
</tr>
<tr>
<td></td>
<td>which are consumable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Normal constituent of human digestive system</td>
<td>Present</td>
<td>No allergic reaction</td>
</tr>
<tr>
<td>4</td>
<td>Ease in isolation and identification</td>
<td>Present</td>
<td>Ease in handling</td>
</tr>
<tr>
<td>5</td>
<td>Growth at wide range of temperature and pH</td>
<td>Present</td>
<td>Ease in cultivation</td>
</tr>
<tr>
<td>6</td>
<td>Provision to exclude competing bacteria</td>
<td>Present</td>
<td>Low chance of contamination, effective probiotic</td>
</tr>
<tr>
<td>7</td>
<td>Growth on agricultural waste</td>
<td>Present</td>
<td>Can be cultivated on waste materials</td>
</tr>
<tr>
<td>8</td>
<td>Ability to survive in human gastrointestinal tract</td>
<td>Present</td>
<td>Effective in action</td>
</tr>
<tr>
<td>S.No</td>
<td>Characters</td>
<td>Result</td>
<td>Inference</td>
</tr>
<tr>
<td>------</td>
<td>--------------------------------------------------------</td>
<td>-------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Probiotic property</td>
<td>Present</td>
<td>Can be taken as probiotics</td>
</tr>
<tr>
<td>2</td>
<td>Natural presence in fermented food products which are consumable</td>
<td>Normally present in soil</td>
<td>May not be taken as such for direct human consumption.</td>
</tr>
<tr>
<td>3</td>
<td>Normal constituent of human digestive system</td>
<td>Absent</td>
<td>May develop allergic reaction</td>
</tr>
<tr>
<td>4</td>
<td>Ease in isolation and</td>
<td>Present</td>
<td>Ease in handling</td>
</tr>
</tbody>
</table>

**Screening results for Bacillus subtilis** : The table 4.2 shows the results for Bacillus subtilis. On the basis of analysis it has been inferred that the strain has many characters which makes it suitable for the studies but this strain is not the normal constituent of human digestive system and also it does not usually occurs in the fermented food products.
<table>
<thead>
<tr>
<th>Identification</th>
<th>Present</th>
<th>Ease in cultivation, 18-43°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Growth at wide range of temperature and pH</td>
<td>Present</td>
<td>Low chance of contamination, Effective probiotic</td>
</tr>
<tr>
<td>6 Provision to exclude competing bacteria</td>
<td>Present</td>
<td>Can be cultivated on waste materials, generally cultivated on selective media (Norris et al., 1981, Sharp et al., 1989)</td>
</tr>
<tr>
<td>7 Growth on agricultural waste</td>
<td>Present</td>
<td>Spores of this organism enable it to pass across the gastric barrier</td>
</tr>
<tr>
<td>8 Ability to survive in human gastrointestinal tract</td>
<td>Present</td>
<td>Spores of this organism enable it to pass across the gastric barrier</td>
</tr>
<tr>
<td>9 Ability to produce antimicrobial substances</td>
<td>Present</td>
<td>Generally used as fungicide (Korsten et al., 1995)</td>
</tr>
<tr>
<td>10 Genome sequenced</td>
<td>Present</td>
<td>Helps in complete information</td>
</tr>
<tr>
<td>11 Sensitivity to common antibiotics</td>
<td>Present</td>
<td>Can be killed easily if any side effect or overdose occurs</td>
</tr>
<tr>
<td>12 Aerotolerancy</td>
<td>Present</td>
<td>Obligate aerobe (need to provide oxygen in the growth media)</td>
</tr>
<tr>
<td>13 Organotropic nutrition</td>
<td>Present</td>
<td>Can utilize waste organic matter</td>
</tr>
<tr>
<td>14 Non pathogenic</td>
<td>Present</td>
<td>No side effects GRAS organism (Djien, K.S. &amp; Hesseltine, 1979)</td>
</tr>
<tr>
<td>15 Ability to produce lactic and acetic acid.</td>
<td>Present</td>
<td>Possibility to control intestinal pH</td>
</tr>
<tr>
<td>16 Important research going on the strain.</td>
<td>Present</td>
<td>This organism is generally used for industrial productions. Harwood, 1989</td>
</tr>
</tbody>
</table>

**Screening results for Bifidobacterium bifidum:** The table 4.3 shows the results for Bifidobacterium bifidum. Table shows that there are many properties making it suitable to be used as
probiotic strain. Also, it is generally preferred for probiotic products. But for our studies, it has been found that the organism does not grow on raw materials. Thus, it is not further utilized for studies.

Table 4.3 Screening results for *Bifidobacterium bifidum*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characters</th>
<th>Result</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Probiotic property</td>
<td>Present</td>
<td>Can be taken as probiotics (Sanders, 1999).</td>
</tr>
<tr>
<td>2</td>
<td>Natural presence in fermented food products which are consumable</td>
<td>Present</td>
<td>Not harmful to humans</td>
</tr>
<tr>
<td>3</td>
<td>Normal constituent of human digestive system</td>
<td>Present</td>
<td>No allergic reaction (Gismondo et al., 1999).</td>
</tr>
<tr>
<td>4</td>
<td>Ease in isolation and identification</td>
<td>Present</td>
<td>Ease in handling</td>
</tr>
<tr>
<td>6</td>
<td>Provision to exclude competing bacteria</td>
<td>Present</td>
<td>Low chance of contamination, Effective probiotic</td>
</tr>
<tr>
<td>7</td>
<td>Growth on agricultural waste</td>
<td>Present</td>
<td>Can be cultivated on waste materials</td>
</tr>
<tr>
<td>8</td>
<td>Ability to survive in human gastrointestinal tract</td>
<td>Present</td>
<td>Effective in action (Schezenmeir and De Vrese, 2001).</td>
</tr>
<tr>
<td>9</td>
<td>Ability to produce antimicrobial substances</td>
<td>Present</td>
<td>Can exclude other organisms (that may be pathogenic) (Ziemer and Gibson, 1998).</td>
</tr>
<tr>
<td>10</td>
<td>Genome sequenced</td>
<td>Present</td>
<td>Helps in complete information</td>
</tr>
<tr>
<td>11</td>
<td>Sensitivity to common antibiotics</td>
<td>Present</td>
<td>Can be killed easily if any side effect or overdose occurs</td>
</tr>
<tr>
<td>12</td>
<td>Aerotolerance</td>
<td>Present</td>
<td>No need to provide oxygen in the growth media</td>
</tr>
<tr>
<td>13</td>
<td>Organotropic nutrition</td>
<td>Present</td>
<td>Can utilize waste organic matter</td>
</tr>
</tbody>
</table>
14 Non pathogenic Present No side effects
15 Ability to produce lactic and acetic acid. Present Possibility to control intestinal pH
16 Important research going on the strain. Present Used as probiotic organism

**Screening results for Enterococcus faecium:** The table 4.4 shows the results for Enterococcus faecium. This organism may or may not be present in the fermented food products and also generally is not a normal constituent of the human digestive tract. Thus it has been inferred that this strain can be used as a probiotic for animal feed products.

Table 4.4 Screening results for Enterococcus faecium

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characters</th>
<th>Result</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Probiotic property</td>
<td>Present</td>
<td>Can be taken as probiotics (Wunderlich PF, 1989, Bellomo G, 1980, Mitra AK, 1990)</td>
</tr>
<tr>
<td>2</td>
<td>Natural presence in fermented food products which are consumable</td>
<td>Present/Absent</td>
<td>Generally not present in fermented food products</td>
</tr>
<tr>
<td>3</td>
<td>Normal constituent of human digestive system</td>
<td>Present/Absent</td>
<td>May develop allergic reaction</td>
</tr>
<tr>
<td>4</td>
<td>Ease in isolation and identification</td>
<td>Present</td>
<td>Ease in handling</td>
</tr>
<tr>
<td>5</td>
<td>Growth at wide range of temperature and pH</td>
<td>Present</td>
<td>Ease in cultivation</td>
</tr>
<tr>
<td>6</td>
<td>Provision to exclude competing bacteria</td>
<td>Present</td>
<td>Low chance of contamination, Effective probiotic</td>
</tr>
<tr>
<td>7</td>
<td>Growth on agricultural waste</td>
<td>Present</td>
<td>Can be cultivated on waste materials</td>
</tr>
<tr>
<td>S.No</td>
<td>Characters</td>
<td>Result</td>
<td>Inference</td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Probiotic property</td>
<td>Present</td>
<td>Can be taken as probiotics</td>
</tr>
<tr>
<td>2</td>
<td>Natural presence in fermented food products which are consumable</td>
<td>Present</td>
<td>Not harmful to humans</td>
</tr>
</tbody>
</table>

**Screening results for Saccharomyces cerevisiae:** The table 4.5 shows the results for Saccharomyces cerevisiae. According to the table the inference can be drawn that Saccharomyces cerevisiae cells does not possesses growth at wide range of temperature and pH and it also does not have the ability to produce lactic and acetic acid. These properties are considered important for economical industrial production and probiotic production respectively.

Table 4.5 Screening results for Saccharomyces cerevisiae
<table>
<thead>
<tr>
<th></th>
<th>Normal constituent of human digestive system</th>
<th>Present</th>
<th>No allergic reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Ease in isolation and identification</td>
<td>Present</td>
<td>Ease in handling</td>
</tr>
<tr>
<td>6</td>
<td>Provision to exclude competing bacteria</td>
<td>Present</td>
<td>Low chance of contamination, Effective probiotic</td>
</tr>
<tr>
<td>7</td>
<td>Growth on agricultural waste</td>
<td>Present</td>
<td>Can be cultivated on waste materials</td>
</tr>
<tr>
<td>8</td>
<td>Ability to survive in human gastrointestinal tract</td>
<td>Present</td>
<td>Effective in action</td>
</tr>
<tr>
<td>9</td>
<td>Ability to produce antimicrobial substances</td>
<td>Present</td>
<td>Can exclude other organisms (May be pathogenic)</td>
</tr>
<tr>
<td>10</td>
<td>Genome sequenced</td>
<td>Present</td>
<td>Helps in complete information (A. Goffeau, 1986)</td>
</tr>
<tr>
<td>11</td>
<td>Sensitivity to common antibiotics</td>
<td>Present</td>
<td>Can be killed easily if any side effect or overdose occurs</td>
</tr>
<tr>
<td>12</td>
<td>Aerotolerancy</td>
<td>Present</td>
<td>No need to provide oxygen in the growth media</td>
</tr>
<tr>
<td>13</td>
<td>Organotropic nutrition</td>
<td>Present</td>
<td>Can utilize waste organic matter</td>
</tr>
<tr>
<td>14</td>
<td>Non pathogenic</td>
<td>Present</td>
<td>No side effects</td>
</tr>
<tr>
<td>15</td>
<td>Ability to produce lactic and acetic acid.</td>
<td>Absent</td>
<td>Causes fermentation</td>
</tr>
<tr>
<td>16</td>
<td>Important research going on the strain.</td>
<td>Present</td>
<td>It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology</td>
</tr>
</tbody>
</table>
Screening results for *Escherichia coli*: The table 4.6 shows the results for *Escherichia coli*. This organism is an opportunistic pathogen thus is usually not taken as probiotic strain.

Table 4.6 Screening results for *Escherichia coli*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characters</th>
<th>Result</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Probiotic property</td>
<td>Present</td>
<td>Can be taken as probiotics</td>
</tr>
<tr>
<td>2</td>
<td>Natural presence in fermented food products which are consumable</td>
<td>Present</td>
<td>Not harmful to humans</td>
</tr>
<tr>
<td>3</td>
<td>Normal constituent of human digestive system</td>
<td>Present</td>
<td>Sometimes may act as opportunistic pathogen</td>
</tr>
<tr>
<td>4</td>
<td>Ease in isolation and identification</td>
<td>Present</td>
<td>Ease in handling</td>
</tr>
<tr>
<td>5</td>
<td>Growth at wide range of temperature and pH</td>
<td>Present</td>
<td>Ease in cultivation</td>
</tr>
<tr>
<td>6</td>
<td>Provision to exclude competing bacteria</td>
<td>Present</td>
<td>Low chance of contamination, Effective probiotic</td>
</tr>
<tr>
<td>7</td>
<td>Growth on agricultural waste</td>
<td>Present</td>
<td>Can be cultivated on waste materials</td>
</tr>
<tr>
<td>8</td>
<td>Ability to survive in human gastrointestinal tract</td>
<td>Present</td>
<td>Effective in action</td>
</tr>
<tr>
<td>9</td>
<td>Ability to produce antimicrobial substances</td>
<td>Present</td>
<td>Can exclude other organisms (May be pathogenic)</td>
</tr>
<tr>
<td>10</td>
<td>Genome sequenced</td>
<td>Present</td>
<td>Helps in complete information</td>
</tr>
<tr>
<td>11</td>
<td>Sensitivity to common antibiotics</td>
<td>Present</td>
<td>Can be killed easily if any side effect or over dose occurs</td>
</tr>
<tr>
<td>12</td>
<td>Aerotolerancy</td>
<td>Present</td>
<td>No need to provide oxygen in the growth media</td>
</tr>
<tr>
<td>13</td>
<td>Organotropic nutrition</td>
<td>Present</td>
<td>Can utilize waste organic matter</td>
</tr>
<tr>
<td>14</td>
<td>Non pathogenic</td>
<td>Present/Absent</td>
<td>Opportunistic pathogen</td>
</tr>
</tbody>
</table>
15 Ability to produce lactic and acetic acid.
Present Produce acids with gas production which may lead to gastric problems.

16 Important research going on the strain.
Present A well known bacterial model

On the basis of all the above tables we have selected *Lactobacillus acidophilus*, and *Enterococcus faecium* for our further studies because they were found to have the majority of criterion selected for the secondary screening. Research shows that *Lactobacillus acidophilus* is used as a probiotic for human consumption while the *Enterococcus faecium* is primarily used as probiotic for animal feed. Therefore the studies were done with both the organisms so that an effective probiotic can be developed for both humans and animals.

4.2 Selection of different waste materials

Different waste materials commonly available in the locality were selected on the basis of following criterions:-

- Easy availability.
- Large volume.
- High organic content.
- High nutrient content.
- Tendency to form suspension.
- Having problematic decomposition.
- Problem in disposal.
- Industrial waste material.

The waste materials selected on the basis of above points were than analyzed for the presence of nutritive substances, mainly carbohydrates and proteins. In the initial steps the qualitative analysis for carbohydrates and proteins were done and the results were as follows:
4.2.1 Studies on waste vegetable peels Table 4.7 shows the results for the qualitative analysis of carbohydrates and proteins in different vegetable peels commonly available in the locality. On the basis of results obtained, inference was drawn whether they can be used for growth of probiotics or not.

Table 4.7 Studies on waste vegetable peels

<table>
<thead>
<tr>
<th>S.No</th>
<th>Vegetable Peel</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potato</td>
<td>Present</td>
<td>Very less</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>2</td>
<td>Cucumber</td>
<td>Very less</td>
<td>Very less</td>
<td>Can't be utilized further</td>
</tr>
<tr>
<td>3</td>
<td>Beans</td>
<td>Very less</td>
<td>Very less</td>
<td>Can't be utilized further</td>
</tr>
<tr>
<td>4</td>
<td>Peas</td>
<td>Very less</td>
<td>Very less</td>
<td>Can't be utilized further</td>
</tr>
<tr>
<td>5</td>
<td>Cabbage</td>
<td>Very less</td>
<td>Very less</td>
<td>Can't be utilized further</td>
</tr>
<tr>
<td>6</td>
<td>Carrot</td>
<td>Very less</td>
<td>Very less</td>
<td>Can't be utilized further</td>
</tr>
<tr>
<td>7</td>
<td>Banana</td>
<td>Present</td>
<td>Very less</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>8</td>
<td>Orange</td>
<td>Present</td>
<td>Very less</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>9</td>
<td>Papaya</td>
<td>Present</td>
<td>Very less</td>
<td>Can be utilized further</td>
</tr>
</tbody>
</table>

4.2.2 Studies on Kitchen-waste materials Table 4.8 shows qualitative analysis of carbohydrates and proteins in Kitchen-waste materials. These wastes may prove to be highly nutritious since they contain a mixture of different food materials but the ratio of these components is not strict and varies according to food type being cooked in the kitchen.

Table 4.8 Studies on Kitchen-waste materials

<table>
<thead>
<tr>
<th>S.No</th>
<th>Kitchen waste</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Peels</td>
<td>Present</td>
<td>Very less</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>2</td>
<td>Waste chapati</td>
<td>Present</td>
<td>Present</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>3</td>
<td>Waste food</td>
<td>Present</td>
<td>Present</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>4</td>
<td>Rice water</td>
<td>Present</td>
<td>Very less</td>
<td>Can be utilized further</td>
</tr>
</tbody>
</table>
4.2.3 Studies on Agricultural-waste materials  Agricultural waste materials are available in large amounts and may contain nutrients for microbial growth thus studies were performed on some of these which are easily available in the locality. Table 4.9 shows studies on agricultural-waste materials.

Table 4.9 Studies on Agricultural-waste materials

<table>
<thead>
<tr>
<th>S.No</th>
<th>Agricultural waste</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rice Husk</td>
<td>Present</td>
<td>Very less</td>
<td>Can’t be utilized further</td>
</tr>
<tr>
<td>2</td>
<td>Wheat Husk</td>
<td>Present</td>
<td>Very less</td>
<td>Can’t be utilized further</td>
</tr>
<tr>
<td>3</td>
<td>Sugarcane waste</td>
<td>Present</td>
<td>Present</td>
<td>Can be utilized further</td>
</tr>
</tbody>
</table>

4.2.4 Studies on Industrial-waste materials  Industrial-waste materials are generated in large amounts and carries a huge problem of disposal. Studies have already shown that these waste materials contains organic substances. Also it is a general observation that when dumped freely in the environment, industrial-waste materials leads to the generation of bad odour and unclean environment. Thus studies were performed on them so that a beneficial usage of them can be suggested.

Table 4.10 Studies on Industrial-waste materials

<table>
<thead>
<tr>
<th>S.No</th>
<th>Industrial waste</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soya waste</td>
<td>Present</td>
<td>Present</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>2</td>
<td>Dairy waste</td>
<td>Present</td>
<td>Present</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>3</td>
<td>Sugar waste</td>
<td>Present</td>
<td>Present</td>
<td>Can be utilized further</td>
</tr>
</tbody>
</table>

Quantitative estimation for carbohydrates and proteins will further provide the amount of these biomolecules in the waste materials. Thus on the basis of qualitative estimation, some of the
waste materials have been selected and then analysed for quantitative estimation. Table 4.11 shows quantitative estimation of selected waste materials:

Table 4.11 Quantitative estimation of selected waste materials

<table>
<thead>
<tr>
<th>S.No</th>
<th>Waste</th>
<th>Carbohydrate (g/100ml)</th>
<th>Protein (g/100ml)</th>
<th>Other nutrients</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potato</td>
<td>50.00</td>
<td>2.50</td>
<td>Lipids &amp; Fibres</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>2</td>
<td>Banana</td>
<td>46.00</td>
<td>1.45</td>
<td>Lipids &amp; Fibres</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>3</td>
<td>Orange</td>
<td>16.86</td>
<td>5.80</td>
<td>Vitamin &amp; Fibres</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>4</td>
<td>Papaya</td>
<td>12.50</td>
<td>57.80</td>
<td>nil</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>5</td>
<td>Rice water</td>
<td>50.35</td>
<td>2.0</td>
<td>Some elements</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>6</td>
<td>Sugar cane waste</td>
<td>negligible</td>
<td>negligible</td>
<td>nil</td>
<td>Can’t be utilized further</td>
</tr>
<tr>
<td>7</td>
<td>Soya waste</td>
<td>35.00</td>
<td>20.50</td>
<td>Minerals</td>
<td>Can be utilized further</td>
</tr>
<tr>
<td>8</td>
<td>Dairy waste</td>
<td>5.8</td>
<td>1.5</td>
<td>Minerals &amp; water</td>
<td>Can be utilized further</td>
</tr>
</tbody>
</table>

After selection of waste from above studies these were utilized for the growth of two selected probiotic organisms *Lactobacillus acidophilus* and *Enterococcus faecium* and the results were as follows:-
Table 4.12 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on some selected waste materials.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Waste</th>
<th><em>Lactobacillus acidophilus</em> (O.D$_{600}$)</th>
<th><em>Enterococcus faecium</em> (O.D$_{600}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potato</td>
<td>0.345</td>
<td>0.453</td>
</tr>
<tr>
<td>2</td>
<td>Banana</td>
<td>1.990</td>
<td>2.203</td>
</tr>
<tr>
<td>3</td>
<td>Orange</td>
<td>1.530</td>
<td>1.726</td>
</tr>
<tr>
<td>4</td>
<td>Papaya</td>
<td>2.112</td>
<td>2.345</td>
</tr>
<tr>
<td>5</td>
<td>Rice water</td>
<td>0.746</td>
<td>0.792</td>
</tr>
<tr>
<td>6</td>
<td>Sugarcane waste</td>
<td>0.234</td>
<td>0.342</td>
</tr>
<tr>
<td>7</td>
<td>Soya waste</td>
<td>1.932</td>
<td>1.978</td>
</tr>
<tr>
<td>8</td>
<td>Dairy waste</td>
<td>1.987</td>
<td>2.211</td>
</tr>
</tbody>
</table>

Fig 4.1 Growth analysis of two probiotic strains on selected wastes
On the basis of qualitative and quantitative analysis of the selected waste materials and finally on the basis of observation of growth of two selected probiotics on the waste materials, the further studies were done on the five selected materials. As seen from the graph 4.1, five waste materials supporting best growth of both the organisms were as follows:

- Banana
- Orange
- Papaya
- Soya waste
- Dairy waste

After this, the studies were done by dividing the selected waste into two different categories:

1) Fruit peels
2) Industrial waste

Fruit peels consists of banana, orange and papaya peels and industrial waste consists of Soya waste and Dairy waste

4.2.5 Studies on Fruit peels

The following figures shows the growth of the Lactobacillus on:

1) Banana peel

Fig 4.2 Growth in Banana peel medium
As observed from the photograph the turbidity is seen in the test tube inoculated with the culture while it is absent in the tube which is not inoculated with organism this is due to growth of organism. It can also be seen that after growth the colour of the banana peel extract changes which is because of formation of acid during metabolism of the organism.

2) Papaya peel

From the above photograph a clear demarcation is seen in the colour of the inoculated and uninoculated test tubes. The control (uninoculated) test tube appear quite clear but the inoculated test tube appears turbid due to growth of organism.

During spectrophotometric analysis a great difference was observed in readings suggesting very high growth in Papaya peel extract.
3) **Orange peel:**

The photograph below shows the growth of probiotic organism on the orange peel extract.

![Growth in Orange peel medium](image)

In the orange peel extract turbidity was present in both the inoculated and uninoculated test-tubes. Since the extract was not clarified. To observe the growth of organisms, spectrophotometric analysis were done and the readings were found to be still higher. But the orange peel extract is not taken in the further studies since it was turbid and difficult to observe.

It is suggested that the orange peel extract can be used as the media if it is clarified by some proper method.

Fig 4.5 shows the results of Spectrophotometric analysis.
Fig 4.5 Growth of Enterococcus and Lactobacillus on different fruit peel extract media

4.2.6 Studies on Industrial waste

For industrial waste the dairy waste were used which is of many types depending upon the product it synthesizes thus in this study two different types of dairy waste were used:

1) Ghee residue

![Ghee residue image](image-url)
2) Whey

Fig 4.7 Dairy waste material ‘whey’ used in the studies

The studies on these materials shows that there is a very less growth of the organism on ghee residue since it has a high fat content and thus it is not taken for further studies.

In case of whey due to high lactose content the growth of both the organisms were found to be high. On the basis of these studies it can be inferred that whey can be utilized as the better media for the growth of probiotic organisms.

The studies were also done on soya waste and growth of organisms were not found too much in it but during studies it has been observed that the growth of organisms in soyamilk causes reduction in bad smell of the soyamilk thus it can be inferred that these organisms can be used for reduction in bad smell of the soyamilk.
4.3 Studies on the characteristics of the probiotics after growth on waste materials:

Different characteristics of the probiotics were determined when they were grown on waste material media:

4.3.1 Results of growth curve tracing:

Bacterial growth differs in several important respects from growth of multicellular organisms. Bacteria display a characteristic four-phase pattern of growth in liquid culture. Bacterial growth under ideal conditions of constant temperature, adequate oxygen, and nutrient excess, is a very peculiar graph. Therefore, studies were done on the growth curve tracing of the organisms when they were grown on the designed media.
Fig 4.9 Growth of *Lactobacillus acidophilus* on different waste materials and MRS Media

Fig 4.10 Growth of *Enterococcus faecium* on different waste materials and MRS Media
The growth curve tracing for both the organisms has shown that the organisms followed their normal growth cycle even when grown on waste materials. For comparison the growth curve tracing were done simultaneously on MRS media also.

4.3.2 Bacteriocin production studies

4.3.2.1 For *Lactobacillus acidophilus*

Both the strains were found to be the effective producers of bacteriocin. *Lactobacillus* were found to inhibit the growth of *Escherichia coli*, *Salmonella* and *Bacillus cereus*. Production studies showed bacteriocin production at stationary phase at 14 h of incubation. In MRS broth 3400 AU/mL was produced by the strain where as in others the production was less.

The bacteriocin activity was tested with different temperature and the activity was found to vary from 1600AU/mL-25000AU/mL, the maximum arbitrary unit was measured as 25600AU/ml at temperature 40°C.

Regarding pH the maximum inhibitory activity was observed at pH 5.0 and minimum was observed at pH 1-3. Regarding various salinity (NaCl %) tested 0.9% NaCl was found to be suitable for bacteriocin production
From the results it can be inferred that this strain can be used in acidic foods like pickle, yogurt etc as the optimum pH for activity was found to be 4.0. According to the graph bacteriocin are the secondary metabolites. Whey seemed to be more suitable medium compared to others for the bacteriocin production.

**4.3.2.2 For Enterococcus faecium** The larger inhibition zones (2-5mm) in comparison with Lactobacillus were observed with Enterococci on the MRS medium. In this case the bacteriocin production goes with the growth of the organism and the culture conditions shows only a slight effect.
From the results it can be inferred that this strain can be used in acidic foods like pickle, yogurt etc as the optimum pH for activity was found to be 4.0. According to the graph bacteriocin are the secondary metabolites. Whey seemed to be more suitable medium compared to others for the bacteriocin production.

4.3.2.2 For *Enterococcus faecium* The larger inhibition zones (2-5mm) in comparison with *Lactobacillus* were observed with *Enterococci* on the MRS medium. In this case the bacteriocin production goes with the growth of the organism and the culture conditions shows only a slight effect.
Fig 4.14 Growth of *Enterococcus faecium* with bacteriocin production
Fig 4.15 Bacteriocin production by *Enterococcus faecium*

4.3.3 Testing for competitive exclusion

Fig 4.16 Competitive exclusion (diagrammatic view)
Competitive exclusion (CE) is a very important probiotic property. Since during its action a probiotic strain establishes itself while excludes the growth of other bacterial species. These bacterial species are generally pathogenic whose colony formation is not allowed by competitive exclusion (CE) of probiotic bacteria.

Experiment were done to check the competitive exclusion (CE) for both the organisms. To be an effective probiotic the organisms must have the ability to exclude the other organisms from gut and maintain its own growth successfully.

Both Lactobacillus and Enterococcus were found to inhibit the growth of other organisms in their vicinity when grown with a mixed population. So many zones of inhibition were observed on the plates.

During studies it was also found that the zones of inhibitions were more pronounced in case Enterococcus then in Lactobacillus.
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During studies it was also found that the zones of inhibitions were more pronounced in case Enterococcus then in Lactobacillus.
Competitive exclusion shown by *Enterococcus* and *Lactobacillus*

**4.3.4 Testing for antibiotic sensitivity**

Fig 4.18 Graphical representation of competitive exclusion shown by *Enterococcus* and *Lactobacillus*

Fig 4.19 Antibiotic sensitivity test for *Lactobacillus acidophilus* with some common antibiotics

Fig 4.20 Antibiotic sensitivity test for *Enterococcus faecium* with some common antibiotics
For checking the antibiotic sensitivity of the two organisms, some common antibiotics were used. Above figure shows that both the organisms are not sensitive to the common antibiotics as there were no inhibitory zone on both the plates.

These results indicate that when these organisms were utilised as probiotics they were not readily killed by the common antibiotics which are generally used for cure of normal intestinal pathogens.

The test were than repeated with vancomycin for both the organisms. Results showed the sensitivity of both the organisms for vancomycin. So it can be said that during any side effect vancomycin can be given for treatment.

![Antibiotic sensitivity test for Lactobacillus acidophilus with Vancomycin](image1)

![Antibiotic sensitivity test for Enterococcus faecium with Vancomycin](image2)
4.3.5 Results of ONPG Assay for Beta-galactosidase:

Fig 4.23 β-gal production by *Lactobacillus acidophilus* on different waste materials and MRS Media

Fig 4.24 β-gal production by *Enterococcus faecium* on different waste materials and MRS Media
On the basis of graph it can be inferred that both the organisms have produced Beta-galactosidase enzyme in the waste material media but the extent of production was less in comparison to MRS media. A considerable enzyme production was observed in Whey, may be due to presence of high amounts of lactic acid.

4.4 Determination of the main factors affecting the growth of probiotics on waste materials

To produce a microorganism in large scale i.e. during industrial production all the major factors affecting its growth have to be well determined. These results are required for increase in yield of the given microorganism.

Thus the experiments have been performed for the highest yield.

4.4.1 Results for *Lactobacillus acidophilus*

![Bar graph](image)

**Effect of pH on growth of *Lactobacillus* in different waste materials**

**Fig 4.25 Effect of pH on growth of *Lactobacillus* in different waste materials**

pH is one of the main factor affecting growth of microorganisms and therefore its effect have to be determined at the time of media designing. The effect should be same as that in its chemically defined media. From the graph it can be observed that Lactobacillus shows highest growth in the pH range of 6-8 and this trend is similar for all the waste material media.
This accounts for no change in the effect of pH on the growth of Lactobacillus on waste material based media.

![Effect of temperature on growth of Lactobacillus in different waste materials](image)

Fig 4.26 Effect of temperature on growth of *Lactobacillus* in different waste materials

From the graph it can be seen that whey based medium follows the same pattern for growth at different temperatures as that of MRS medium. In all the cases the organism shows highest growth at 37 °C. Now since the research have shown that Lactobacillus is an organism capable of growing at higher (45 °C) and lower (8°C) temperatures, the results of present study also goes with the same. On all the selected waste material media the organism was observed to be growing at higher and lower temperatures even though the extent of growth was found to be less.

![Effect of salt concentration on growth of Lactobacillus on waste materials](image)
Fig 4.27 Effect of salt concentration on growth of *Lactobacillus* on waste materials

*Lactobacillus* is quite resistant to the osmotic effects. Research studies have shown that it can tolerate salt concentration up to 2.00% or more. In the present study, it has been observed that lactobacillus shows growth to a lesser extent at 2.00% salt concentration in all the waste material media.

Most optimum results were shown by whey. In BPE and PPE, the organism was found to be growing optimally up to 1.00% salt concentration but the growth was found to be decreased at 1.5% and higher %.

The study supports the property of *Lactobacillus* that it is osmotic resistant.

Fig 4.28 Effect of methylene blue concentration on growth of *Lactobacillus* on waste materials

![Graph showing the effect of methylene blue concentration on growth of *Lactobacillus*](image-url)
In all the three selected media the lactobacillus were found to be growing even at 0.4% methylene blue concentration. The growth was found to be increasing with the percentage of methylene blue. This criterion can be used for selection of Lactobacillus and can avoid contamination during large scale production because all the common organisms are generally sensitive to methylene blue.

![Effect of inoculation volume on growth of Lactobacillus in waste materials](image)

Fig 4.29 Effect of inoculation volume on growth of *Lactobacillus* in waste materials

For industrial production of any microbial metabolite, the proper inoculum volume have to be determined. It has been studied that as the inoculum volume is increased the growth of microbial biomass also increases but after a certain extent the further increase in the inoculums volume does not causes increase in the biomass.

The same trend were observed in the present study for Lactobacillus. From the graph it can be observed that 10% inoculum was optimum for most of the media and after this any further increase in the inoculum volume did not gave a considerable increase in the biomass.
Effect of incubation time on the growth of *Lactobacillus* in waste materials

As studied from the growth curve the Lactobacillus starts its death phase after 18 hrs of incubation. Thus the proper incubation time for it is upto 24 hrs only after which the growth starts decreasing.

Fig 4.31 Effect of Flask volume on growth of *Lactobacillus* on waste materials

Growth (O.D.600)

<table>
<thead>
<tr>
<th>Incubation time (in hrs)</th>
<th>MRS</th>
<th>BPE</th>
<th>PPE</th>
<th>WHEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>18</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>24</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>48</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>72</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Growth (O.D.600)

<table>
<thead>
<tr>
<th>Flask volume (ML)</th>
<th>MRS</th>
<th>BPE</th>
<th>PPE</th>
<th>WHEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ML</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>300 ML</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>500 ML</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Study have suggested that the flask volume has an important effect on the growth. From the graph it can be observed that the growth was slight less in 100ml flask which was increased when the flask volume was increased to 300ml. The biomass was not much increased in 500ml flask.

Thus the inference can be drawn that for a constant inoculums volume, an initial increase in the flask volume results in the increase in yield but beyond a certain limit (about 300 ml, here) the flask volume does not characteristically affect the growth and yield.

Thus at industrial level the volume of the production tank does not affect the growth until worked out by using a standard pre-studied inoculums volume.

Almost all the media showed the same result.

4.4.1 Results for Enterococcus faecium

![Effect of pH on growth of Enterococcus in different waste materials](image)

Fig 4.32 Effect of pH on growth of Enterococcus in different waste materials
Enterococcus were found to be effective in growth than Lactobacillus. May be because it is less fastidious than Lactobacillus. Effect of pH follows the same pattern as that of Lactobacillus but the range has been increased in this case that is from 6.0-10.0 pH.

As the graph shows, that Enterococcus was found to be more resistant to temperature than Lactobacillus. The organism showed almost same growth at 25 °C and at 45 °C. The highest growth were at 37°C. Thus it can be inferred that when this organism were to be grown at larger scale, the temperature maintenance is not required upto some extent. This can further reduce the cost of production.
Fig 4.34 Effect of salt concentration on growth of Enterococcus on waste materials

In MRS and whey based medium the same trend of growth were observed that is the organisms were found to be growing at even higher concentrations (2.00%) of salt. For PPE and BPE the growth was found to be decreased after 1.5% salt concentration.

Fig 4.35 Effect of methylene blue concentration on growth of Enterococcus on waste materials
The Enterococcus were also found to be resistant for even 0.4% methylene blue concentration and the trend were same for all three selected media.

![Effect of inoculation volume on growth of Enterococcus in waste materials](image)

Fig 4.36 Effect of inoculation volume on growth of Enterococcus in waste materials

The growth pattern of Enterococcus was found to be almost same at 10%, 20% and 50% inoculation volume, which suggests that 10% is the optimum inoculum volume.

After further increase in the inoculation volume the growth was not found to increase in considerable manner. The pattern was found to be similar for all the three media and goes along with the MRS medium.

Enterococcus is a fast growing organism and thus its 10% inoculums is sufficient for the required yield. Higher inoculums volumes can be used for more larger flasks.
The growth of Enterococcus was found increasing up to 18-24 hours of incubation after which the growth showed decline. After 72 hours there were almost negligible organisms. This shows that during industrial production the process should be terminated at or before 24 hours of production.
As in case of Lactobacillus, here also on increasing the flask volume up to an extent that is from 100 ml to 300 ml, the growth was increased and after that in 500 ml flask the growth was found to be either same or less than that in 300 ml flask. This shows that the inoculum volume and the flask volume should be considerably adjusted according to the type of organism used.

4.5 Results for optimisation of the waste materials to be used as the growth media for probiotics

The optimization of all the three suggested waste materials were done in three series of steps:
1) With respect to the optimum concentration of the waste material
2) With respect to the optimum nutrients
3) With respect to the optimum concentration of the nutrients

4.5.1 Optimisation results for Banana Peel Extract (BPE) Media

![Graph showing growth of Lactobacillus acidophilus and Enterococcus faecium on different BPE concentrations.](image)

Fig 4.39 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different conc. of BPE

Results have shown a logithermic increase in growth of both the organisms when the banana peel concentration was increased from 2% - 10%, but after that in 20% media the increase in growth was not much pronounced. Therefore the 10% BPE were selected for further studies.

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Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different nutrients with 10% BPE

When the 10% BPE was enriched with different nutrients, the highest growth has been observed in 1.0% glucose. For Enterococcus the growth was found to decrease when 1.0% yeast extract were added in the medium. Peptone and sucrose also supported the growth but to a lesser extent than glucose.

Fig 4.41 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different conc. of glucose with 10% BPE
Further studies were done on the optimization of glucose concentration and it has been found that the growth was similar in even higher concentrations of glucose. The growth was found to decrease at 5.0% glucose. Thus 1.0% glucose was suggested for optimum media.

### 4.5.2 Optimisation results for Papaya Peel Extract (PPE) Media

#### Fig 4.42 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different conc. of PPE

Growth of both the organisms were analyzed at different concentrations of papaya peels extract and on the basis of results, the 10% media were taken for further studies.

#### Fig 4.43 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different nutrients with 10% PPE

Growth of both the organisms were analyzed at different concentrations of papaya peels extract and on the basis of results, the 10% media were taken for further studies.
For Enterococcus the highest growth was observed in 1.0% glucose but for Lactobacillus the 1.0% glucose, 1.0% yeast extract and 1.0% peptone have given almost same results. It may be because the yeast and extracts and peptone provide sources of carbon, nitrogen and vitamins for general bacterial growth. The yeast extract also contains vitamins and amino acids specifically required by *Lactobacilli*.

The growth extent for Enterococcus was found to much more higher than that of Lactobacillus.

![Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different conc. of glucose with 10% PPE](image)

Fig 4.44 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different conc. of glucose with 10% PPE

For Enterococcus the highest growth were observed in 2.0% glucose while for Lactobacillus the growth was almost same in 1.0% and 2.0%. In both the cases the growth was found to decrease at higher concentrations of glucose. It may be attributed to decrease in bacterial growth at higher sugar concentrations due to change in osmotic pressure of the cells.
4.5.3 Optimisation results for Whey Media

Fig 4.45 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different conc. of whey.

For whey the similar results were obtained for both the organisms at 10 and 20% concentrations. Thus it has been suggested that the concentration of whey should be selected on the basis of some other criterions such as easy availability, transport facility and problems of disposal.

Fig 4.46 Growth of *Lactobacillus acidophilus* and *Enterococcus faecium* on different nutrients with 10% whey.
The extent of growth was almost similar for both the organisms with slight increase for Enterococcus. All the four tested nutrients have shown same results with a little more growth in case of glucose.

![Growth of Lactobacillus acidophilus and Enterococcus faecium on different conc. of glucose with 10% whey](image)

For Enterococcus the results were good in case of 2.0% glucose while for Lactobacillus both 1.0% and 2.0 % have shown the same results. Thus 1.0 % concentration is suggested for optimization of media.

During optimization of all the three waste media it has been found that about 10 % waste materials enriched with 1% Glucose is the highly optimized medium. For whey 20% waste material also gives the good results but to make it economical 10% can be used. If whey is available in large quantities then 20% can be preferred over 10%.

A comparative study was done to select the best possible waste material media.
Comparision of Growth of *Lactobacillus* and *Enterococcus* on the three optimised media

As observed from the figure almost all the waste materials supports the growth of both the organisms to the same extent. But a slight increased growth was observed in case of whey. This is because whey is a natural complex medium for microorganisms and, it contains approximately 50% of the nutrients present in original milk such as soluble proteins (6-8 gL⁻¹), lactose (44-52 gL⁻¹) and minerals (4.3-9.5 gL⁻¹). As a carbon and energy source mainly lactose is present and as a nitrogen source serves the various proteins. Thus, the microbes need extracellular β-galactosidase and protease/peptidase activities when growing on whey media and both the organisms under study produces β-galactosidase enzyme for successful utilization of whey based media.

Thus depending upon the cost and availability any of the three optimized media can be utilized for production of Enterococcus and Lactobacillus.
4.6 Comparison of the growth of probiotics on waste materials and the standard media available in markets:

A final study was done for the comparison of growth of the two selected organisms on the three optimized media with that of chemically defined media MRS. As shown in the graph it has been observed that in comparison to MRS media the highest growth of both organisms were observed in whey.

Papaya peel extract (PPE) and Banana peel extract (BPE) also showed significant growth. Results of standard plate count method also supported the same results.

Fig 4.49 Comparison of growth of Lactobacillus and Enterococcus on MRS and three optimised media
Fig 4.50: Growth on MRS Media

Fig 4.51: Growth on Banana peel extract (BPE) Media
Fig 4.52: Growth on Papaya peel extract (PPE) Media

Fig 4.53: Growth in Whey Media
4.7 Checking for the contamination of the media designed by waste materials:

The checking for contamination was performed to determine the shelf life of the media and to observe the possible contaminants which may contaminate and prevail easily in the three optimized media.

4.7.1 Contamination check for Banana peel extract (BPE) Media

Fig 4.54 Plates showing contamination on Banana peel extract (PPE) media

4.7.2 Contamination check for Papaya peel extract (PPE) Media

Fig 4.55 Plates showing contamination on Papaya peel extract (PPE) media
4.7 Checking for the contamination of the media designed by waste materials:

The checking for contamination was performed to determine the shelf life of the media and to observe the possible contaminants which may contaminate and prevail easily in the three optimized media.

4.7.1 Contamination check for Banana peel extract (BPE) Media

![Fig 4.54 Plates showing contamination on Banana peel extract (BPE) media](image)

4.7.2 Contamination check for Papaya peel extract (PPE) Media

![Fig 4.55 Plates showing contamination on Papaya peel extract (PPE) media](image)
4.7.3 Contamination check for Whey Media

The results have shown that whey media is highly prone for contamination both for bacteria and fungus may be due to the presence of high amounts of sugar and other nutrients. Papaya peel extract(PPE) and Banana peel extract(BPE) also showed significant growth of contaminants. On both the media more fungal contamination was observed than bacterial contamination. In comparison to BPE more contaminants were observed on whey and PPE Media. So it can be inferred that these two media can support the growth of various other organisms also and thus have to properly stored.

4.8 Determination of reduction in environmental pollution by the use of waste materials as Growth Media.

Studies have been done on the effect of all the selected waste materials in general environment. When these were dumped freely in the soil there were observed some changes in the physical, chemical and biological properties of that soil. These change in properties were studied and their possible impact on the soil and in turn on environment were determined.
4.8.1 Studies on Banana peel

Physical properties of soil

Texture - Clay of size <0.002 mm were taken for the studies and the particles size were found to be increased after 7 days of addition of banana peels. This may be due to the clogging of the particles because of the slight moisture addition which was confirmed in following studies.

Structure – Clay particles were taken for the studies having maximum aggregation properties and organic materials, especially microbial cells and waste products, which aggregate to increase their strength. Aggregation of soil has increased after addition of banana peels.

Consistence – Soil consistence, and its description, depends on soil moisture content. Thus the moisture content of soil were determined using following formula:

\[
W = \frac{w_2 - w_3}{w_3 - w_1} \times 100
\]

\(W\) = moisture content percent  
\(w_1\) = weight of container with lid (in gms)  
\(w_2\) = weight of container with lid with wet soil (in gms)  
\(w_3\) = weight of container with lid with dry soil (in gms)

Moisture content percent of normal soil

\[
W = \frac{78.977 - 75.581}{73.581 - 45.906} \times 100
\]

\[= 3.396 \times 100\]

\[= 27.675\]

\[= 12.271\%\]

Moisture content percent of banana peel enriched soil

235
The results have shown the great increase in the moisture content of the soil when banana peels were added to it.

**Color** – The color of the soil were found to be darker than the original soil taken. This may be due to increase in moisture content and organic matter.

**Chemical properties of soil**

**Major Elements** – Due to increase in organic content the major elements such as carbon hydrogen and nitrogen were also increased in banana peel enriched soil.

**Soil pH** – The pH of the original soil were 6.5 and it were found to be decreased to 4.3 after 7 days of incubation with banana peels

**Organic matter** – The biological studies have shown that the organic content of soil has been greatly increased after addition of banana peels.

**Plant nutrients** – Studies have been done on the growth of a very well known plant that is Aloevera on the waste laden soil and the results have shown that the growth of the plant has increased in that soil. This shows that some of the plant nutrients have increased due to addition of banana peels in soil system.
Biological properties of soil

- Bacterial content

Fig 4.57 Plates showing bacterial content of soil laden with banana peels

As shown in the photograph the bacterial content of the soil were found to be greatly increased in the waste laden soil suggesting the high nutrient content of the soil.

4.8.2 Studies on Papaya peel

Physical properties of soil

Texture – Less clogging were observed in case of papaya peels may be due to their less moisture content.

Structure – Aggregation were found but to a lesser extent than that of banana peels laden soil

Consistence -

\[
W = \frac{80.403 - 74.653}{74.653 - 45.901} \times 100
\]

\[
= \frac{5.75}{28.752} \times 100
\]

\[
= 19.99\%
\]
As per the calculation, moisture content was found less as compared to banana peels burdened soil.

**Color** - The color was found darker than normal but lighter than that of the soil laden with banana peels

**Chemical properties of soil**

**Major Elements** – Since the organic content was less thus it has been estimated that papaya peels contribute less elements to the soil.

**Soil pH** – Studies showed decrease in pH from 6.8 to 3.9

**Organic matter** – As per the biological studies it has been estimated that papaya peels contributes less organic matter to the soil.

**Plant nutrients** – No major change in the growth of the Aloevera plant was observed when it was grown on the soil laden with papaya peels.

**Biological properties of soil**

**Bacterial content**

![Plate showing bacterial content of soil laden with papaya peels](image)

Fig 4.58 Plate showing bacterial content of soil laden with papaya peels
As seen in the photograph the number of bacterial colonies were found lesser than that of banana waste laden soil

4.8.3 Studies on Whey

Physical properties of soil

Texture – The texture of the soil was greatly influenced after the addition of whey into it. The particles size were found to increase greatly after 7 days of addition of whey addition. This may be due to the clogging of the particles because of the high amount of moisture addition which was confirmed in following studies.

Structure – The soil particles were found to greatly aggregate after the addition of waste whey since it was containing a huge amount of water in it. After 7 days of incubation huge clumps of soil were formed in the test tray.

Consistence

\[ W = \frac{84.664 - 70.393}{70.393 - 45.908} \times 100 \]

\[ = \frac{14.271}{24.485} \times 100 \]

\[ = 58.284\% \]

The calculation shows that incorporation of whey into the soil greatly increases the moisture content of the soil.

Color – The colour of the whey laden soil has darken but after 7 days of incubation the tray was completely covered by fungal growth due to which it appeared turbid white in color.

Chemical properties of soil

Major Elements – After 7 days, a huge fungal growth was observed on the tray laden with whey. This suggests that the incorporation of all the major nutrients of whey into the soil. It is also
to be noted that whey contains high amounts of sugar which also contributes to the major elements in the soil.

**Soil pH** - The pH of the soil with whey was found to decrease from 6.5 to 5.6

**Organic matter** – The biological studies and heavy fungal growth suggests that the organic content of the soil was greatly increased after enrichment with whey. A high content of sugar in whey is also the suggestive measure of high organic content.

**Plant nutrients** – The growth of Aloevera plant was highly increased when it was sown in the soil incorporated with whey. This suggests that whey contributes a large amount of plant nutrients to the soil.

**Biological properties of soil**

**Bacterial content**

Fig 4.59 Plate showing bacterial content of soil laden with whey

The photograph shows large number of bacterial colonies in the soil laden with whey.
On the basis of soil analysis with the different waste materials, it can be inferred that the soil having these waste materials has undergone various physical, chemical and biological changes, some of which are beneficial while a large of them are harmful. The studies on physical parameters suggest that a completely loosen soil changes to the clumped soil. Due to clumping, the porosity of soil decreases since the particle size increases. The waste materials have contributed to the darkening of the soil and have greatly increased the moisture content of it.

The biological studies have shown that when soil contains waste materials having high organic content, the bacterial and fungal content of the soil has greatly increased. The major soil bacteria on the plate were identified as Pseudomonas, Serratia, Enterobacter, E.coli, Bacillus, and Salmonella. Almost all of these are known to have pathogenic properties.

Fig 4.60 Moisture content of the normal and soil with three different waste materials (in %)
Chapter 5
Conclusion

The relationship between food and health has long been known to exist, and today the fundamental concept of food is changing from its involvement in the maintenance of life to the promotion of better health and quality of life by prevention of diseases. The increasing interest in health provides investment opportunities in health food categories in many countries including India. Functional foods which are already available in Indian markets include those with removal of an allergic protein (gluten free atta), those containing live bacteria (probiotics, prebiotics) such as Yakult health drink and Amul’s butter milk or those containing some health nutrients such as energy bars, juices and soy based products. Developing countries are a rich source of raw materials for functional food products because of their vast biodiversity and cost advantages in crop production, therefore functional food industry development in these countries is relatively easier.

Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific research for their ability to prevent and cure a variety of diseases. This has led to the coining of the term probiotics. Today probiotics are available in a variety of food products and supplements, and have got wide applications in the control of cholesterol, cancers, allergies, etc. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods, is the small but rapidly expanding area of probiotics – live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance. 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".

The development of suitable technology for probiotic production, taking into account viability and stability, is a key area of research for industrial production. Production of probiotics should be based on the microbial criteria, and the ability to withstand stress during processing and storage of probiotic products is important. Thermophilic probiotics are of great interest in this area as they can have all the desired characteristics. This study gives some key points for probiotic selection studies. The development of suitable probiotics in food and feed needs good
proof of their efficacy and function in order to be accepted as a valuable product. Developing probiotic food and feed is a key research and development area for future functional food markets. The use of probiotics to improve quality of life-forms is currently generating a great deal of interest.

Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as complementary and alternative medicine (CAM). 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.

Some probiotic foods date back to ancient times, such as fermented foods and cultured milk products. Interest in probiotics in general has been growing nowadays.

Since the probiotics have many applications, therefore their large scale production is required. The production will be effective and economical if it is done on a cheaper media. The companies associated with probiotic production are using skimmed milk and milk medium for their production which is expensive.

Waste materials (such as fruit peels, plant extracts, agricultural waste and industrial waste) are usually organic substances rich in nutrients but when dumped freely in the environment in large amounts, their by-products can lead to environmental pollution. If we could produce valuable products from these wastes, by-products polluting environment could be converted into products with a higher economic values. The present study suggests an easier method for this purpose. The study deals with the use of waste materials as nutritive media for growth of Probiotics.

This study discusses the selection criteria, desirable characteristics and some new developments in the field for screening new probiotic strains with suitable wastes (raw materials) for their large scale industrial production.

Work is carried out on the growth of probiotics on waste materials.

Our aim in this work was to study the growth of probiotics on waste materials and to study the biochemical properties of the organisms when grown on waste materials.
Studies on the growth of probiotics on waste materials helped to develop a better economic media for their growth and also gives a suggestive measures for the reduction of environmental pollution caused by waste materials.

The study was aimed at developing an economical media for probiotics without sacrificing their natural characteristics. The study has lead to the development of a cheaper, economical media for the growth of probiotics that can be used in the industries for their large scale production.

The study also contributes towards the control of environmental pollution giving a suggestive measure to use the waste materials for some useful purposes.

**Selection of appropriate probiotic strain**

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 first objective of the study was to select an appropriate probiotic strain. Six different strains have been taken for studies namely *Lactobacillus acidophilus, Bacillus subtilis, Bifidobacterium bifidum, Enterococcus faecium, Saccharomyces cerevisiae* and *E.coli*. All these strains have been screened on the basis of different probiotic properties. On the basis of research we have fixed sixteen different parameters by which the above strains were analysed. After a step-wise process of screening through sixteen fixed characteristics, two microorganisms have been selected for further studies. These were *Lactobacillus acidophilus* and *Enterococcus faecium*.

The present studies have selected the 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.

For successful delivery of probiotics in products to the intestine, the beneficial microorganisms must survive food processing, storage during product maturation and shelf life, as well as the stress conditions in the host gastrointestinal tract system. The selection of probiotics with all
these characteristics is a technological challenge and is evaluated according to the viability and stability of probiotics in the final probiotic product. The criteria for the selection of probiotics thus also includes acid tolerance, bile tolerance, heat tolerance, and ability to metabolize prebiotics, adherence and colonization to intestinal epithelium/tissue, stimulating immune response, antimicrobial activity/antagonisms to pathogens, improving host digestion, etc. Fundamental knowledge of specific characteristics of proteolytic activity, stress response, etc. of thermophilic LAB can be applied for the improvement of T-LAB and other mesophilic probiotics.

It has also been determined that the development of probiotic strain for human use should be such that the particular selected probiotic occurs constantly in the natural foods so that it does not adversely affects the host organism.

Study of functionality of multistrain probiotics has been shown to be more effective and more consistent than that of monospecies probiotics. Thus the studies have incorporated two probiotic strains. Factors related to feed technology for development of probiotics in animal feed are to be taken into account for the future probiotic products. Overall choice of suitable probiotics which can survive stresses during processing, and passage through alimentary canal followed by their proliferation and studying their growth on raw materials is important for these probiotics to be applied successfully.

The study was done on the selected probiotics which showed some characteristic growth on waste materials. The growth was determined by spectrophotometric analysis (Standard Methods, 1992). This step involved the screening of different probiotics and selection of those showing growth to a higher extent than other.

**Selection of appropriate waste material**

If it is to be used as probiotics the Lactic acid bacteria have to be cultivated in large amounts. Studies have shown that about 60% of the cost of any industrial production using microorganisms depends on the cost of material used for the growth of selected microbial species. Therefore for their economic production in large scale some cheap source of nutrients is required. For this purpose use of waste materials is suggested in present studies.
Different waste materials such as fruit peels, kitchen waste, agricultural waste, and industrial waste were tested for supporting the growth of probiotics. The selection parameter also involved the criteria of their easy availability.

Thus the second objective of the study was to find an appropriate waste material for the best possible growth of selected microbes. These studies were undertaken on the basis that waste materials contain high amounts of organic content which can be utilized by microbes as nutrients for their growth. There were some factors fixed for the selection of waste materials before using them in studies. One of the main factors was their high and easy availability. About 20 different waste materials have been analysed which includes fruit and vegetable peels, agricultural waste, and industrial wastes. During preliminary studies, nine waste materials have been selected and taken for further studies.

**Analysis of the composition of the selected waste materials**

The next objective was to analyse the composition of the selected waste materials. This step was necessary for determination of nutritive factors that are present in waste materials and available for growth of microbes. Therefore, the chemical analysis was done for general nutritive chemicals such as proteins and carbohydrates etc. In the first step, the selected nutrients were analysed qualitatively to determine their presence or absence in the waste materials, and in the next step, quantitative estimation was done. On the basis of these tests, the waste materials having the highest content of useful nutrients were selected. The waste materials supporting the growth were analysed for their components which are acting as the nutritive substances, by qualitative and quantitative analysis, for Carbohydrates by Cole's Method, (Cole, 1933) and for Proteins by Biuret Method, (Reigler, 1914).

On the basis of results obtained, the useful waste materials were found to be fruit peels such as Papaya peels (12.50 g/100ml carbohydrates and 57.80 g/100ml proteins), Orange peels (16.86 g/100ml carbohydrates and 5.80 g/100ml proteins), and Banana peels (46.00 g/100ml carbohydrates and 1.45 g/100ml proteins), dairy industrial waste, Whey (5.8 g/100ml carbohydrates and 1.5 g/100ml proteins). It is a by-product of the dairy industry and contains usually high levels of lactose (4-4% w/w/g), low levels of nitrogenous compounds and small
amounts of vitamins and minerals. Lactose, the main nutrient in whey, can be economically utilized by microbes for their growth.

**Studies on the growth of probiotics on selected waste materials**

After finding the waste materials having highest content for carbohydrates and proteins, they were analysed for the growth of selected probiotic strains. The growth was determined by **spectrophotometric analysis** (Standard Methods, 1992). For *Lactobacillus acidophilus* banana peels showed 1.990 (O.D$_{600}$), Papaya peels showed 2.112 (O.D$_{600}$), and Dairy waste showed 1.987 (O.D$_{600}$).

For *Enterococcus faecium* banana peels showed 2.203 (O.D$_{600}$), Papaya peels showed 2.345 (O.D$_{600}$), and Dairy waste showed 2.211 (O.D$_{600}$).

Thus the above waste materials were further taken for studies.

**Studies on the characteristics of the probiotics after growth on waste materials**

The probiotics have some peculiar properties (such as β- galactosidase production, Bacteriocin production etc.) which are important for their functions and which can be exploited for beneficial purposes. The studies also involved the determination of these properties on waste materials. This step involved the "**ONPG Assay**" Craven et al. (1965), and "**Bacteriocin assay**" methods (Tagg & McGiven, 1971).

After the selection of waste materials the studies have been done on the characteristics of probiotics after their growth on waste materials. These studies were done simultaneously with chemically defined media (MRS Media) for both the organisms. This was to compare the normal characteristics of the organisms with the characters when they were grown on waste materials.

For initial investigation the growth curve tracing for both the organisms were performed. According to these studies the growth percentage was found to be less in waste materials with only slight fluctuation in their peculiar properties. The growth curve tracing for both the organisms has shown that the organisms followed their normal growth cycle even when grown
on waste materials. The other main properties under study included β- galactosidase production and bacteriocin production.

**Bacteriocin production (Lactobacillus acidophilus):** Both the strains were found to be the effective producers of bacteriocin. Lactobacillus were found to inhibit the growth of *Escherichia coli*, *Salmonella* and *Bacillus cereus*. Production studies showed bacteriocin production at stationary phase at 14 h of incubation. In MRS broth 3400 AU/ml was produced by the strain where as in others the production was less. The bacteriocin activity was tested with different temperature and the activity was found to vary from 1600 AU/ml to 25000 AU/ml, the maximum arbitrary unit was measured as 25600 AU/ml at temperature 40°C. Regarding pH the maximum inhibitory activity was observed at pH 5.0 and minimum was observed at pH 1-3. Regarding various salinity (NaCl %) tested 0.9% NaCl was found to be suitable for bacteriocin production. From the results it can be inferred that this strain can be used in acidic foods like pickle, yogurt etc as the optimum pH for activity was found to be pH 4.0. According to the graph obtained, bacteriocin are the secondary metabolites. Whey seemed to be more suitable medium compared to others for the bacteriocin production.

**Bacteriocin production (Enterococcus faecium):** The larger inhibition zones (2-5mm) in comparison with Lactobacillus were observed with Enterococci on the MRS medium. In this case the Bacteriocin production goes with the growth of the organism and the culture conditions shows only a slight effect.

The extent of both the properties were found to be only slightly less than their normal media.

**Competitive exclusion by Lactobacillus and Enterococcus:** Experiment were done to check the competitive exclusion (CE) for both the organisms. To be an effective probiotic the organisms must have the ability to exclude the other organisms from gut and maintain its own growth successfully.

Both Lactobacillus and Enterococcus were found to inhibit the growth of other organisms in their vicinity when grown with a mixed population. So many zones of inhibition were observed on the plates. During studies it was also found that the zones of inhibitions were more pronounced in case Enterococcus then in Lactobacillus.
Antibiotic sensitivity test for *Lactobacillus acidophilus* and *Enterococcus faecium*:- Since the probiotics are the live cultures to be administrated directly, thus their antibiotic sensitivity have to be determined. This makes their consumption safe. For checking the antibiotic sensitivity of the two organisms, some common antibiotics were used. Results showed that both the organisms are not sensitive to the common antibiotics as there were no inhibitory zone on both the plates.

This results indicated that when these organisms were utilised as probiotics they were not readily killed by the common antibiotics which are generally used for inhibition of normal intestinal pathogens.

The test were than repeated with vancomycin for both the organisms. Results showed the sensitivity of both the organisms for vancomycin. So it can be said that during any side effect vancomycin can be given for treatment.

One of the very important property of probiotic organism is that they can help in the treatment of lactose intolerance. This occurs due to the production of Beta –galactosidase enzyme. Thus the Beta –galactosidase enzyme production was also studied and according to the results it was produced in the waste material media but the extent of production was less in comparison to MRS media. A considerable enzyme production was observed in Whey, may be due to presence of high amounts of lactic acid.

Thus it is concluded that only the growth is less in waste materials and organism does not sacrifices its peculiar properties when grown on them. This less extent of growth can be further increased by optimization process after studying the main factors which are affecting the growth of probiotics on waste materials.

**Determination of the main factors affecting the growth of probiotics on waste materials**

For undertaking the next objective, different factors affecting growth of probiotics were determined. During this the standards were fixed as of the growth of same probiotics on their usual media. The main factors under study includes effects of temperature, pH, methylene blue concentration and salt concentration. The results showed temperature in the mesophillic to slight thermophillic range. Since the organisms were found to be able to grow at wide range of temperature (i.e. from 25-45 °C) thus they can be effectively cultivated on large scale without
strict adjustment for temperature. Both the organisms were found to growing at pH nearly neutral in the range of 6.0-8.0. This pH can be very effectively maintained during industrial production. During study it has been found that the growth of both the organisms were not effectively declined due to change in pH so while cultivating the organisms on large scale a slight change in pH will not cause a major decrease in yield. Both the organisms were found to be resistant to about 0.4% methylene blue and above 2% salt concentration. These properties can further be proved beneficial for industrial production since by enriching the media with these components, chances of contamination can be greatly reduced.

For industrial production of any microbial metabolite, the proper inoculum volume have to be determined. It has been studied that as the inoculum volume is increased the growth of microbial biomass also increases but after a certain extent the further increase in the inoculums volume does not causes increase in the biomass. It has been found that on increasing the inoculum volume up to 10% the growth of both the organisms were increasing and after that there was no considerable increase in growth even on increasing the inoculums volume.

Effect of incubation time on growth of both the organisms were determined and found that these organisms undergoes growth maximally upto 24 hours after which their growth starts decreasing. This data can be used for determination of fermentation time during their large scale production. On further studying the effect of flask volume on growth, it has been found out that for a standard inoculum volume the flask volume affects the yield and yield can be increased by increasing the flask volume up to an extent.

The main factors affecting the growth of probiotics on waste materials was effectively determined and the conditions for better growth was standardized.

Optimisation of the waste materials to be used as the growth media for probiotics

In the next step the optimization of selected waste materials have been done. Some standard nutrients which were found to be less or absent in given waste material during quantitative analysis were added in the different concentrations and the best concentration supporting growth was determined.
Experiments have been repeated with different nutrients and then with different concentrations of the same nutrient. After selecting and standardizing the best nutrient and its concentration the optimization studies were done on their combination with the waste materials under study. The best possible combination of nutrients with the waste materials were determined and taken for further studies. The optimization of all the three suggested waste materials were done in three series of steps:

- With respect to the optimum concentration of the waste material
- With respect to the optimum nutrients
- With respect to the optimum concentration of the nutrients

For banana peel media and papaya peel media about 10% waste material was found to be effective while for whey about 20% waste supports the growth. In all these the enrichment with 1.0% glucose was further found to increase the growth.

**Comparison of the growth of probiotics on waste materials and the standard media available in markets**

In the next objective the finally designed media was compared for growth and normal characteristics with chemically defined media available. The growth was found to be about 10-25% less in waste materials with about the same percentage of decrease in their properties. It is inferred that since the growth was found to be less, the extent of properties were also less. Thus conclusion can be drawn that properties of the culture remains unaffected when they are grown on waste materials. Only the extent of growth was decreased slightly.

Out of the three suggested and optimized media, the highest growth of both organisms were observed in whey.

The comparison was done by studying the growth on both the media by spectrophotometric analysis in liquid media and by forming the agar media from waste material and then performing the standard plate count method. (American Public and health Association (APHA, 1993)

**Checking for the contamination of the media designed by waste materials**

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When the designing of media was done, the next objective was to check for its contamination. This step was done to observe the shelf life of designed media. This step also suggested the possible contaminants which can easily thrive on designed waste materials. The results have shown that whey media is highly prone for contamination both for bacteria and fungus may be due to the presence of high amounts of sugar and other nutrients.

Papaya peel extract (PPE) and Banana peel extract (BPE) also showed significant growth of contaminants. On both the media more fungal contamination was observed than bacterial contamination. In comparison to BPE more contaminants were observed on whey and PPE Media. So it can be inferred that these two media can support the growth of various other organisms also and thus have to properly stored.

Thus it is inferred that the designed media should be taken care of from such organisms during storage.

Contamination check was done by the incubation of the un-inoculated media under proper conditions for proper time.

**Determination of reduction in environmental pollution by the use of waste materials as Growth Media**

The final objective of the study was to determine the reduction in environmental pollution since the study makes beneficial use of waste material which may otherwise cause increment on environmental pollution by some or the other way. For this purpose the studies were done on the physical, chemical and biological parameters of soil.

In the first step all the characters were studied for normal soil devoid of the waste materials under study. These were kept as standards.

In the next step the parameters were studied for the soil containing waste materials. The color of the soil was found to be dark in waste laden soil which may be due to the presence of high humus into it. The chemical property shows increase in organic matter content while decrease in inorganic materials. This may be attributed to utilization of inorganic contents by microbes for their growth thereby imparting organic matter content to soil. The biological properties shows
the presence of heavy microbial population in the soil having waste materials. Mostly these organisms include the normal indigenous microbes found in soil but in higher percentage.

Thus it can be further concluded that when these waste materials were thrown as such on the soil they causes increase in its nutritive content and making it more favorable for the growth of microbes. These microbes may be pathogenic in nature and may cause the incidence of certain diseases in the area.

Study of change in physical, chemical and biological properties of waste materials shows the reduction in environmental pollution.

Study of parameters was be done according to the methods given by American Public and health Association (APHA, 1986):

**Physical properties of soil**

Texture - Clay of size <0.002 mm were taken for the studies and the particles size were found to be increased after 7 days of addition of waste materials. This may be due to the clogging of the particles because of the slight moisture addition which was confirmed in following studies.

Structure - Since the clay particles were taken for the studies which are known to have maximum aggregation properties and organic materials, especially microbial cells and waste products, act to cement aggregates and thus to increase their strength. Thus the aggregation of soil increases after addition of waste materials.

Consistence - Soil consistence, and its description, depends on soil moisture content. Thus the moisture content of soil were determined. Moisture content percent of normal soil were found to be 12.271 %, Moisture content percent of soil enriched with banana peel were found to be 41.019 %, Moisture content percent of soil enriched with papaya peel were found to be 19.99 % and Moisture content percent of soil enriched with whey 58.284 %. According to results the moisture content of soil was found to be greatly increased when the selected waste materials were dumped into it.
Color - The color of the soil were found to be darker than the original soil taken. This may be due to increase in moisture content and organic matter.

**Chemical properties of soil**

Major Elements – Due to increase in organic content the major elements such as carbon hydrogen and nitrogen were also increased in enriched soil.

Soil pH – The pH of the original soil were 6.5 and it were found to be decreased to 4.3 after 7 days of incubation with banana peels

Organic matter – The biological studies have shown that the organic content of soil has greatly been increased after studies

Plant nutrients – Studies have been done on the growth of a very well known plant alocvera on the waste laden soil and the results have shown that the growth of the plant is increased in the waste laden soil. This shows that some of the plant nutrients have increased in this soil.

**Biological properties of soil**

The biological studies have shown that when soil contains waste materials having high organic content the bacterial and fungal content of the soil has greatly increased. The major soil bacteria on the plate were identified as Pseudomonas, Serratia, Enterobacter, E.coli, Bacillus and Salmonella. Almost all of these are known to have pathogenic properties.

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. As there is no study on the economical production of probiotics and to assess the efficacy and the properties of these products, at present, it was considered necessary to provide some useful studies to evaluate and suggest general guidelines for their production. 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 economically produce them.

Probiotics should not be considered as harmful substances for health, but can be incorporated into a balanced and varied diet to maximize good health. Several beneficial health effects of probiotics have been determined and thus they should be made available to each and every person. To make them available for common man their cost in market have to be decreased which can be achieved by economical production of probiotics.

Complex media commonly employed for growth of lactic acid bacteria are not economically attractive due to their high amount of expensive nutrients such as yeast extract, peptone and salts. Nevertheless, much effort in process optimization and at the engineering and biological levels has been done, and for some applications probiotics can be produced from several inexpensive waste substrates, thereby decreasing their production cost.

This study suggests the most effective way for this purpose. It can be concluded from study that there are some probiotics which are less fastidious in their growth requirements and can be grown simply on raw materials available. The waste materials from different sources can be used for the same purpose which can further solve the problems related to their disposal. The papaya peels, banana peels and dairy industrial waste, whey are having sufficient nutrients for the growth of probiotic organisms. The growth can further increased if some nutrients are added in adequate amounts to enrich the above waste materials. All the important properties of the probiotic organisms are maintained during this production.

The utilization of waste materials for this kind of beneficial purpose further reduces their content from the environment which in turn helps in removal of their ill-effects from ecosystem.