6. CONCLUSIONS

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With the increased in changing lifestyle and pollution, man are prone to many kind of infectious diseases and moreover with the increase incidence of antibiotic resistance of bacterial pathogen, antibiotics are less effective in curing the diseases. The solution for the existing problem lies in searching for the alternative therapy which should be effective and should not have any side effects.

Probiotic is the most promising solution for these existing problems. Probiotic which is a relatively new term meaning 'for life' is associated with those microorganisms which are beneficial for maintaining good health in human and animals. The concept of existence of the probiotics dates back to 19th century, when the Russian Scientist Eli Metchnikoff one of the founders of modern immunology stated that the longevity of the Bulgarian people were linked with their consumption of yoghurt. Over the past few decades, there is an increasing interest in the field of probiotics. The interests in probiotics also stems from the growing universal awareness among the consumers regarding the safety aspects related with chemical drugs. The emerging need of safe and natural therapeutics without any adverse effects could be one of the main reasons behind the expanding market of probiotics. Probiotics being endowed with large number of beneficial attributes offer tremendous opportunities for their extensive application in almost all segments including food, pharma and cosmetics. The WHO declared probiotics to be the next most important immune defense system when antibiotics are useless

LAB are the main probiotics groups of microorganisms. They can be isolated from the different sources as they are ubiquitous in nature and generally associated with habitats rich in nutrients such as various food products such as milk, meat, vegetables etc., normal flora of the mouth, intestine and vagina of mammals but for human application they should preferably of human origin. LAB are present in the gastrointestinal tract of the man. They are normal resident in the GIT of man and played a very beneficial role in the well being of the host. GIT of infant is a source of probiotic rich LAB. Limited studies are available on the probiotic characterization of LAB of human origin. From the scientific data, it is also evident that probiotic properties
are strain specific. In order to act as a probiotic the microbes should meet certain criteria such as the microbes should be able to resist the stress factor of low pH of stomach and the bile in the intestine. They should possess antimicrobial property and also should be least resistance to the commonly used antibiotics. They should also be able to adhere to the intestinal epithelium. They should also influence the human metabolic activities (cholesterol assimilation, vitamin production, etc). Therefore taking into account that there are different effects between the probiotic strains and those different strains which belong to the same species function differently with regards to physiological characteristics rendering them different or improved probiotic properties against other bacteria, the identification and characterization of the probiotic strains is very important.

Considering the importance of characterization of probiotics strains of the same species and its limited studies on LAB of human origin for its human application the present research work was focussed with the following objectives:

(i). To isolate and identify the Lactic Acid Bacteria (LAB) from the infant faeces by using conventional methods.

(ii). To determine the tolerance of low pH, bile acid and determine the production of biogenic amines.

(iii). To examine the antipathogenic activities of LAB on certain pathogen like *E. coli* and to study the antibiotic susceptibility of the selected LAB.

(iv). To evaluate the *in vivo* properties of LAB using animal model and quantitative estimation of effects of selected LAB on cholesterol of mice.

(v). To identify the probiotic LAB by using molecular technique.

Isolation and identification of LAB from the infant faeces in the age group of 1-1.8 years was carried out by using conventional phenotypic methods such as microscopic examination, catalase test, gas production from glucose, growth at different temperature and NaCl concentration, arginine hydrolysis test and sugar fermentation test. In this study 66 isolates were identified upto species and 3 isolates could not be identified upto species and one isolates was doubtful of being LAB so could not be identified. The
The present research finding was the identification of *L. acidophilus* as the dominant LAB in the faeces of infant which is in consistent with the previous finding.

After the identification of the lactic acid bacteria by using conventional methods, the isolates were subjected to invivo probiotic characterization by applying different tests such as low pH, bile salt, biogenic amine, antimicrobial activity and antibiotic susceptibility. *In vitro* assays can give an indication of probiotic properties and can be the first part of the selection process. The isolates which were able to grow at low pH and bile underwent biogenic amine test. Biogenic amine test is also an important probiotic selection criteria as biogenic amine are harmful to health. Then the isolates which were negative to biogenic amine were tested for the antibacterial activities by using pathogenic *E. coli* as the indicator organism. Probiotic microorganism should possess antibacterial property to at least one of the common pathogen. Antibiotic susceptibility test was also done for the bacterial isolates which showed zone of inhibition in the antibacterial test. In the recent years, there is concerned over the possible spread of antibiotic resistance determinants from bacteria used in probiotic products so, it is important for the testing of the antibiotic susceptibility of the bacterial isolates which are used in the probiotic as a safety measures.

The best five isolates - SW8, ST9, SC10, SH10 and SN2 fulfil all the criteria of *in vitro* probiotic property and they were selected for the *in vivo* test and feeding of the bacterial isolates to the mice took place. Among the five isolates only the two isolates, SW8 and ST9 were able to adhere the mouse’s intestine as these isolates were detected in the faeces of mice which were fed with these isolates. The quantitative estimation of the cholesterol level indicated that the cholesterol level of the mice which fed the isolates SW8 and ST9 were lower than the mice which fed the isolates SC10, SH10, SN2 and the control mice.

Probiotics effects are known to be strain specific. Therefore, strain identity becomes important to associate a specific effect with a particular strain. Therefore, it is very important to perform proper identification in order to associate a specific effect with a particular strain. Molecular identification of the two probiotic strains SW8 and ST9 was done by using 16S rDNA sequence analysis and identified as *Bacillus coagulans* and *Weissella paramesenteroides strain FMA204* respectively.
The present research work isolated and identified a new isolate *Bacillus coagulans* SW8 as a probiotic. Even though the research study focussed on the isolation of LAB, the probiotic isolates SW8 was identified as lactic acid forming *Bacillus coagulans* by molecular characterisation. Nearest homolog of SW8 was found to be *Lactobacillus thermophilus* (GeneBank Accession Number: M58832.1 LBARR16SAD). In the phenotypic identification, SW8 could not be identified as it is difficult to identify *Bacillus coagulans* by only phenotypic identification. *Bacillus coagulans* was originally known as *Lactobacillus. sporogenes*. The *Lactobacillus sporogenes* or as it should be classified, *Bacillus coagulans*, represents the archetypal misidentified probiotics and its annoveration among lactobacilli has often been matter of debate. In fact, since this bacterium shows characteristics of both genera *Lactobacillus* and *Bacillus*, its taxonomic position between the families Lactobacillaceae and Bacillaceae has often been discussed. They are not normal inhabitant of GIT. Being a spore former this strain could be of great commercial value because of their survivability in acidic, alkaline, heated and cold preserved foods and beverages as well and could open a new horizon in the field of biotherapy. *Bacillus coagulans* is found in several probiotic supplements currently available in the market. It is considered to be a superior probiotic than the other probiotic supplements due to its spore forming capacity, it has higher resistance to technological stresses during production and storage processes. The other probiotic strain (ST9) which was identified was *Weissella paramesenteroides* which is formerly known as *Leuconostoc paramesenteroides*. The other close homologs which were 0.99 alignment were *Weissella sp.* (GeneBank Accession Number: JX193634.1). They are generally found in plants and in dairy products and not the normal resident of GIT. They are transit microorganisms. This microbe was present in the GIT of infant due to the consumption of dairy products by the infant. At the best of my knowledge, this is the first research finding in India in which the probiotic strain *Weissella paramesenteroides* from the infant faeces was isolated and characterised as probiotic.

The characterization of the new probiotic strains *Bacillus coagulans* SW8 and *Weissella paramesenteroides* ST9 in the present research work would open a new horizon in the field of biotherapy. These two novel probiotics would act as a superior probiotics supplement for the human consumption. However, large numbers of controlled clinical trials and technological properties such as strain stability, viability in products are needed for the commercialization of these probiotics.