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A STUDY OF ISOLATION OF LACTIC ACID BACTERIA FROM FOOD SOURCES

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ABSTRACT

Lactic acid bacteria are the most important microbes that find application in food industries for food fermentation, to enhance texture and taste, especially for fermented food products and are normal constituents of intestinal microflora. They are a group of gram-positive, cocci or rods, non-spore forming, catalase-negative, chemoorganotrophytes with low pH tolerance. The DNA of LAB has a low G+C content. The main attraction of LAB is the production of lactic acid from glucose and many growth inhibition substances like bacteriocins, diacetyls hydrogen peroxide, etc., which has the ability to prevent the proliferation of food spoilage bacteria and pathogens. Some bacilli such as bifidobacteria also form lactic acid as a major end product. LAB is grouped into the Clostridium branch of Gram-positive bacteria which is related to bacilli, whereas Bifidobacterium belongs to Actinomycetes (Hati et al., 2019).Due to their permeating occurrence in food and their contribution to the healthy microflora of human mucosal surfaces, they are generally acknowledged as safe (GRAS microorganisms).

KEYWORDS: Isolation, Lactic Acid Bacteria, Food Sources, helicobacter pylori infection, pathogenic microorganisms

INTRODUCTION

The present study is designed to obtain lactic acid bacteria (LAB) from various food sources that could be used against the antibiotic resistant bacteria (ARB) and to investigate the efficiency of the isolated probiotic strains through acid tolerance, bile tolerance, auto aggregation, hydrolysis and hemolytic test. On the other hand, bacteria from poultry waste were isolated for antibiotic susceptibility test using disc diffusion method. From the susceptible test results, the multiple antibiotic susceptible strains were tested against the probiotic bacteria isolated from food source. The well cut method was used to determine the antimicrobial of bacteriocin against indicator strain (antibiotic resistant bacteria from poultry waste). To improve the antagonistic substance production of probiotic bacteria against the antibiotic resistant bacteria, various parameters which includes incubation time, temperature and nutrient composition were studied. In addition, the stability of the antagonistic substance was also studied at different temperatures.

Probiotics are live microorganisms that have beneficial effects on their host's health. The word 'probiotic' comes from Greek language 'pro bios' which means 'for life'. The majority of the microorganisms used for probiotic purposes belong to two types, Bifidobacterium and lactic acid bacteria

(LAB). LAB includes a wide group of Gram-positive fermenting bacteria which are generally non-sporulating and non-motile.

They comprise of both cocci and bacilli belonging to Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella genera (Hofvendahl and Hahn-Hägerdal, 2000). Probiotics have been used for about 100 years to treat a variety of mucosal surface infections, such as those in the gut and vagina, but the use of these traditional treatments diminished after the advent of antibiotics. However, these agents are now being reconsidered as alternatives to antibiotics because of the rise in antibioticresistant strains of bacteria (D'Souza et al., 2002).

The aim of this study is to the India are traditionally rich in fermented foods but the nature of products and the base material varying from region to region. Appam or hoppers from fermented batter, morkuzhambu from curd or buttermilk are the common type of food in South India cuisine especially in Tamil Nadu and Kerala. Some of the traditional fermented dairy products of Inner Mongolia are "Airag" (also called Koumiss, a traditional fermented beverage made from mare's milk), "Byaslag" (a traditional cheese made from milk of cattle) and "Tarag" a traditional yogurt made from cow, goat or camel milk. In Taiwan, pickled cabbage is a popular traditional snack. Popular traditional white cheeses in Bulgaria have been described as the most suitable carriers for the administration of such bacteria. Currently a wide range of probiotic dairy product is available in markets like pasteurized milk, ice cream, fermented milks, cheeses and baby feed milk powder. Many researchers studied the health benefits of fermented foods and probiotics but did not use sufficient test subjects or used microorganisms which were not clearly identified. Owing to this a number of studies have been established only partially. But some can be regarded as well-established and clinically well documented for specific strains. Probiotics effects the control of lactose intolerance, improves the immune system, prevents colon cancer, reduces cholesterol and triacylglycerol plasma concentrations (weak evidence), lowers blood pressure, reduces inflammation, reduces allergic symptoms, has beneficial effects on mineral metabolism particularly bone density and stability, reduces helicobacter pylori infection, suppresses pathogenic microorganisms (antimicrobial effect), prevents osteoporosis and urogenital infections.

HISTORY OF PROBIOTICS

The history of probiotics began when man (especially the Greeks and the Romans) started to consume fermented foods (Gismondo et al., 1999; Guarner et al., 2005). In 1899, Tissier (Pasteur Institute, France) isolated Bifidobacteria from the stools of breast fed infants and found that they were a predominant component of the intestinal flora in humans (Ishibashi and Shimamura, 1993). Tissier recommended the administration of Bifidobacteria to infants suffering from diarrhoea, 'believing' that the Bifidobacteria would displace putrefactive bacteria responsible for most intestinal disorders, while re-establishing themselves as the dominant intestinal microorganism (O'Sullivan et al., 1992).

At the beginning of the 20th century, the Russian bacteriologist ElieMetchinikoff (Pasteur Institute, France) was the first person who identified the beneficial effects of lactic acid bacteria present in fermented milk. He attributed the good health and longevity of Bulgarians to their large consumption of

fermented milk known as yogurt. In 1908, he postulated a 'longevity without aging' theory. The principle of this theory was that the lactic acid bacteria caused the displacement of toxin producing bacteria normally present in the intestine which could be responsible for longevity. Metchnikoff stated that lactic acid and other products produced by lactic acid bacteria in sour milk inhibited the growth and toxicity of anaerobic, spore forming bacteria in the large intestine (Hughes and Hoover, 1995).

The first clinical trials for the effect of probiotics on constipation were conducted in the 1930s. In the 1950s, a probiotic product was licensed by the United States Department of Agriculture (USDA) as a drug for the treatment of scour (Escherichia coli infection) among pigs (Orrhage et al., 1994).

The beneficial properties of live microbial food supplements such as fermented milk have been documented over many centuries (Oberman, 1985). Moreover, scientists such as Hippocrates considered fermented milk not only as a food product but also as a medicine and prescribed it for disorders of the gastrointestinal system (Oberman, 1985). During the last century, different microorganisms were employed to prevent and cure diseases, leading to the coining of the generic term, probiotics (Lidbeck et al., 1992).

Studies on lactic acid producing bacteria in food continued throughout the 20th century with each yielding variable reports on the benefits of consuming probiotics. Earlier research dealt with the use of fermented milk in the treatment of intestinal infections, however recent studies have focused on other aspects of potential health, strain selectivity to ensure survival of these bacteria in the gastrointestinal tract and carrier food.

In 1994, the World Health Organization (WHO) deemed probiotics to be the next most important immune defense system when commonly prescribed antibiotics are rendered ineffective through antibiotic resistance (Levy, 2000). The use of probiotics where antibiotic resistance occurs is known as microbial interference therapy.

PROBIOTICS AND TRADITIONAL FOOD

India is traditionally rich in fermented foods but the nature of products and the base material vary from region to region. Appam or hoppers from fermented batter, morkuzhambu from curd or buttermilk are the common type of food in South India cuisine especially in Tamil Nadu and Kerala (Kumar et al., 2010). Some of the traditional fermented dairy products of inner Mongolia are "Airag" (also called Koumiss, a traditional fermented beverage made from mare's milk), "Byaslag" (a traditional cheese made from milk of cattle) and "Tarag" a traditional yogurt made from cow, goat or camel milk (Kumar et al., 2010). In Taiwan, pickled cabbage is a popular traditional snack (Giraffa et al., 2010). Popular traditional white cheeses in Bulgaria have been described as the most suitable carriers for the administration of such bacteria (Liu et al., 2011). Currently a wide range of probiotic dairy product is available in markets like pasteurized milk, ice cream, fermented milks, cheeses and baby feed milk powder (Georgieva et al., 2009).

LACTIC ACID BACTERIA (LAB)

LAB shows its presence in foods (milk products, fermented meat and vegetables, sour dough, beverages, silage etc.), sewage, in plants and within the respiratory, intestinal and genital tracts of humans and animals (Schleifer et al., 1995; Hammes et al., 1991). Together with acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide and diverse metabolites. LAB produces a variety of low molecular mass compounds.

Lactic acid bacteria produce a variety of antagonistic factors that consists of end products which are produced metabolically, like bactericidal proteins and antibiotic-like substances termed bacteriocins. The rate of inhibitory activity by bacteriocins of lactic acid bacteria can be either narrow; inhibiting only those strains that are closely related to the producer organism or broad; inhibiting a diverse group of Grampositive microorganisms. Bacteriocins of LAB are considered as safe natural preservatives or biopreservatives, because it is assumed that they are destroyed by the proteases in gastrointestinal tract (Cleveland et al., 2001).

1 Historical background of lactic acid bacteria

Lactic acid- manufacturing fermentation is a recent invention. Varied cultures in various features of the planet have used fermentation to enhance, the storage qualities and nutritive worth of perishable foods like milk, vegetables, meat, fish and cereals. The organisms that manufacture this kind of fermentation lactic acid bacteria had a very important role in preserving foods. In developed world, lactic acid bacteria are mainly related to fermented dairy products like cheese, buttermilk and yogurt. The utilization of dairy starter culture has become the trade throughout this century.

The concept of the cluster name _lactic acid bacteria‘ was created for bacteria inflicting fermentation and coagulation of milk, and defined as those that manufacture lactic acid from lactose. The family name Lactobacteriaceae was applied by (Orla-Jensen, 1919) to a physiological cluster of bacteria manufacturing lactic acid alone or acetic and lactic acids, alcohol and carbondioxide. Today, lactic acid bacteria are considered synonymous with the family Lactobacteriaceae (Breed et al., 1957).

Ever since the period of Russian scientist Metchnikoff, lactic acid bacteria have conjointly been related to helpful health effects. Today, an increasing range of health food and so called purposeful foods similarly as pharmaceutical preparation are promoted with health claims base mostly on the characteristics of sure strains of lactic acid bacteria. Most of those strains, however, haven‘t been completely studied and consequently the claims aren‘t well substantiated. Furthermore, health edges are judged mainly using subjective criteria. Additionally, the particular bacterial strains employed in the studies are typically poorly defined. The majority of data regarding the health effects of lactic acid bacteria is therefore unreliable.

Lactic acid bacteria are a gaggle of gram-positive bacteria united by a constellation of morphological, metabolic, and physiological characteristics. They are non-sporing, carbohydrate fermenting lactic acid bacteria producers, acid tolerant of non-aerobic habitat and catalase negative. Usually they are non-

motile and don‘t scale back nitrite. They are subdivided into 4 genera: Streptococcus, Leuconostoc, Pediococcus and Lactobacillus. Latest taxonomic revisions advice that lactic acid bacteria cluster can be comprised of genera, Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, and Vagococcus. Originally, Bifidobacteriawere included within the genus, Lactobacillus and therefore the organisms were spoken as Lactobacillus bifidus. The term lactic acid bacteria were used synonymously with ―milk souring organisms‖. Important progress in the classification of these bacteria was made when the similarity between milk-souring bacteria and other lactic acid producing bacteria of other habitats was recognized (Axelesson, 1993).

Lactic acid bacteria are generally associated with habitats rich in nutrients, such as various food products(milk, meat, vegetables), but some are also members of the normal flora of the mouth, intestine and vagina of mammals.

2 Classification at genus level

The source for the classification of lactic acid bacteria in different genera has essentially remained unchanged since the work of (Orlajensen, 1919). Although their morphology is regarded as questionable as a key character in bacterial taxonomy (Woese, 1987),it is still very important in the current descriptions of the lactic acid bacteria genera.

Lactic acid bacteria can be divided into rods (Lactobacillus and Carnobacterium) and cocci (all other genera). An important characteristic used in the differentiation of lactic acid bacteria genera is the mode of glucose fermentation under standard conditions, that is, non- limiting concentrations of glucose and growth factors(amino acids, vitamins and nucleic acid precursors) and limited oxygen availability. Under these conditions, lactic acid bacteria can be divided into two groups: homofermentative, which convert glucose almost quantitatively to lactic acid, and heterofermentative, which ferment glucose to lactic acid, ethanol/acetic acid and carbon dioxide. In practice, a test for gas production from glucose will distinguish between the groups. Leuconostoc and a subgroup of Lactobacillus are heterofermentative; all other lactic acid bacteria are homofermentative.

Lactic acid bacteria are a genus with some 50species. They occur on plants and dairy products and in man; they form a part of normal flora of gut and vagina. Apart from their association with dental caries, Lactobacilli are generally regarded as non- pathogenic, but there are reports of their involvement in human disease. Lactobacillus plantarum have been occasionally seen associated with bacterial endocarditis.

3 Metabolism of lactic acid bacteria

The essential feature of lactic acid bacteria metabolism is efficient carbohydrate coupled to substrate - level phosphorylation. The generated ATP is subsequently used for biosynthesis purposes.

Lactic acid bacteria as a group exhibit an enormous capacity to degrade different carbohydrates and related compounds. Generally, the predominant end product is of course, lactic acid. It is clear however, that the lactic acid bacteria adapt to various conditions and change their metabolism accordingly. This may lead to significantly different endproduct patterns.

4 Taxonomic diversity of lactobacillus

The human gastrointestinal tract contains hundreds of different bacterial species (Tannock, 2000). Members of the genus Lactobacillus are commonly present as members of microbial communities and have received considerable attention with respect to their putative health conferring properties as probiotics. Lactobacillus has worldwide industrial use as starters in the manufacturing of milk products. Moreover, some of the Lactobacillus strains have probiotic characteristics and are therefore included in fresh fermented products or used in capsular health products such as freeze-dried powder. The use of some Lactobacillus strains as probiotics is based on studies that show that these species belong to the normal intestinal flora and that the strains have beneficial effects on human and animal health (Salminen et al., 1996).

Organisms of the human gastrointestinal tract include diverse bacterial genera or families, and are divided into following three groups:

1. Lactic acid bacteria in a broad sense, including Bifidobacterium, Lactobacillus and Streptococcus (including Enterococcus);

2. Anaerobic group, including Bacteriodaceae, Eubacterium, Peptococcaceae, Veillonella, Megasphera, Hemmiger, ClostridiumandTreponema.

3. Aerobic group, including Enterobacteriaceae, Staphylococcus, Bacillus, Corynebacterium, Pseudomonas and yeasts.

The species composition of Lactobacillus has changed considerably in the last decade, many new species have been described, but others have reduced in rank.

CONCLUSION

This study deals with the isolation of lactic acid bacteria from food sources for a study on its antagonistic activity against the antibiotic resistant bacteria isolated from poultry waste. Experiments were also conducted to check the characterization and optimization of LAB against antibiotic resistant bacteria. Fourteen colonies were isolated from various food sources namely, cow milk, goat milk and idli batter. Among these isolates, only four bacteria which produce lactic acid in MRS media with 1 % of calcium carbonate were selected for antagonistic activity using well diffusion method. The isolate had high antagonistic activity against the Staphylococcus aureus MTCC 96, Klebsiellapneumoniae MTCC 530, Shigellaflexneri MTCC 1457 and Pseudomonas aeruginosa MTCC1688. A lesser antagonistic activity was observed against Salmonella typhii MTCC 531 and Vibrio cholerae MTCC 3906. The four bacterial

strains were identified as Weissella sp. to generic level according to Bergey's Manual. By sequencing the 16S rRNA genes and using BLAST similarity search accessed through the NCBI database, the four isolates were identified as Weissellacibaria strain. The amplified products were supported with Weissellacibaria with boost trap value of 69 %, 98 %, 95 % and 94 %.The 16S rRNA sequences of Weissella sp. showed 99 % of sequence similarity with Weissellacibaria strain II-I59. Thus these isolates were designated as Weissellacibaria KTSMBNL 27, Weissellacibaria KTSMBNL 28, Weissellacibaria KTSMBNL 29 and Weissellacibaria KTSMBNL 30 and deposited in NCBI under the accession number; KC987948.1, KC987949.1, KC987950.1 and KC987951.1, respectively.

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