REVIEW ON *Lactobacillus fermentum*

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ABSTRACT

Milk and other dairy products are considered as the most abundant sources of LAB (Lactic Acid Bacteria), which is also a member of the gut micro biota of man.The genus Lactobacillus consists of a diverse group of rod-shaped, Gram-positive, non-spore forming, non-pigmented, catalase negative and microaerophilic to strictly anaerobic organisms. Lactobacillus fermentumis a gram positive rod shaped bacterium and a heterofermentative organism, found abundantly in fermented food and vegetables. It is catalase negative and exhibits various enzymatic, amylase and antimicrobial activities. The bacterium also produces bacteriocins, which are peptides that can act as food preservatives as well as substitute to antibiotics.The main aim of this review article is to study, analyse and interpret various information on Lactobacillus fermentumhighlighting its sources, isolation and characterisation methodology, its potential in fermentation and the possible applications of the same in various sectors.

KEYWORDS:-*Lactobacillus fermentum, Heterofermentative bacteria, probiotics, Exopolysaccharide, Bacteriocin*

INTRODUCTION

Lactobacillus fermentum, a rod shaped, gram positive bacteria, categorised as an obligateheterofermentative bacteria[17], is a major species of the human gut and can easily survive in the harsh environment of the animal stomach and intestinal tract.Its coloniesgrow well at 45°C but not at 15°C[6]. The *Lactobacillus fermentum*rods are of variable length. It is a catalase negative, non-motile and non-sporing organism. *Lactobacillus fermentum* uses several carbohydrates such as arabinose, cellobiose, galactose, maltose, mannose, melibiose, raffinose, ribose, sucrose, trehalose, and xylose[19], but fermentation is strain dependent. It has 52–54% G+C content (mol %) and the d-aspartyl-l-ornithine-type peptidoglycan. The bacterium produces lysozyme, gas from glucose and ammonia from arginine[27]. True catalase activity to remove hydrogen peroxide is also exhibited. [13] It is found that many LAB including several strains of *Lactobacillus fermentum*can produce EPS (exopolysaccharides)[37], which can essentially impact on an enhanced perception of taste**[16]**. Moreover, *Lactobacillus fermentum*is considered as GRAS (Generally Considered as Safe) for usage as both food and feed. It can also be used as a probiotic[35], whose supplementation can improve immunity, fight digestive diseases and reduce cholesterol levels (LDL).

Lactobacillus fermentum, due to its enzymatic activity and hence it is used in preparation of various fermented products (used in starter cultures). The strains of *Lactobacillus fermentum*shows proteolytic activity as well as alkaline phosphatase, esterase lipase, leucine-aminopeptidase, valine-aminopeptidase, acid phosphatase, phophohydrolaze, α-galactosidase and β- galactosidase activity [25].

It can also be safely used for medical and veterinary applications as the bacteria shows susceptibility to antibiotics [7]. Several LAB, including *Lactobacillus fermentum*and its metabolites have been shown to play an important role in improving microbiological quality and shelf life of many fermented food products and provide a good example of bio preservation[36]. There are various research papers inferring that some strains of *Lactobacillus fermentum*can produce bio-surfactants that shows a wide range of anti-microbial activity against many bacterial pathogens.This bacterium produces peptides, defined bacteriocins which exhibit good antibacterial activity having therefore, good probiotic features and finally, be safe towards microglial cells[9].

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS

Lactobacillus fermentum is the major heterofermentative *Lactobacillus* species of the human gut. The major physiological trait of this bacterium is that it can grow even in the complex and harsh environment of the animal and human stomach and intestinal tract. The bacterium grows on a variety of media including MRS (Man, Rogosa, and Sharpe) agar where they appear as white, usually mucoid colonies. Identification and differentiation between various*Lactobacillus* species is preferably done by molecular means (16S rRNA genes) as phenotypic identification is generally unreliable.

Lactobacillus fermentum is a gram positive rod shaped bacteria. The length of the rods generally varies with strain. They have approximate dimensions of 0.5-0.9 x 3.0µm. They are non-motile and occurs single or in pairs. Colonies are generally flat, circular or irregular to rough, convex, often translucent and are mostly non-pigmented. Some colonies of certain strains of the bacterium shows orange pigmentation. It has a dispersed growth in broth (liquid medium). Colonies of the bacterium grows well at 41-42℃ (for freshly isolated strains) and does not exhibit growth at 15℃. The important growth factor requirements for *Lactobacillus fermentum* are calcium panthothenate, niacin and thiamine among others. They are non-motile.

SOURCES

Lactobacillus fermentum is a probiotic and gut friendly bacterium usually inhabiting the oral cavity, gastrointestinal tract and the vaginal tract of humans. It is also present in fermenting plant and animal materials.It is more survivable in acid and bile than other probiotic strains. It is also one of the few strains particularly beneficial for dealing with issues more prevalent in women, like yeast infections and UTIs.*Lactobacillus fermentum*is also present in normal cow or buffalo milk and dairy products like yoghurt (Chobani), cheese, etc. The bacteria can also be isolated from human breast milk [21]as well as faecal samples of human[15]. It is also sometimes isolated from the intestine and/or caeca of chicken[8], pigs[39],mouse or rats, etc. Sometimes it can also be isolated from manure[31] and sewage.

ISOLATION AND CHARACTERISATION

Serial dilutions of the collected samples need to be prepared in normal saline and pour plated on MRS agar medium (with the following composition: peptone 1%, yeast extract 0.5%, beef extract 0.5%, glucose 2%, dipotassium phosphate 0.2%, dibasic ammonium citrate 0.2%, sodium acetate 0.5%, magnesium sulphate 0.01%, manganese sulphate 0.005%, Tween 80 (0.1%) and agar 3% (for solid medium)). This has to be later incubated at 36℃ for a period of 24-48 hours. When the colonies become predominant and morphologically distinctat the end of the incubation period, the colonies need to be streaked in fresh MRS agar to obtain pure cultures of the isolates[29].Identification of the pure isolates is subjected to the Bergey's Manual of Determinative bacteriology and even some software likePIBWin and IDENTAX. Cultural and morphological characters are to be examined. Colour, shape, size and texture of the colonies is to be observed and recorded.

The isolates are to be done gram staining and studied under the microscope. *Lactobacillus fermentum*is found to be a gram-positive bacterium which is also catalase negative. Further characterization[2]including the biochemical tests of fermentation of different carbon sources, gas production from glucose, growth at different temperatures, tolerance to inhibitory substances such as bile, phenol and sodium chloride, production of ammonia from arginine, etc.**.**[1] needs to be done.

Certain research regarding the yield of *Lactobacillus fermentum*in a medium other than the MRS medium has also come up. It was found that the bacterium, for its growth, prioritizes peptone, urea and yeast extract. The following composition of the fermentation medium was estimated to be the most economical formula (per litre): 30g corn syrup, 15g glucose, 14·4g peptone, 7g $(NH_4)_2SO_4$, 0·5g urea, 3g sodium acetate, 4g sodium citrate, 0·1g $MnSO_4 \cdot 4H_2O$, 0·5g $MgSO_4 \cdot 7H_2O$, 7·3g yeast extract, 0·5g K_2HPO_4. On comparison, it was found that the yield of *Lactobacillus fermentum*in this medium is 64% higher than that of the yield in the traditional MRS agar and that the cost of production is also 89% lower than the MRS medium.This research indicates that it is possible to increase bacterial yield by using inexpensive materials.[38]

BIOCHEMICAL PROPERTIES AND TESTS FOR CHARACTERISATION

*Lactobacillus fermentum*is a catalase negative bacterium. Catalase test is performed with the help of hydrogen peroxide(3% H2O2 reagent grade). *Lactobacillus fermentum*can ferment carbohydrates (sugars) effectively giving out ethanol and CO_2 along with lactic acid as the products. Carbohydrate utilization (fermentation) is determined in 1% (w/v) MRS broth containing the specific sugar and devoid of glucose and beef extract containing phenol red as indicator. The sugars that can be used for checking fermentation includes L-arabinose, cellobiose, mannitol, mannose, melebiose, raffinose, ribose, salicin, lactose, sorbitol, xylose, trehalose.

Acid and gas production from glucose is determined with the help of inverted Durham's tube containing 1% (w/v) glucose in MRS broth without beef extract. The bacterium is positive for gas production. Nitrate test is done in Nitrate broth and tested with alpha-naphthylamine and sulphanilic acid.

Exopolysaccharide productioncan be determined by streaking the bacterial cells (fresh overnight cultures on MRS agar) on LTV agar [0.5 g/l tryptone, 10 g/l meat extract, 6.5 g/l NaCl, 8 g/l potassium nitrate, 8 g/l sucrose, 0.1 % (v/v) Tween 80, 17 g/l agar, pH 7.1 ± 0.2] and incubated at 35 °C for 48 h. The stickiness of colonies to be determined by the inoculating loop method. Isolates are tentatively considered positive for exopolysaccharide if the length of slime was above 1.5 mm. Positive isolates are confirmed using MRS – sucrose broth without glucose and peptone as follows: [1 % (w/v) meat extract, 5 g/l yeast extract, 50 g/l sucrose, 2 g/l $K_2HPO_4{\cdot}3H_2O$, 5 g/l sodium acetate trihydrate, 2 g/l triammonium citrate anhydrous, 0.2 g/l $MgSO_4.7H_2O$, 0.05 g/l manganese (II) sulphate monohydrate, 0.1 % (v/v) Tween 80, pH 5.0 ± 0.2]. The isolates were then incubated at 30 °C for 24 h. A volume of 1.5 ml of the 24 h culture was centrifuged at 5000 *g* for 10 min (4 °C). About 1 ml of the supernatant is to be put in a glass tube and an equal volume of ethanol (99 %) is required to be added. In the presence of EPSs, an opaque link is formed at the interface.[11]

Amylase activity of the bacterium by point-inoculating the active cultures on modified MRS agar without glucose but with potato soluble starch as the sole carbon source [28]. The media composition as follows: [10 g/l tryptone, 10 g/l meat extract, 5 g/l (w/v) yeast extract, 20 g/l potato-soluble starch, 2 g/l $K_2HPO_4.3H_2O$, 5 g/l sodium acetate, 2 g/l triammonium citrate, 0.2 g/l $MgSO_4.7H_2O$, 0.05 g/l manganese (II) sulphate monohydrate, 0.1 % (v/v) Tween 80, pH 5.0 ± 0.2]. Inoculated plates needs to be incubated anaerobically at 35 °C for 48 h. The culture plates can be covered by spraying with Lugol's iodine [0.33 % (w/v) iodine, 0.66 % (w/v) potassium iodide] to detect starch hydrolysis. Un-degraded starch stains blue-black while the presence of a clear halo zone around a tested colony to be taken as indication of starch degradation and therefore the production of α-amylase.

OTHER PROPERTIES OF *Lactobacillus fermentum*

USE AS PROBIOTICS

A microorganism is considered a probiotic by meeting certain characteristics, such as being of human origin, non-pathogenic, having high resistance to passing through the intestine, and being beneficial to the immune system. In general, they are beneficial to the host's body and the human health. *Lactobacillus fermentum* has been identified as a potential probiotic.[14] It is also involved in reducing cholesterol content and boosting immunity. It also offers antimicrobial activity thus, protecting against various diseases.

LOW pH RESISTANCE

This can be determined by [20] and[40]. Bacterial cells from fresh overnight (18 h) cultures has to be harvested (10,000 x g, 5 min, 4 °C), washed twice with PBS buffer (pH 7.2), re-suspended (2 %) in PBS solution and adjusted to pH 2.5. Resistance can be assessed in triplicates in terms of viable colony counts and enumerated on MRS agarafter incubation at 37 °C for 4 h, reflecting the possible time spent by food in the stomach.

BILE SALT RESISTANCE

Bacterial cells from overnight (18 h) cultures to be harvested (10,000 x g, 5 min, 4 °C), washed twice with PBS buffer (pH 7.2), before inoculating in PBS solution (pH 8.0), containing 0.3 %, 0.5 %, 1 % and 2 % (w/v) bile salt. Resistance can be assessed in triplicates in terms of viable colony counts and enumerated after incubation at 37 °C for 4 h.[11]

SURVIVAL RATE

Survival rates for *Lactobacillus fermentum*strains can be estimated after their growth in low pH (pH 2.5) and different bile salt concentrations (0.3 %, 0.5 %, 1 % and 2 %).[11]

ANTIMICROBIAL ACTIVITY ASSAY

Antibacterial assay has to be done using well diffusion method. Mueller-Hinton agar plates to be prepared, and the plates need to be seeded with Escherichia coli and Staphylococcus aureus separately using McFarland standard 0.5. Following this 5 mm well should be prepared in each plate, and 10 µl of cell-free culture filtrate of the isolate can be loaded in each well. The plates are to be incubated for 24 hrs at 35±2°C[34].

ANTIBIOTIC RESISTANCE

For antibiotic resistance test, *Lactobacillus fermentum*strains has to be inoculated (1 % v/v) in MRS broth supplemented with 9 different antibiotics (Ampicillin, Chloramphenicol, Tetracycline, Erythromycin, Streptomycin, Kanamycin, Gentamycin, Quinupristin/Dalfopristin, Clindamycin) at various final concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 µg/ml) and examined in triplicate for growth in a microplate reader (OD at 610 nm) following a 24 h incubation period at 35 °C.[11]

ENZYMATIC ACTIVITY

The enzyme profiles of various strains of *Lactobacillus fermentum*shows β-glucoronidase, α-glucosidase and β-glucosidase activity. They exhibit proteolytic activity as well as alkaline phosphatase, esterase lipase, leucine-aminopeptidase, valine-aminopeptidase, acid phosphatase, phophohydrolaze, α-galactosidase and β-galactosidase activity.[25]

These bacteria also exhibit amylolytic activity.The enzyme was able to hydrolyze blocked p-nitro phenyl methyl heptaoside, releasing a yellow compound (p-nitro phenol) with maximum absorption at 530 nm. This result was a proof that amylase from *Lactobacillus fermentum*(04BBA19) is an endo acting amylase (α-amylase). Results from a certain research shows the thermostable characteristics of the amylase enzyme from the bacterium, this property is quite unique among lactic acid bacteria.Due to its high thermostability, α-amylase from *Lactobacillus fermentum*could be highly competitive in industrial bioconversion reactions.[4]

Ferulic acid is a well-known phenolic phytochemical present in plant cell walls. Various studies have indicated that ferulic acid has physiological functions in the prevention of oxidative stress and some chronic diseases. Ferulic acid can also be used as cosmetic additives to prevent lipid peroxidation [10]as well as to promote insulin production and prevent diabetes[3]. It has been shown to play an important chemoprotective role in degenerative diseases. Ferulic acid also shows strong antioxidant[32] and nitrite-scavenging potential and anticarcinogenic and anti-inflammatory properties[5]. The acid extracted from certain strains of *Lactobacillus fermentum*has great potential not only in biofuel production but also in pharmaceutical, polymer, oleo chemical, cosmetic additive, and detergent industries, as well as human health-related applications, including food flavour, functional foods, probiotic agents, preventive medicine, and animal feed.[30]

APPLICATIONS OF *Lactobacillus fermentum*

Being a probiotic bacterium and safe for consumption, *Lactobacillus fermentum* finds a wide range of applications in many sectors mainly in the chemical, beverages, food and medical and pharmaceutical sectors.The potential industrial application of certain strain could be the bioconversion of inexpensive raw material as starch into lactic acid in single step process. They are also potential candidates for food industries (making of high density gruels, baking, brewing) and for the production of biodegradable plastic from starchy raw material.

MEDICAL AND PHARMACEUTICAL INDUSTRY

Lactic acid-producing bacteria are the most used probiotics that play an important role in protecting the host against harmful microorganisms, strengthening the host immune system, improving feed digestibility, and reducing metabolic disorders. *Lactobacillus fermentum* is a Gram-positive bacterium belonging to Lactobacillus genus, and many reportedly to enhance the immunologic response as well as prevent community-acquired gastrointestinal and upper respiratory infectionsand to potentially help prevent alcoholic liver disease and colorectal cancer among humans. It is also one of the few strains particularly beneficial for dealing with issues more prevalent in women.Probiotic utilization is becoming increasingly popular in veterinary

medicine.*Lactobacillus fermentum*(AD1) survive transit through the canine gastrointestinal tract and populate the colon and probably increased absorption of some nutrients[33].

*Lactobacillus fermentum*may assist in reducing the development of obesity by modifying the way energy is handled within the host. This suggests potential to create a microbiome that favours fat oxidation (breakdown) over fat storage by populating the gut with protective bacteria and preventing the proliferation of pathogenic bacteria.[24]

*Lactobacillus fermentum*may inhibit the growth of *Candida albicans* and demonstrates the capability to prevent yeast infections. Other studies have shown that *Lactobacillus fermentum*could be effective in helping to reduce the likelihood of UTI recurrences in women.

*Lactobacillus fermentum*exhibits some antimicrobial and antioxidative properties.[22] It has demonstrated the ability to suppress growth of harmful bacteria, like staphylococci, toxic enterobacteria, and common hospital-acquired infections like *S. aureus* and *P. aeruginosa*. Recent studies also suggest that it could improve resistance against influenza infections. According to this study, *Lactobacillus fermentum*could be helpful in maintaining remission and preventing relapse of ulcerative colitis. It could also reduce the duration or severity of some infections like respiratory illness.

*Lactobacillus fermentum*may be able to help ease some of the negative effects of the aging process, particularly immunosenescence, which is the multi-faceted decline in the function of the immune system that occurs during progressive aging. According to the test results, *Lactobacillus fermentum*helps to resist infections and improve antioxidant capacity, which signify its potential in supporting healthy aging. As with most probiotics, research is ongoing for*Lactobacillus fermentum*, but there are already a wide variety of proven benefits to supplementation.

FOOD INDUSTRY

*Lactobacillus fermentum*is a bacterium that is essentially a part of various fermented food and feed items. Fermentation is considered the most economical way by which food products can be preserved and saved from spoilage. In addition to preservation, fermented foods can also have added benefits of enhancing flavour, increased digestibility, and improving nutritional and pharmacological values [12]. Many lactic acid bacteria are essentially involved in the fermentation of food products.

Lactobacillus fermentum is named 'fermentum' due to its role in fermentation. *Lactobacillus fermentum* is a heterofermentative organism which produces ethanol and CO2 as the primary product along with lactic acid and other metabolites. It can be used in fermentation of various food products including milk, sorghum (to Ting), cereal based foods (like, European sour rye bread, Asian salt bread, sour porridge, and African based millet dough) and the traditional sourdough cultures.

International[18] fermented foods include, Ca muoi (eggplant) – Vietnam, Duamuoi (mustard/ beet) – Vietnam, Paocai (Cabbage, celery, cucumber, and radish) – China, Sinki (raddish) - India, Nepal, and Bhutan, Tempoyak (Duriyan) – Malaysia.

Indian[26] fermented foods include, Cereal based food (Koozhu – Tamil Nadu, Kallappam – South India, Dhokla – South India, Ambali – India), Milk based food (Curd – India, Churrpi – Arunachal Pradesh), Vegetables and fruits (Gundruk – Arunachal Pradesh, Sinki – North east India, Eup – Arunachal Pradesh), Pulse based food (Wadi – Punjab), etc.

Some strains of the bacterium produce diverse and potent antimicrobial peptides- bacteriocins, which can be applied as food preservative agents or as alternatives to antibiotics. This bacterium is a key microorganism in sourdough technology, contributing to flavour, texture, or health-promoting dough ingredients, and has recently been used to develop new foods stuffs such as fortified and functional foods with beneficial attributes for human health. Development of such new foodstuffs are currently taking important proportions of the food industry market. Furthermore, an increasing awareness of the consumers prompts the food-makers to implement alternative environmentally friendly solutions in the production processes and/or suitable biological alternative to limit the use of antibiotics in feed and food. Thus, this bacterium acts a potential bio-preservative and aids in increasing the shelf-life of various products.[23]

CONCLUSION

Lactobacillus fermentum is a member of the *lactobacillus* genus which has immense potential in fermentation. It is an abundantly found bacteriumin fermenting plant and animal products as well as in system's microbiota. It has various probiotic and antimicrobial properties-produce a protein called bacteriocin, which inhibits the growth of many microorganisms. It can easily be cultured, and characterisation can be performed.Lacticacid bacteria exert a strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens. Production of the primary metabolite lactic acid and the resulting pH decrease is the main preserving factor in food fermentation. Increasing consumer demand for 'natural' and 'additive-free' products has led to greater interest in the application of natural inhibitory substances as foodpreservatives,which could replace or reduce the use of chemical additives.Since only a little is known about this bacterium in general, various advancements in research regarding this particular bacterium is coming up. Exploitation of this bacterium for major applications beneficial for the human race has a promising scope.

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