AN OVERVIEW OF PROBIOTIC LACTOBACILLUS SPECIES

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ABSTRACT

Probiotics plays a vital role in our life. Lactobacillus sp. used as a common probiotic around the world. In many food and fermentation industries lactobacilli used to prevent food from spoilage against a variety of food spoilage bacteria. Lactobacilli have two pathways for fermentation metabolism: homofermentative and heterofermentative. They are classified according to their metabolism and lactic acid isomer form. Lactobacilli are able to inhibit / prevent the growth of many bacteria, moulds, pathogens due to their antibacterial / antifungal / antimicrobial properties. Lactobacillus sp. have beneficial evidence on human health because of preventive / curative effect on various infectious diseases such as gastrointestinal, urogenital infection, diarrhea and even carcinogenesis.

Keywords: probiotic, lactobacillus, fermentation, food spoilage, antibacterial, antifungal

INTRODUCTION

The word 'probiotics' comes from two greek words i.e. 'pro & bios' which means 'for life'. Probiotics means live micro-organisms that exist in the digestive, genital and urinary tract where they make small groups of flora and they must also have beneficial / positive / protective effect on the host **(Gismondo *et al.*, 1999) .** They're also present in a number of consumable fermented milk products, cheeses, yogurts and dietary supplements. Probiotics are a group of food and feed products that are also called as direct fed microbials (DFM) designed to deliver potentially beneficial bacterial cells to the microbiotic ecosystem of humans and other animals. Genera of lactic acid bacteria are the most common microbes employed as probiotics like Lactobacillus, Bifidobacterium, Lactococci, some Enterococci and some Streptococci. In this review we only discuss about the genus lactobacillus. Importantally, Lactobacillus genus is one of the most common probiotics which is largely used in many food and fermentation industries around the world. Each species of Lactobacillus has its own specific properties. Lactobacillus is gram (+), catalase negative, non spore forming, facultative anaerobic or microaerophilic rod-shaped bacteria are generally recognized as safe (GRAS) micro-organisms and play a vital role in food fermentation

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industries belongs to the beneficial and non pathogenic lactic acid bacteria group which are able to produce lactic acid as main end-product of the fermentation of carbohydrates. GC content of DNA base composition of genome of lactobacillus show lower than 54 mol% **(Felis *et al.*, 2007)**. Lactobacilli are components of the normal microflora are found in respiratory, gastrointestinal and genital tracts of humans and animals. They are also found in carbohydrates (food) like fermented dairy products, beverages, fruits, vegetables, meat, plant material and in sewage. Many of lactobacillus species have been isolates from human, animals, food and environmental sources. Lactobacillus genus recently having more than 180 species and included a large variety of organisms. Lactobacillus bacteria are major interest of bacteria because of their inhibitory growth of various food contaminated bacteria. They also have been shown potential effect of antibacterial / antimicrobial / antifungal on selected food spoilage bacteria. Lactobacillus bacteria have many clinical evidence such as its effect on diarrhea, lipid metabolism, and carcinogenesis **(Roos *et al*., 1999) ,** urogenital tract infection **(Reid, 2001).**

METABOLISM

Lactobacilli are chemotrophic in nature so that they utilized energy for their metabolism by the oxidation of chemical compounds. Lactobacilli have two distinct metabolic pathways for fermentation : Homafermentative pathway and heterofermentative pathway.

Homofermentative pathway

By the homofermentative pathway, lactobacilli only produce lactic acid from sugars, especially convert glucose into lactic acid. The homofermentative pathway follows the glycolysis, in this pathway cleavage of fructose 1-6 bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetonephosphate by aldolase, then oxidation of glyceraldehyde 3 phosphate into 1-3 bisphophoglycerate by glyceraldehydes 3 phophodehydrogenase, now phosphoryl group transfer from this 1-3 bisphosphoglycerate to ADP and form 3 phosphoglycerate by phosphoglycerate kinase, and this 3 phosphoglycerate convert in 2 phosphoglycerate by phosphoglycerate mutase then dehydration of 2 phosphoglycerate into phosphoenolpyruvate by enolase and again phosphoryl group transfer from phosphoenolpyruvate to ADP and form pyruvate which is then reduced to lactic acid (Figure 1) **(Axelsson, 1998).**

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Homofermentation in equation form:

1 Hexose + 2ADP + 2Pi $\longrightarrow$ 2 Lactate + 2 ATP

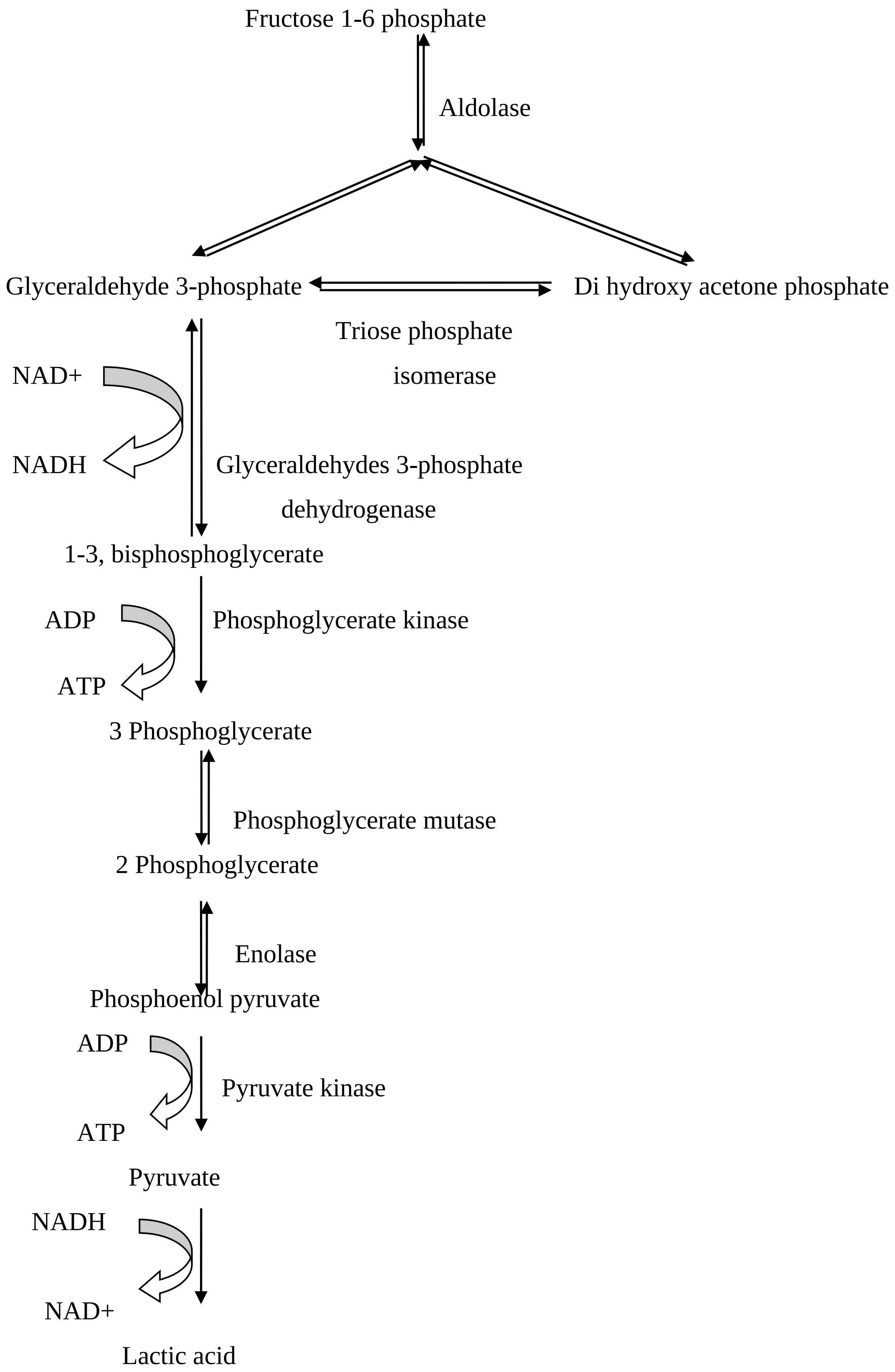

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Figure 1 Flow chart of Homofermentative pathway

Heterofermentative pathway

By heterofermentative pathway lactobacilli convert sugar into alcohol and lactic acid. The heterofermentative pathway follows the pentose phosphate pathway / phosphogluconate pathway / hexose monophosphate pathway.

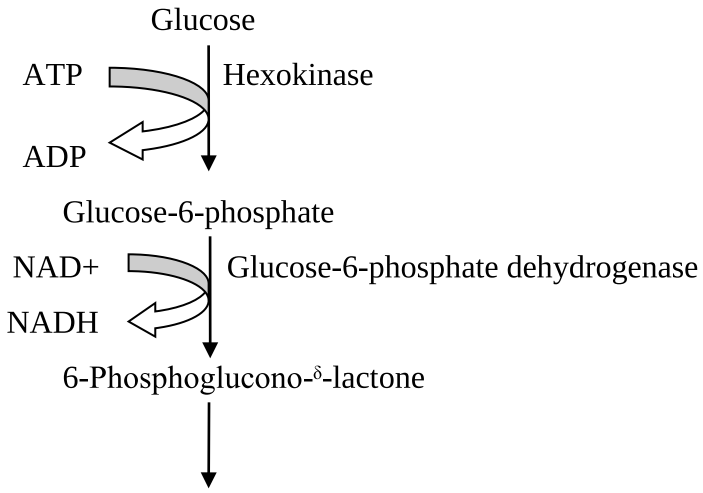
In this pathway glucose is oxidized into D- ribose 5 phosphate. In this process of forming D-ribose 5 phosphate from glucose two oxidation reactions occur : first is 6 phosphoglucono $^{\delta}$ lactone forms from glucose 6 phosphate by dehydrogenase. The location of this glucose 6 phosphate is completely different from the glucose 6-P in the homofermentative pathway. Second is D ribulose 5 phosphate forms from the 6 phosphogluconate by dehydrogenase. In both these oxidation reactions NADPH is generated. (See fig 2). D ribulose 5 phosphate is then epimerized into ribose 5-phosphate or xylulose 5-phosphate. The pentose sugar xylulose 5 phosphate is cleaved into glyceraldehyde 3- phosphate and acetyl phosphate in the presence of phosphoketolase. The glyceraldehyde 3- phosphate is split into lactic acid as same as in the homo fermentative pathway. The acetyl phosphate metabolized either into ethanol or acetic acid it depends on the environmental conditions or ability to use external electron acceptors to regenerate NADH. If lactobacilli have ability to use external electron acceptors to regenerate NADH, so it gains more energy and acetic acid will be formed otherwise ethanol will be formed (Figure 2) **(Axelsson, 1998).**

Heterofermentation in equation form:

1 Hexose + 1 ADP + Pi $\longrightarrow$ Lactate + Ethanol + CO2 + 1 ATP

or (if external e- acceptor)

1 Hexose + 2 ADP + Pi $\longrightarrow$ Lactate + Acetate + CO2 + 2 ATP



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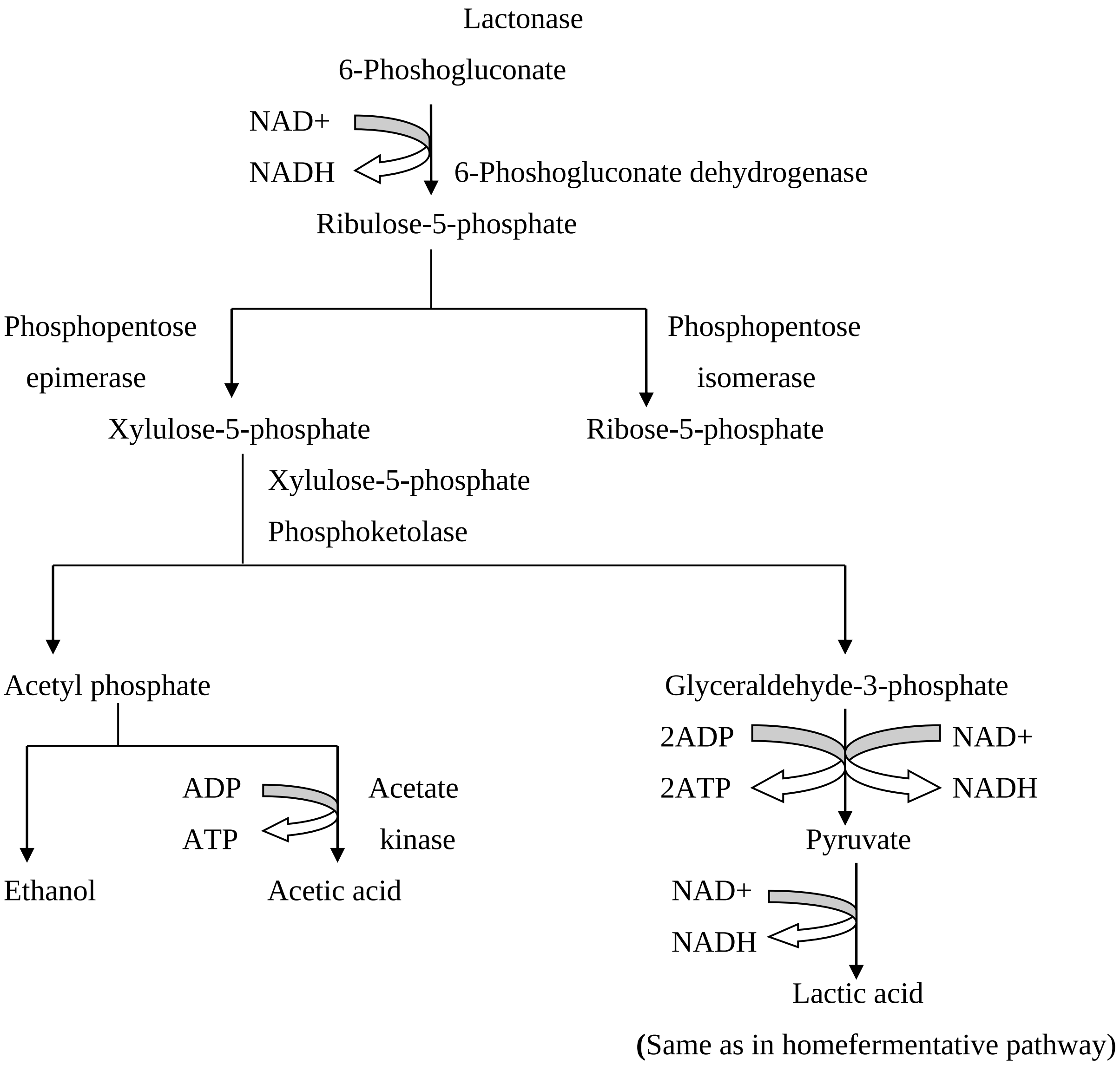

Figure 2 Flow chart of Heterofermentative pathway

CLASSIFICATION

Orla Jenson 1919, had a great work on the classification of lactic acid bacteria (LAB) which is being very useful in the systematic study of LAB. Orla Jenson classified LAB by using some important characters such as morphology, type of glucose fermentation, growth at certain temperature, lactic acid isomers produced through fermentation and range of sugar utilization **(Axelsson, 1998).** The main classification of lactic acid bacteria includes four genera : Lactobacillus, Leuconostoc, Pediococcus and Streptococcus. According to classification of genus lactobacillus belongs to the kingdom : bacteria, phylum : firmicutes, class : bacilli, order : lactobacillales, family : lactobacillaceae.

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Many phenotypic methods have been used for the identification of the lactobacillus. Recently 16S rDNA sequencing have been used for the determination of individual strain of lactobacillus more accurately and simply **(Schleifer *et al*., 1995).** More recently, genetic techniques such as RAPD fingerprinting and POPGENE analysis have been used in the classification of lactobacillus species **(Tafvizi *et al.*, 2012).**

According to metabolism

On the basis of metabolism lactobacilli classified into three categories:

Obligately homofermentative lactobacilli (Group I) : In this group those lactobacilli are include which ferment only hexoses into lactic acid with the help of Embden–Meyerhof–Parnas (EMP) / glycolysis pathway while not able to ferment pentoses and gluconate because they lack of phophoketolase.

Facultatively heterofermentative lactobacilli (Group II) : In this group those lactobacilli are include which ferment hexoses into lactic acid with the help of Embden–Meyerhof–Parnas (EMP) / glycolysis pathway and also ferment pentoses and sometimes gluconate because they have aldolase and phosphoketolase.

Obligately heterofermentative lactobacilli (Group III) : In this group those lactobacilli are include which ferment both hexoses and pentoses with the help of phosphogluconate pathway. By the fermentation of hexoses with the help of phosphogluconate pathway lactate, ethanol or acetic acid and CO2 are produced.

Table 1 classification on the basis of metabolism

Obligately homefermentative lactobacillus sp.(Group I)	Facultatively heterofermentative lactobacillus sp. (Group II)	Obligately heterofermentative lactobacilli (Group III)
L. acidophilus (Moro 1900) Hansen and Mocquot 1970	*L. acetotolerans* Entani *et al*. 1986	*L. acidifarinae* Vancanneyt *et al*. 2005
L.vitulinus Sharpe *et al*. 1973	*L. algidus* Kato *et al*.2000	*L. antri* Roos *et al*., 2005
L. amylophilus Nakamura and Crowell 1981	*L. fuchuensis* Sakala *et al*. 2002	*L. brevis* (Orla-Jensen 1919) Bergey *et al*. 1934
L. amylotrophicus Naser *et*	*L. acidipiscis* Tanasupawat *et*	*L. buchneri* (Henneberg

al. 2006	*al*. 2000	1903) Bergey *et al*. 1923
L. amylovorus Nakamura 1981	*L. alimentarius* Reuter 1983	*L. collinoides* Carr and Davies 1972
L. animalis Dent and Williams 1983	*L. apodemi* Osawa *et al*. 2006	*L. diolivorans* Krooneman *et al*. 2002
L. aviarius subsp. *araffinosus and aviarius* Fujisawa *et al*. 1986	*L. bifermentans* Kandler *et al*. 1983	*L. fermentum* Beijerinck 1901 emend. Dellaglio *et al*. 2004
L. catenaformis (Eggerth 1935) Moore and Holdeman 1970	*L. casei* (Orla-Jensen 1916) Hansen and Lessel 1971	*L. fructivorans* Charlton *et al*. 1934
L. concavus Tong and Dong 2005	*L. coleohominis* Nikolaitchouk *et al*. 2001	*L. frumenti* Müller *et al*. 2000
L. crispatus (Brygoo and Aladame 1953) Moore and Holdeman 1970	*L. composti* Endo and Okada 2007	*L. gastricus* Roos *et al*., 2005
L. delbrueckii subsp. *bulgaricus* (Orla-Jensen 1919) Weiss *et al*. 1984	*L. coryniformis* subsp. *coryniformis and torquens* Abo-Elnaga and Kandler 1965	*L. hilgardii* Douglas and Cruess 1936
L. iners Falsen *et al*. 1999	*L. farraginis* Endo and Okada 2007	*L. ingluviei* Baele *et al*. 2003
L. delbrueckii subsp. *indicus* Dellaglio *et al*., 2005	*L. curvatus* (Troili- Petersson 1903) Abo-Elnaga and Kandler 1965 emend. Klein *et al*. 1996	*L. kefiri* Kandler and Kunath 1983
L. delbrueckii subsp. *lactis* (Orla-Jensen 1919) Weiss *et al*. 1984	*L. fornicalis* Dicks *et al*. 2000	*L. malefermentans* Farrow *et al*. 1989
L. equi Morotomi *et al*. 2002	*L. agilis* Weiss *et al*. 1982	*L. lindneri* Back *et al*. 1997
L. farciminis Reuter 1983	*L. graminis* Beck *et al*. 1989	*L. mucosae* Roos *et al*. 2000
L. gallinarum Fujisawa *et al*.	*L. hammesii* Valcheva *et al*.	*L.kunkeei* Edwards *et al*.

1992	2005	1998
L. gasseri Lauer and Kandler 1980	*L. hamsteri* Mitsuoka and Fujisawa 1988	*L. namurensis* Scheirlinck *et al*. 2007
L. helveticus (Orla-Jensen 1919) Bergey *et al*. 1925	*L. harbinensis* Miyamoto *et al*. 2006	*L. oligofermentans* Koort *et al*. 2005
L. delbrueckii subsp. *delbrueckii* (Leichmann 1896) Beijerinck 1901	*L. homohiochii* Kitahara *et al*. 1957	*L. oris* Farrow and Collins 1988
L. johnsonii Fujisawa *et al*. 1992	*L. intestinalis* (ex Hemme 1974) Fujisawa *et al*. 1990	*Lactobacillus panis* Wiese *et al*. 1996
L. kalixensis Roos *et al*., 2005	*L. jensenii* Gasser *et al*. 1970	*L. parabrevis* Vancanneyt *et al*. 2006
L. kefiranofaciens subsp. *kefiranofaciens* Fujisawa *et al*. 1988	*L. kimchii* Yoon *et al*. 2000	*L.parabuchneri* Farrow *et al*. 1989
L. kefiranofaciens subsp. *kefirgranum* (Takizawa *et al*. 1994 Vancanneyt *et al*. 2004)	*L.kitasatonis* Mukai *et al*. 2003	*L.paracollinoides* Suzuki *et al*. 2004
L. mali Carr and Davies 1970, emend. Kaneuchi *et al*., 1998	*L. murinus* Hemme *et al*. 1982	*L. parakefiri* Takizawa *et al*. 1994
L. manihotivorans Morlon-Guyot *et al*. 1998	*L. nantensis* Valcheva *et al*. 2006	*L. pontis* Vogel *et al*. 1994
L. mindensis Ehrmann *et al*. 2003	*L. paracasei* subsp. *paracasei and tolerans* Collins *et al*. 1989	*L. sanfranciscensis* Weiss and Schillinger 1984
L. nagelii Edwards *et al*. 2000	*L. parafarraginis* Endo and Okada 2007	*L. reuteri* Kandler *et al*. 1982
L. pantheris Liu and Dong 2002	*L. paralimentarius* Cai *et al*. 1999	*L. rossiae* Corsetti *et al*. 2005
L. ruminis Sharpe *et al*. 1973	*L. paraplantarum* Curk *et al*. 1996	*L. psittaci* Lawson *et al*. 2001

L. saerimneri Pedersen and Roos, 2004	*L. pentosus* Zanoni *et al*. 1987	*L. siliginis* Aslam *et al*. 2006
L. salivarius Rogosa *et al*. 1953 emend. Li *et al*. 2006	*L. perolens* Back *et al*. 2000	*L. suebicus* Kleynmans *et al*. 1989
L. satsumensis Endo and Okada, 2005	*L. plantarum* (Orla-Jensen 1919) Bergey *et al*. 1923	*L. vaccinostercus* Kozaki and Okada 1983
L. sharpeae Weiss *et al*. 1982	*L. plantarum* subsp. *argentoratensis* Bringel *et al*. 2005	*L.vaginalis* Embley *et al*. 1989
L. ultunensis Roos *et al*., 2005	*L. rennini* Chenoll *et al*. 2006	*L. zymae* Vancanneyt *et al*. 2005
L. versmoldensis Kröckel *et al*. 2003	*L. rhamnosus* (Hansen 1968) Collins *et al*. 1989	
L. amylolyticus Bohak *et al*. 1999	*L. sakei* subsp. *carnosus* Torriani *et al*. 1996	
	Lactobacillus zeae Dicks *et al*. 1996	
	Lactobacillus secaliphilus Ehrmann *et al.* 2007	
	Lactobacillus sobrius Konstantinov *et al*. 2006	
	Lactobacillus spicheri Meroth *et al*. 2004	
	Lactobacillus vini Rodas *et al*. 2006	
	Lactobacillus sakei subsp. *sakei* Katagiri *et al*. 1934 emend. Klein *et al*. 1996	

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According to lactic acid isomer forms

On the basis of lactic acid isomer production through fermentation lactobacilli are classified into three categories:

D lactic acid isomer form lactobacilli : These type of lactobacilli produced lactic acid in which hydroxyl group is on the chiral centre at right side in a projection formula that has carboxylic group at the top.

L lactic acid isomer form lactobacilli : These type of lactobacilli produced lactic acid in which hydroxyl group is on the chiral centre at left side in a projection formula that has carboxylic group at the top.

DL lactic acid isomer form lactobacilli : These type of lactobacilli can produced both forms of

PROPERTIES OF LACTOBACILLUS SPECIES

Lactobacilli have many properties of antibacterial, antifungal, antimicrobial. There have been many researches done on effect / activities / properties of lactobacillus sp. that shows potential control of a variety of food spoilage bacteria, moulds, fungus and also pathogens that leads to cause many diseases and (or) infections like diarrhea, gastrointestinal and urogenital infections. Lactobacilus sp. can be isolated from different different things and organisms also.

Antibacterial property of lactobacillus sp.

As we discuss lactobacillus sp. has antibacterial properties and a lot of research work have been done on the effect of lactobacillus sp. on large number of bacteria that causes diseases and food spoilage. Authochlorous lactobacillus sp. isolated from traditional yogurts for their antibacterial effect (**Ebtehaj *et al.*, 2009**) . Bacterocin producing Lactobacillus sp., isolated from traditional milk products such as Curd, Cheese, Butter, Milkpeda and Ghee for their antibacterial activity **(Arokiyamary *et al.*, 2011).** Four lactobacillus strains (Lactobacillus salivarius CECT5713, Lactobacillus gasseri CECT5714, L. gasseri CECT5715 and Lactobacillus fermentum CECT5716) isolated from fresh human breast milk for antimicrobial potential and this milk was evaluated and compared with a reuterin-producing strain Lactobacillus coryniformis CECT5711 from an artisan goat's cheese **(Olivares *et al* , 2005).** L. acidophilus CYC 10051 and L. kefiranofaciens CYC 10058 isolated from kefir for in vitro antimicrobial characteristics **(Santos *et al.*, 2003).** L. casei shows in vitro antibacterial activity against various enteropathogenic bacteria **(Amdekar *et al.*, 2010).** Lactobacillus spp. JK-8 and JK-11 has been shown potential of removing pathogenic bacteria and N2 **(Ma *et***

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***al.*, 2008).** Many Lactobacillus sp. Isolated from Goat's Milk shows antimicrobial activity against *Staphylococcus aureus* **(Anas *et al.*, 2008).**

Antifungal property of lactobacillus sp.

Lactobacillus sp. has antifungal properties also because of producing antifungal metabolites. Lactobacilli are able to control mycotoxinogenic mould growth such as Aspergillus, Fusarium and Penicillium. There are many factors have been considered which effects the antifungal activity of Lactobacilli including temperature, growth medium, incubation time, pH and nutritional values **(Dalie *et al*., 2009).** Lactobacilli produces numerous antifungal compounds including fermented products such as organic acids (lactic acid , acetic acid) and other end products; proteinaceous compounds such as bacteriocins and low molecular weight compounds such as reuterin, hydroxyl fatty acids, hydrogen peroxide, phenolic compounds, cyclic dipeptides **(Magnusson, 2003).** Bacteriocins of lactobacillus generally divided into three categories: class I includes lantibiotics, class II includes heat stable unmodified bacteriocins, class III includes larger heat stable bacteriocins **(Nes, 1996; Nes & Holo, 2000).** Lactic acid reduced the growth of *Aspergillus parasiticus* **(Reiss, 1976).** Antimicrobial peptide nisin produced by a strain of *Lactococcus lactis* **(Hirsch *et al.*, 1951).** Sodium acetate was involved in the inhibitory action of *Lactobacillus rahmnosus* against several moulds includes Penicillium sp., Aspergillus sp., Fusarium sp., Alternaria sp. **(Stiles *et al.*, 2002).** Antifungal effect of PentocinTV35b from L. pentosus against Candida albicans has been reported **(Okkers *et al.*, 1999).**

CLINICAL EVIDENCE OF USEFULNES

The challenges of control and treatment of infectious diseases includes the development of antibiotic resistance, increased intensity of opportunistic infections in immunocompromised patients and emergence of new type of pathogens, results increase antibiotic exposure in the population which produces resist strains of pathogens. There is requirement to develop new therapies or methodologies or techniques to control, prevent and treat the infectious diseases. Probiotics have preventive as well as curative effects on various types of infectious diseases includes urinary tract infection, gastrointestinal infection, genital tract infection and this is called 'bacteriotherapy'. Many research work have been done on effects of probiotics on human health. In these researches probiotic Lactobacillus sp. shows curative and preventive potential on many infectious diseases includes diarrhea, lipid metabolism, and even carcinogenesis **(Nicole *et al.*, 1999).** Michael well reviewed the

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preventive effect of probiotics on several types of diarrhea includes osmotic diarrhea, secretory diarrhea, inflammatory diarrhea, motility associated diarrhea **(Vrese *et al.*, 2007).** Effect of lactobacillus sp. on immune system has also been reported **(Herich *et al.*, 2002).** Lactobacilli have also been useful to protect urogenital tract infection **(Gregor, 2001).**

CONCLUSION

Lactobacillus sp. have enormous benefits in food fermentation industries and human health. It plays important role in food industries as it prevents food from spoilage against many sensitive food spoilage bacteria and pathogens as well as in the preservation of food also. It actively takes part in the production of beverages such as wine (red, white and sparkling) production and juices production. Lactobacillus bacteria have great evidence in clinical area as it displays inhibitory / preventive action against several bacteria and pathogen causes infectious diseases. Thus, Lactobacillus bacteria as probiotics are perfectly advantageous for our environment and health.

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