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REVIEW ARTICLE

Lactic Acid Bacteria and its Antimicrobial Properties: A Review

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ABSTRACT

Fermented food products being rich source of nutrients for human, can also serve as a good medium for the growth and multiplication of microorganisms. The most lactic acid bacteria (LAB) inhibit pathogenic, non-pathogenic and spoilage organism in fermenting foods and beverages. Bioactive compounds from plant by-products act as good preservatives. Antioxidants poly phenolic fraction from plant by-product are possible alternatives to synthetic antimicrobial agent can be easily degraded by living organisms. They are based on renewable raw materials (Protein, oils) and constitute eco-friendly alternatives to synthetic antimicrobial surfactants. Some of the microbes are best sources of bio-active compounds as they synthesize as secondary metabolites for their self defense against other competitive microorganisms. Lactic acid bacteria have been used successfully in all fermented food products. The literature shows that the "Microbial fermentation and production of fermented food products" are briefly reviewed in this paper.

Key words: Lactic acid bacteria, Fermentation, Bacteriocins and Antimicrobial activity.

1. INTRODUCTION

Lactic acid bacteria have been used for thousands of years in food and alcoholic fermentations. Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations. The bacteriocins from the lactic acid bacterial isolates generally recognized as safe (GRAS) lactic acid bacteria (LAB) have arisen a great deal of attention as a novel approach to control pathogens in food-stuffs. Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram negative bacteria. The antimicrobial effect of lactic acid bacteria has been appreciated by man for more than 10000 years and has enabled him to extend the shelf life of many foods through fermentation processes [1].
Lactic acid bacteria have been used in food fermentations for more than 4000 years. It is important to acknowledge that the widespread term "Lactic acid bacteria" have no official status in taxonomy and is only a general term of convenience used to describe the group of functionally and genetically related bacteria. Lactic acid bacteria consist of bacterial genera within the *Firmicutes* comprised of about 20 genera. The main members of the Lactic acid bacteria are genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Carnobacterium*, *Aerococcus*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella*. *Lactobacillus* is the largest genus of this group, comprising around 80 recognized species. Lactic acid bacteria have a long history of use in a variety of cereal fermentations, especially in the manufacture of baked goods. It has been reported that around 50 different species of Lactic acid bacteria have been isolated from sourdough. *Lactobacillus* strains are the most frequently observed bacteria in this matrix, but the species belonging to the genera *Leuconostoc*, *Weissella*, *Pediococcus*, *Lactococcus* or *Enterococcus* have been isolated as well [2, 3].

The ability of the Lactic acid bacteria to prevent and cure a variety of diseases has lead to the coining of the term probiotics or pro-life. The most important role of lactic acid bacteria is its protective role against infections and colonization

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of pathogenic microorganisms in the digestive track. In most of the cases inoculums passively transits the gastrointestinal track. The probiotics can influence the unspecific immunity, which consists of T- lymphocytes and B-lymphocytes. The increase in the specific immune response corresponds with the activity of B and T-lymphocytes, which leads to an increase of interleukin and the level of circulating antibodies [2].

A major development in the distribution and storage of food came in 1940 with the availability of low cost home refrigerator and freezers. Other development includes the artificial; drying, vacuum packing, ionizing radiation, chemical preservation etc. nowadays consumers are concerned about the synthetic chemicals used as preservative in food. Moreover the side effects like cancer, cardiovascular diseases and aging pore a major threat to consumers. Despites, improved manufacturing facilities and implementations of effective process control procedures such as HACCP (Hazards Analysis and Critical Control Point) in the food industries, these number of food borne illness is in increased trend. Modern day consumers are more favorable to preservatives of natural origin than that of chemical origin [3].

The lactic acid bacteria (LAB) comprise a clade of Gram positive, acid tolerant, non-sporulation, non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics. These bacteria are usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end product of carbohydrate fermentation. This trait has historically linked LAB with food fermentation as acidification inhibits the growth of spoilage agents. The LAB group comprises the genera *Lactobacillus, Streptococcus, Lactococus, Leuconostoc, Pediococcus, Aerococcus, Alloicoccus, Dolosigranulum, Enterococcus, Globicatella, Lactospaera, Oenococcus, Carnobacterium, Tetragenococcus, Vagoccus* and *Weissella*. Historically, the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* form the core of the LAB group.

Members of these genera *Lactobacillus* plays an essential role in the fermentation of food and feed. The most important characteristics of the lactic acid bacteria are their ability to ferment sugars to lactic acid. This may desirable in making products and these organisms have been isolated and screened by using fermented foods such as curd, buttermilk, cheese and yoghurt. Different antimicrobials such as lactic acid, acetic acid, hydrogen peroxide, carbon-di-oxide and bacteriocins produced by these bacteria can inhibit pathogenic and spoilage microorganisms extending the shelf-life and enhancing the safety of food products.

There are many potential applications of protective cultures in various food systems. These organisms have been isolated from grains, dairy and meat products, fermenting vegetables and the mucosal surface of animals. The lactic acid bacteria not only have their effect on food and flavor but they are also known to produce and excrete compounds with antimicrobial activity, such as bacteriocins. Bacteriocins of LAB are considered as safe natural preservatives or biopreservatives as it is assumed that they are degraded by the proteases in gastrointestinal tract.

Bacteriocins are generally defined as extracellular released peptide or protein that shows a bactericidal activity against more distantly related species. The inhibitory spectrum of some bacteriocins also include food spoilage and for food borne pathogenic microorganisms. The discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative dates back of to the first half of last century but research on bacteriocin of lactic acid bacteria has expanded in the last two decades, searching for novel bacteriocin producing strains from dairy, meat and plant products, as well as traditional fermented products. Among the Gram positive bacteria, bacteriocins produced by many lactic acid bacteria used in food fermentation and dairy products, including strains in the genera *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc*.

2. LACTIC ACID BACTERIA

Lactic acid bacteria were first discovered by Scheele [4] from sour milk. Pasteur discovered in 1857, that the souring of milk was caused by the microorganisms. Lactic acid was first produced commercially by M/s Clinton processing company, USA. Lactic acid bacteria have been widely used for the fermentation of many fermented product such as cheese, sourdough, buttermilk, brined vegetables, yoghurt and sauerkraut [5].

Lactic acid bacteria are widespread in nature and predominant microflora of milk and its products. Lactic acid bacteria are one of the important groups of microorganisms in food fermentation. A wide variety of strains are routinely used as starter cultures to manufacture dairy products such as curd, cheese, whey and yoghurt [6, 7]. These bacteria produce organic acid hydrogen peroxide and several enzymes during fermentation [8-10]. These compounds not only contribute to desirable effect on food flavor and texture, but also inhibit undesirable microflora and extending shelf life of products [11]. Growth of spoilage and pathogenic bacteria in the fermented foods were inhibited due to the production of antimicrobial substances by lactic acid bacteria as their competition for nutrients [12].

3. FERMENTATION BY LACTIC ACID BACTERIA – HOMOFERMENTATION & HETEROFEREMNTATION

The homofermentative rod shaped lactic acid bacteria with optimum temperature around 40°C (104°F) as thermobacterium, where as those with optimum temperature near 30°C (86°F) were *Streptobacteria* [13]. Homofermentative bacteria such as *Lactococcus* and *Streptococcus* yield two lactates from one glucose molecule where as heterofermentative (*Leuconostoc* and *Wiessella*) transform a glucose molecule into lactate, ethanol and carbondioxide [14].

Toshinobs [15] reported that homofermentative they produced appreciable amounts of volatile products including alcohol, in addition to lactic acid. The homofermentative *Lactobacillus* with optimal temperature of 35°C or above include *Lactobacillus bulgaricus, Lactobacillus helviticus, Lactobacillus delbrueckii, Lactobacillus acidophilus*, *Lactobacillus thermophilus* and *Lactobacillus fermentum* is the chief of a heterofermented *Lactobacillus* growing well at high temperature.

4. TAXONOMICAL CLASSIFICATION OF LACTIC ACID BACTERIA

Lactic acid bacteria consist of a number of bacterial genera within the phylum fumicutes. The genera *Enterococcus, Lactobacillus, Lactococcus, Lactosphera, Leuconostoc, Milissococcus, Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* are recognized as lactic acid bacteria [16]. Lactic acid bacteria is a non sporulating, catalase negative dvoid of cytochromes, but aerotolerent, fastidious, acid tolerant and ferment carbohydrates into energy and lactic acid depending on the organism, metabolic pathways differ when glucose is the main carbon source [17].

5. GENERAL POSITION OF SPECIFIC LACTIC ACID BACTERIA

5.1. *Lactobacillus*

The characteristics of *Lactobacillus* are rods, usually long and slender, that forms chains in most species. They are microaerophilic, but some are strict anaerobe is knowing, catalase-negative and Gram positive, and they ferments sugars to yield lactic acid as the main product. They ferment sugars chiefly to lactic acid if they are homofermentative, with small amount of acetic acid, carbon-di-oxide and trace products, if they are heterofermentative, they produce appreciable amounts of volatile products, including alcohol, in addition to lactic acid. Most species of this non spore forming bacterium ferment glucose into lactate hence the name *Lactobacillus* is industrial production of fermented food production of fermented food products [18].

5.2. *Streptococcus*

The characteristics of cocci occur in pairs, short chains or in long chain, depending upon the species and the conditions of growth and all are homofermentative. *Streptococcus* lactic grows well in milk and ferment the lactose to 0.8 to 1.0 percent acid of which L(+) lactic acid constitutes nearly all of the acid formed, although traces of acetic and propionic acid may be present. Optimum temperature of 30°C and a temperature range of 10°C to 40°C were required [19].

5.3. *Pediococcus*

The characteristics of cocci occur in single, in pairs or in short chains or in tetrads and are Gram positive, catalase - negative and microaerophilic. They are homofermentative, fermenting sugars to yield 0.5 to 0.9 percent acid, mostly lactic and they grow fairly well in salt brines upto 5.5 percent and poorly in concentrations of salt upto about ten percent. They grow in the temperature 45°C but the best in 32°C. *Pediococcus* have been found growing, during the fermentation of brined vegetables [20].

6. OCCURRENCE OF LACTIC ACID BACTERIA IN NATURE

Lactic acid bacteria was first isolated from milk [21-24] had isolated lactic acid bacteria from curd which were tested for inhibitory activity against psychrophilic pathogens *via*, *Escherichia coli, Staphylococcus aureus, Lactobacillus plantarum* had been isolated from various habitats *via*., cheese and milk [25, 26]. Wild lactic acid bacteria strains were isolated from Egyptian raw milk and were screened based on their yield of biomass production during fermentation process [27]. Eight stains of lactic acid bacteria were isolated from brukino faso fermented milk sample [28]. The isolated *Lactobacillus plantarum* strain from boza, a traditional drink produced by fermentation of different cereals with yeast and lactic acid bacteria [29]. Salim Ammor *et al*. [30] reported the occurrence of lactic acid bacteria (36 *Lactobacillus* sake, 22 *Enterococcus faecium*, 16 *Lactobacillus graviae*, 11 *Vagococcus caniphilus* and 2 *Enterococus* sp.).

7. ANTIMICROBIAL PROPERTIES OF LACTIC ACID BACTERIA

Tag *et al*. [31] reported that the difference in the spectrum of inhibition of pathogenic microorganism by the cell free culture supernatants of the *Lactobacillus* isolates might be due to the activity of particular antibacterial substance synthesized by the organism and partly due to the presence of appropriate receptor sites in the cell wall of the susceptible organisms. The *Lactobacillus* culture filtrates showed an increased trend in inhibition of all the test organisms, from 24 to 72 hours of incubation. The antimicrobial activity of all the *Lactobacillus* was found to decrease significantly at 96 hours incubation against the test organism *Staphylococcus aureus* [32, 33].

The most bacteriocins produced by LAB appear to have relatively narrow inhibitory spectrum. Some bacteriocins such as nisin and pediocin are active against a wide range of bacterial spectra. In general, bacteriocins are sub-divided into 3 classes based on their mode of action and their structure. Class I bacteriocins including lanthionine as such in nisin, class II bacteriocins of small, heat-stable, non-lanthionine containing peptides and Class III bacteriocins of relatively large molecular weight and heat stability was suggested [34].

The antimicrobial effect by lactic acid bacteria was the production of lactic acid and reduction of pH. In addition of lactic acid produced, various antimicrobial compounds which can be classified as low molecular mass (LMM) compounds such as hydrogen peroxide, carbon-di-oxide, di-acety (2,3-butanoide), uncharacterized compounds like bacteriocins are produced by lactic acid bacteria was reported [35]. Gilliland and Walker [36] reported the factors that have to be considered when selecting a culture of *Lactobacillus acidophilus* as a probiotic several criteria include biosafety aspects, the methods of administering the probiotic, the location in the body where the microorganisms of the probiotic product must be active, survival and colonization in the host and the tolerance for bile.

Blazeka Suskovic and Matosic [37] reported that nine strains lactic acid bacteria are commonly used as started cultures for the dairy industry and ensiling six *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Streptococcus lactis* and *Streptococcus faecium* had antimicrobial activity. The most sensitive strains are *Staphylococcus aureus* was used as a target microorganism. The cultures of *Streptococcus faecium* and *Lactobacillus plantarum* gave the most intense antimicrobial activity by adding $CaCO_3$ to the medium (to bind accumulated lactic acid) increased the antibiotic activity of the lactic acid bacteria.

Biswas *et al*. [38] proved that Lactococcin produced by *Lactococcus lactis* reached a maximum activity at early stationary phase. The highest production was obtained in MRS broth at 6.5-7.0 initial pH values, 30°C temperature and 18-24 hours incubation time. Mortvedt *et al*. [39] demonstrated the purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus* sake L_{45}. It has been purified by ion exchange, hydrophobic interaction, reverse phase chromatography and gel filtration. Lactocin S contained approximately 33 amino acid residues, of which about 50% were non polar amino acids alanine, valine and leucine.

Bacteriocin producers have attracted considerable interest in recent years and several works have focused on the isolation and development of new strains of bacteriocin-producing bacteria. The detection rate of bacteriocin producing strains from LAB isolates can be as low as 0.2% and therefore needs a large number of isolates from food sources [40]. Chauviere *et al*. [41] studied the antibacterial effect of the adhering human *Lactobacillus acidophilus* strain. The strain produces an antibacterial activity against a wide range of Gram negative and Gram positive pathogens. In contrast, it did not inhibit *Lactobacilli* and *Bifidobacteria*.

Marie Helene Cocoonier *et al*. [42] observed the antimicrobial effect of the adhering human *Lactobacillus acidophilus* strain against a wide range of Gram negative and Gram positive pathogen like *Staphylococcus aureus*, *Salmonella typhimurium*, *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas aeruginosa*. Farida Khalid *et al*. [43] isolated a new bacteriocin, lactocin LC-90, isolated from a clinical sample was inhibitory against many species of *Lactobacilli* and other

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Gram positive bacteria. The bacteriocin was inactivated by protease treatment. Curing of LC-90 with acridine orange, ethidium bromide and by elevated temperature resulted in mutants defective in bacteriocin production.

Rongguang Yang and Yanling [44] reported the cell growth of *Lactobacillus acidophilus* was higher than *Pediococcus acidilactici* in dairy based media and the low level of multiplication of *Pediococcus acidilactici* was due to inability or poor ability to utilize lactose. *Lactobacillus acidophilus* is lactose fermenters. Maximum production of bacteriocin was observed after 8 hours of incubation for both *Lactobacillus acidophilus* and *Pediococcus acidilactici*, *Lactobacillus acidophilus* showed maximum bacteriocin activity than the *Pediococcus acidilactici*.

Lactic acid bacteria (LAB) are a group of catalase negative, Gram positive bacteria which have played a very important role in fermentation of food. They often produce a variety of antimicrobial compounds including lactic acid and acetic acids, alcohol, aldehyde and bacteriocins. Among them, bacteriocins have attracted a great interest in food industry due to their potentiality in food preservation [45]. Elilzete de Raque *et al*. [46] performed the isolation, identification and physiological study of *Lactobacillus* ferment for use as probiotic in chickens. Selection of strains included various criteria such as agreement with bio safety, viability during storage, tolerance to low pH/gastric juice, bile and antimicrobial activity. Cleveland *et al*. [47] discovered nisin, the first bacteriocin used on a commercial scale as a food preservative. Research on bacteriocins of LAB has expanded in the last two decades, searching for novel bacteriocin producing strains from dairy, meat and plant products as well as traditional fermented products.

Many bacteriocins of LAB are safe and effective natural inhibitors of pathogenic and food spoilage bacteria. Nisin is the classical example of a commercially successful, naturally produced inhibitory agent [48]. Adesogan *et al*. [49] studied the effect of *Lactobacillus buchneri, Lactobacillus fermentum, Leuconostoc mesenteroides* inoculants or a chemical additive on the fermentation, aerobic stability and nutritive value of crimped wheat grains. The ability of the inoculants was compared and in conclusion, bacterial inoculants containing *Lactobacillus buchneri* are promising preservatives for crimped wheat grains.

Yukio Yamamoto *et al*. [50] demonstrated the purification of bacteriocin produced by *Enterococcus faecalis*. LAB *Enterococcus faecalis* isolated from rice bran exhibited a wide spectrum of growth inhibition in various Gram positive bacteria. A bacteriocin purified from culture fluid was heat stable and was not sensitive to acid and alkali, but it was sensitive to several proteolytic enzymes. Girum Tadesse *et al*. [51] *Lactobacilli* isolates were separately grown in MRS and LAPTg (Lactose propyl thiogalactosidase) broth and their antimicrobial activity was tested against the test strains using the well diffusion method. All isolated, except *Escherichia coli* showed additional 3 to 4 mm of inhibition zone. This was <3 mm for *Escherichia coli*. *Lactobacillus* isolates were the inhibitor to the test strains followed by *Pediococcus*, *Streptococcus* and *Leuconostoc*. *Eshcerichia coli* was the least sensitive in all cases.

Nowroozi *et al*. [52] reported that lactic acid bacteria isolated from sausage had antibacterial activity and it was done by an agar spot, well diffusion and blank disc method. The antibacterial activity was stable at 100°C for 10 minutes and at 56°C for 30 minutes but actively was lost after autoclaving. The maximum production of plantaricin was obtained at 25 - 30°C at pH 6.5. Anne Vaughan *et al*. [53] investigated the antimicrobial efficacy of a bacteriocin for the development of microbiologically stable beer. *Lactococcus lactis* was shown to produce the antimicrobial activity during growth under specific conditions. The capacity of the bacteriocin to prevent microbial spoilage of bacteriocin containing beer at 30°C or room temperature resulted in antimicrobial activity.

Aly Savadogo *et al*. [54] isolated eighty strains of lactic acid bacteria producing bacteriocin were isolated from Burkina Faso fermented milk samples. These strains were identified to species *Lactobacillus fermentum, Pediococcus* sp., *Leuconostoc mesenteroides* and *Lactococcus.* Isolated bacteriocin exhibited antibacterial activity against *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* using the agar drop diffusion test. The inhibition diameters obtained with bacteriocin are between 8 mm and 12 mm. Gram positive indicator bacteria were most inhibited.

Eva Rodriguez *et al*. [55] demonstrated the combined effect of high pressure treatments and bacteriocin producing lactic acid bacteria on inactivation of *Escherichia coli* in raw milk

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cheese, pasteurization and addition of bacteriocin such as nisin, enterocin produced by LAB completely inactivated *Escherichia coli*. Strompfova *et al*. [56] studied the effect of probiotic strain *Lactobacillus fermentum* on Japanese quail. The results demonstrated that the 4 day application of this strain significantly increased the population of lactic acid bacteria like *Lactobacilli* and *Enterococci* and decreased the counts of *Escherichia coli* in faeces. The index of phagocytic activity of leucocytes was significantly improved.

Dickson *et al*. [57] developed a species – specific PCR assay for identifying *Lactobacillus fermentum*. PCR primers specific for *Lactobacillus* were identified by alignment of bacterial 16S rRNA genes and selection of sequences specific for *Lactobacillus fermentum* at their 3´ ends. This PCR assay provides a more rapid, specific and sensitive alternative to conventional culture methods for the identification of *Lactobacillus fermentum.* Padmanabha Reddy *et al*. [58] selected the *Lactobacillus acidophilus* cultures based on their antibacterial activity against *Shigella dysenteriae*, *Escherichia coli*, *Salmonella typhi*, *Yersinia enterocolitica* using agar well assay technique for determining their possible use as dietary adjutants in probiotics dairy products.

Ogunshe *et al*. [59] isolated 50 bacteriocin producing *Lactobacillus* strains from some Nigerian indigenous fermented foods and beverages and characterized as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus lactis* and *Lactobacillus plantarum* were screened for these inhibitory potentials against food borne pathogenic from the same or similar to fermented food sources and against clinical indicator bacterial isolates. The survival rates of the pathogenic indicator bacteria in the fermented food sources were between 8 and 14 days while the clinical isolates survived in simulated fermented food samples between 5 and 9 days.

Lue De Vuyst and Frederic Leroy [60] described the production, purification and food applications of bacteriocins from lactic acid bacteria. In fermented foods, lactic acid bacteria display numerous antimicrobial activities due to the production of organic acids and other compounds such as bacteriocins. Michael Baker Diop *et al*. [61] evaluated that bacteriocin produced by *Lactobacillus lactis* and *Enterococcus faecium* show antimicrobial activity against *Listeria monocytogenes* and *Bacillus coagulans* whereas only that produced by *Lactococcus lactis* has an activity against *Bacillus cereus*. Bacteriocin producing *Lactococcus lactis* strains were found in variety of traditional foods indicating a high potential of growth of this strain in variable ecological complex environment and has been selected for application in food preservation.

Adetunji and Adegoke [62] demonstrated bacteriocin and cellulose production by lactic acid bacteria isolated from West African soft cheese. All the LAB used in this study produced cellulose. The correlation between cellulose productions and bacterial growth was highly significant after 72 hours of incubation. The bacteriocin produced by the strains could be good for biopreservation. Mechai Adbelbasset and Kirane Djamila [63] isolated twenty samples of traditional milk “Raib” were collected in eastern Algeria from individual household. From 13 of these samples 52 strains of Lactic acid bacteria were isolated and shown to exhibit inhibitory activity against the indicator strain *Listeria monocytogenes*.

Adesokan *et al*. [64] isolated seven species of lactic acid bacteria (LAB) namely *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus delbureckii*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus brevis* and *Leuconostoc mesenteroides* were isolated from ogi, burukutu and retted cassava. The isolates were screened for quantitative production of lactic acid using normal MRS broth and modified MRS broth under varying conditions of growth such as temperature and pH and influence of carbon and nitrogen sources. It was observed that all the test isolates best utilized glucose and yeast extract at concentrations of 20g/lit and 5g/lit respectively for production of 30°C and pH 5.5. *Lactobacillus plantarum* produced the highest quantity of lactic acid production. Rowaida Khalil *et al*. [65] reported the isolation and characterization of a bacteriocin produced by a newly isolated *Bacillus megaterium* 19 strains. The strain isolated from a fermented vegetable wastes produced a bacteriocin that displayed a wide spectrum antimicrobial activity against food spoilage microorganisms and possessed a bactericidal mode of action.

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