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Therapeutic applications of lactic acid bacteria based on the nano and micro biosystems

Mehran Alavi[1,2*], Mahendra Rai[3,4], Irwin Rose Alencar de Menezes[5]

[1]*Department of Biological Science, Faculty of Science, Kurdistan University, Sanandaj, Kurdistan, Iran*
[2]*Nanobiotechnology Department, Faculty of Innovative Science and Technology, Razi University, Kermanshah, Iran*
[3]*Nanobiotechnology Laboratory, Department of Biotechnology, SGB Amravati University, Amravati-444 602, Maharashtra, India*
[4]*Department of Microbiology, Nicolaus Copernicus University, 87-100 Toruń, Poland*
[5]*Department of Biological Chemistry, Regional University of Cariri, Brazil*

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ABSTRACT

A plethora of natural metabolites has been identified as antimicrobial and antioxidant agents. Pathogenic and spoilage microorganisms can be inhibited by antimicrobial agents produced by lactic acid bacteria (LAB) via strong antagonistic activity toward them. These bacteria can synthesize bioactive metabolites such as bacteriocins, reutericyclin, reuterin, hydrogen peroxide, carbon dioxide, acetoin, diacetyl, ethanol, acetaldehyde, and organic acids (acetic and lactic acid) as antimicrobial agents. Both Gram-negative and Gram-positive pathogenic bacteria have shown different levels of sensitivity towards these materials. In addition, chelating ferrous ions as well as degrading cholesterol and nitrite have been found for LAB as its main antioxidant activity. These bacteria may be isolated from fermented foods including stinky tofu, corn noodle, chili sauce, glutinous rice dough, meat, vegetable, yogurt, sauerkraut, cultured butter, cheese, cocoa, and potherb mustard pickles. These bacteria can be employed to produce minicells and SimCells in nanosized drug delivery systems to load a range of different chemotherapeutic drugs as a new technology that is functionalized by specific ligands to active targeting tumor cells with minimum toxicity against healthy cells. Moreover, LAB is able to synthesize various types of organic and inorganic nanomaterials as nano-bioreactors.

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1.Introduction

Inactivation or removing pathogenic microorganisms has met a hindrance of antibiotic resistance [1, 2]. There are various microbial (spirotetronate polyketides and bacteriocins) and herbal (flavonoids, saponins, alkaloids, and terpenoids) metabolites for hindering and inactivation of pathogenic microorganisms (Figure 1) [3-5]. Probiotics are live microbial food supplements that have beneficial effects by improving the balance of intestinal microbial flora [6]. This group of bacteria has an effect on the consumer's health in different parts of the body, especially the mouth, digestive system, organs, and genitals [7]. Additionally, among probiotic microorganisms, lactic acid bacteria (LAB) play an important role in preventing virus infections [8]. Cell-free supernatant from LAB can have antibacterial and antifungal activities because of proteinaceous compounds, fatty acids, and organic acids [9]. These bacteria can produce antimicrobial metabolites such as bacteriocins, reutericyclin, reuterin, hydrogen peroxide, carbon dioxide, acetoin, diacetyl, ethanol, acetaldehyde, and organic acids (acetic and lactic acid) (Figure 2) [10, 11]. Moreover, degrading cholesterol and nitrite and chelating ferrous ions have been found for LAB as its main antioxidant activity [12, 13]. Growth of pathogenic such as *Klebsiella* and spoilage microorganisms can be inhibited by antimicrobial agents produced by LAB isolated from cow faeces including *Enterococcus hirae*, *Enterococcus durans*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Weissella confuse* [14]. Other genera including

*Corresponding author. E-mail: mehranbio83@gmail.com

Vagococcus, *Tetragenococcus*, *Lactobacillus*, *Pediococcus*, *Oenococcus*, *Carnobacterium*, *Lactococcus*, *Leuconostoc*, and *Streptococcus* are used to produce lactic acids [15]. LAB is Gram-positive, free of spores and negative catalase products and the main result of sugar fermentation by them is lactic acid. Antimicrobial activity of LAB is caused by their production of organic acids and hydrogen peroxide [16, 17]. In another study, the growth of *Bacillus cereus* Frankland (CICC 20551), *Escherichia coli* (ATCC 8739), *Salmonella enterica* (ATCC14028), and *Staphylococcus aureus* (ATCC 6538p) were inhibited by the supernatant of some strains isolated from fermented foods [18].

Mechanistically, heterofermentative bacteria have biochemical rout involving enzymes as flavoprotein oxidases and catalyze that cause increase in accumulation of hydrogen peroxide (H_2O_2) that promotes the increment of oxidative stress contributing to antimicrobial activity [19]. Other products formed by the metabolism of glucose such as formic acid, lactic acid, acetic acid, acetaldehyde, and diacetyl also possess antimicrobial activity [20].

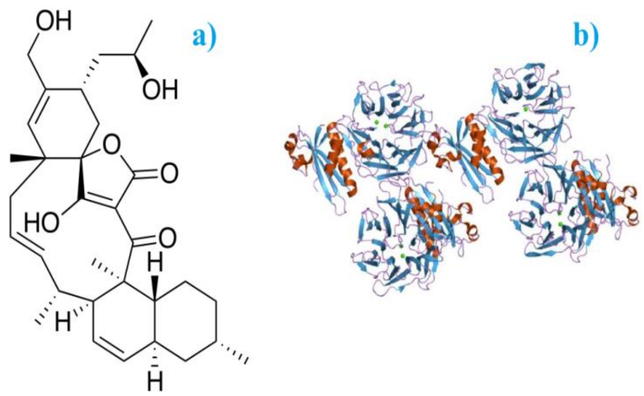

Fig. 1. Maklamicin ($C_{32}H_{44}O_6$) [21] and colicin (PDB code: 2ivz) [22] related to the classes of spirotetronate polyketides and bacteriocins, respectively.

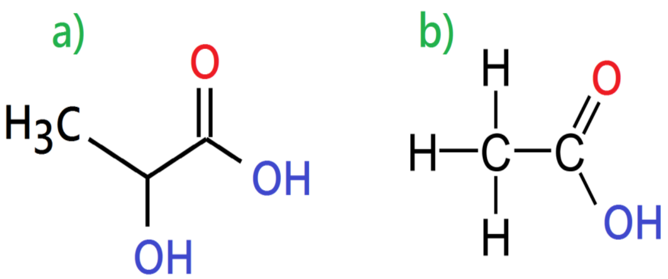

Fig. 2. Lactic acid (a) and acetic acid (b) as the main antimicrobial organic acids produced by LAB.

These bacteria were first isolated extensively from milk as a starter crop in the dairy, meat, vegetable, yogurt, sauerkraut, cultured butter, cheese, sour cream, cucumber pickles, cocoa, sausages, stinky tofu, corn noodle, chili sauce, glutinous rice dough, and grain industries [18, 23]. Many species are used and generally recognized as safe (GRAS), but it should be noted that not all LAB can be found as GRAS [15]. Moreover, by the safety authorities, several species of LAB probiotics are recognized as safe European food (EFSA) [15]. In order to create beneficial effects in the body, they must be able to grow in the stomach and the intestine and the ability to connect to the intestinal wall and live [24]. In addition, for this purpose, the necessary resistance must be faced with hydrochloric acid in the stomach and bile salts in the intestine. There are a lot of advantages to use bacteria as a probiotic [25, 26]. These bacteria should be in sufficient quantity and as required alive, present in the product and during the storage period. In recent years of consumption, they do not lose their genetic stability and such products of LAB increased because of their suitable health effects. Genetic engineering of LAB is the challenging issue and various novel methods such as clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 may be applied to modify these bacteria [15]. LAB can be enriched by some biocompatible elements to increase antimicrobial capacities. In this respect, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* were enriched by sodium selenite at a concentration of 80 µg/mL. The accumulation amounts of selenium were 11.56 µg/mL and 12.05 µg/mL for *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, respectively. Morphology changes including wrinkled structure and cytoplasmic leakage were observed for *Listeria monocytogenes*, *S. aureus*, *Salmonella typhimurium*, and *Escherichia coli* under treating by cell-free culture supernatant of selenium-enriched LAB. This study showed that morphology change was more greater as the dissolution of the cell wall bacteria for Se-enriched *S thermophilus* [27]. In another investigation, the cell-free supernatant of *Lactobacillus plantarum* strains including TA4, SC8, FF2, and MPJ10 showed growth inhibition against *E. coli*, *Salmonella* sp., *S. aureus*, and *Staphylococcus epidermidis*. Diameters of inhibition zone were

19.33, 16.67, 19, and 17.67 mm toward *E. coli*, *Salmonella* sp., *S. aureus,* and *S. epidermidis*, sequentially [28].

2. Nano aspects

Organic and inorganic nanomaterials have received significant attention particularly for drug delivery applications in the various diseases [29]. Nanosized drug delivery systems (NDDS) derived from microorganisms specifically bacteria cells have been applied to loading a range of different chemotherapeutic drugs as a new technology, which may be functionalized by specific ligands such as epidermal growth factor to active targeting cancer cells without toxicity against healthy cells. Minicells as one of the NDDS have protein and chromosomal RNA without or little chromosomal DNA [30, 31]. Several bacterial species related to Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*, *Shigella flexneri*, *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* have been used to generate mnicells in size of 400 nm [32, 33]. As illustrated in Figure 3, minicells are produced during the cell division cycle from cytokinesis and division septum. In addition, SimCells are made from these bacteria by removing bacterial chromosomes and replacing by synthetic genes (Figure 4) [34]. *Lactobacillus* minicells can load hydrophilic and hydrophobic compounds based on their various functional groups involving hydroxyl, carboxyl, phosphoryl, amide, phosphate, and carbohydrate. In this regard, anticancer drugs of paclitaxel and cisplatin can bind to dd-transpeptidase/teichoic acid and exopolysaccharide (EPS) of bacterial membrane [31].

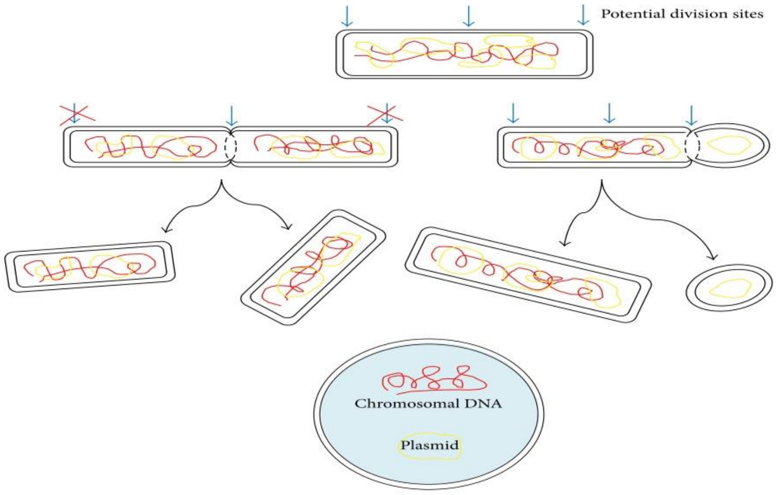

Fig. 3. Generation of minicells from bacterial cells caused by cell division inhibitor genes of ftsZ, minBCD locus, which can lead to asymmetrical division of cells [31].

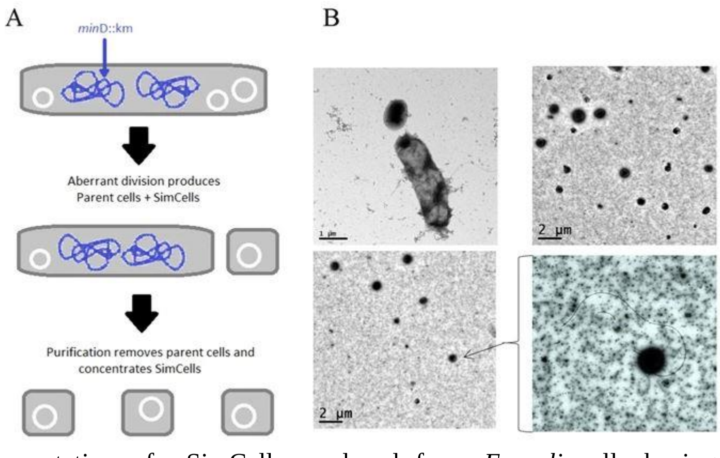

Fig. 4. (A) Schematic representation of SimCells produced from *E. coli* cells having plasmids without bacterial chromosomes (B) Electron images illustrating *E. coli* generating SimCells of different morphologies and sizes [33].

Synthesis of nanomaterials can be carried out by bacterial biomass as a green simple method [3, 35-39]. Three species of LAB including *Lactobacillus fermentum*, *Lactobacillus farciminis*, and *Lactobacillus rhamnosus* had the ability to produce AgNPs in different sizes of 11.2, 17, and 15.7 nm, respectively. This study also showed the concentration of silver recovered depended on the species applied, as *L. fermentum* and *L. fructivorans* recovered 83% and 73%, respectively [40]. In the case of other nanomaterials, carbon dots by with high-hydroxylated groups and a mean size of 2.8 nm were prepared and functionalized by hydrothermal method from bacteria-free supernatant of *Lactobacillus acidophilus*. Carbon dots were loaded on bacterial nanocellulose by value of 71.74 mg/cm^2. CDs-bacterial nanocellulose at this concentration showed diameter zones of inhibition of 18.44 mm and 12.82 mm against *Listeria monocytogenes* and *E. coli*, respectively [41]. As mentioned in previous review, several advantages have been reported for bacterial nanocellulose including mechanical robustness, high water uptake capacity, permeability to water and gases, high porosity, and purity [42]. In addition, biological macromolecules of LAB may be utilized to prepare NPs. For instance, by the method of submerged fermentation, exopolysaccharides of *Lactobacillus fermentum* (LPF6) and *Lactobacillus casei* (LPW2E) were employed to synthesize aggregated AgNPs with a size range of 0.2 -10 nm. These NPs were able to hinder *Bacillus* sp., *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella* sp., and *Pseudomonas aeruginosa* by inhibition diameter zones of 15, 10, 11, 9, and 8 mm, respectively [43]. Postbiotics of *L. plantarum* was incorporated in bacterial nanocellulose to produce nanopaper with antibacterial activity against *Listeria monocytogenes* [44]. Different strains of *Lactobacillus* were employed to synthesize AgNPs in different sizes. There were smaller and larger NPs for *L. rhamnosus* CCM 1825^T and *L. acidophilus* NCS with mean diameters of 19.21 and 36.99 nm, respectively. Additionally, in compared to AgNPs prepared by *L. plantarum* 92T with a size of 19.92 nm, the synthesized NPs with large diameter 30.65 nm via *L. acidophilus* 58p illustrated higher antibacterial activity toward *Shigella sonnei*, *Shigella flexneri*, *S. epidermidis*, *K. pneumonia*, and *E. coli* [45]. Based on bioremediation [46], resisting metal ion and synthesizing NPs may be simultaneously carried out by some LABs such as *L. plantarum* strain TA4. ZnONPs with the mean hydrodynamic diameter of 124.2 nm with the polydispersity index (PDI) of 0.36 were obtained by the cell biomass of *L. plantarum* strain TA4 in aqueous medium having Zn^{2+} at amount of 500 mM [28].

3. Conclusions

Various natural metabolites isolated including pigments, antibiotics, and alkaloids from microorganisms have shown antimicrobial, anticancer and antioxidant activities. Cell suspension and cell-free supernatants are two main sources of LAB with antimicrobial activity. LAB as one of the main nonpathogenic bacteria can produce antimicrobial metabolites such as bacteriocins, reutericyclin, reuterin, hydrogen peroxide, carbon dioxide, acetoine, diacetyl, ethanol, acetaldehyde, and organic acids (acetic and lactic acid), which can hinder Gram-negative and Gram-positive pathogenic bacteria in a different efficiency. In addition, LAB can be enriched by some biocompatible elements such as selenium to increase antibacterial effects on Gram-negative and Gram-positive bacteria. As the major antimicrobial mechanism, heterofermentative bacteria have biochemical pathway involving enzymes as flavoprotein oxidases and catalyze that lead to accumulation of hydrogen peroxide that augments antimicrobial activity. As nano aspect, LAB has ability to generate functionalized minicells and SimCells by specific ligands to encapsulating various anticancer drugs as an effective strategy to active targeting tumor cells. These bacteria also can be used to as bioreactors synthesis of nanomaterials as reducing and stabilizing agents in the intracellular or extracellular ways.

Study Highlights

- Cell suspension and cell-free supernatants are two main sources of LAB with antimicrobial activity.
- LAB can produce antimicrobial metabolites such as bacteriocins and organic acids (acetic and

lactic acid), which can hinder Gram-negative and Gram-positive pathogenic bacteria.

- LAB can be enriched by selenium to increase antibacterial effects on Gram-negative and Gram-positive bacteria.
- Heterofermentative bacteria have biochemical pathways involving enzymes as flavoprotein oxidases and catalyze that increase antimicrobial activity.
- LAB has ability to produce functionalized minicells and SimCells by specific ligands to encapsulating various anticancer agents.
- LAB also can be employed to as bioreactors synthesis of nanomaterials as reducing and stabilizing agents.

Abbreviations

LAB: Lactic acid bacteria
GRAS: Generally recognized as safe
EFSA: European food safety authority
NDDS: Nanosized drug delivery systems
EPS: Exopolysaccharide
CRISPR: Clustered regularly interspaced short palindromic repeats
PDI: Polydispersity index

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with animals or human participants performed by any of the authors.

Authors' contribution

MA: conceptualization and preparing the first drafting; MR and IRAM: revising the manuscript.

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