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Microbiology

Antibiotic Resistance of Probiotic Lactic Acid Bacteria Isolated from Fruits

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Microorganisms with probiotic properties isolated from fruits are relevant for the production of functional juices. Such beverages are good alternatives to dairy based probiotics for those who are lactose intolerant. One of the important probiotic characteristics is the resistance of lactic acid bacteria to antibiotics. The goal of our work was to isolate indigenous lactic acid bacteria (LAB) from fruits collected in various regions of Georgia, study their morphological characteristics and susceptibility to antibiotics. Based on a multi-step screening procedure and a range of probiotic characteristics (ability to growth at low pH, tolerance to bile salts, and antibacterial activity), 10 LAB isolates were selected out of 110 ones and their antibiotic susceptibility was assessed. All isolates displayed resistance to ciprofloxacin, nalidixic acid (DNA gyrase inhibitor) and vancomycin (cell wall synthesis inhibitor). The isolate G-99 was distinguished by antibiotic resistance to approximately 80% of antibiotics. Most isolates (90%) showed resistance to streptomycin, neomycin, and kanamycin (protein synthesis inhibitors). The selected antibiotic-resistant LAB isolates (G-87, G- 88, G-89, G-91, G-92, G-95, G-99, G-100, G-101, G-105) with probiotic properties are relevant candidates for the development of bacterial consortia for producting functional fruit juices. The potential of probiotics as antibiotic alternatives makes them very important nowadays, since infectious diseases have become more difficult to treat due to antimicrobial resistance. *© 2025 Bull. Georg. Natl. Acad. Sci.*

lactic acid bacteria, antibiotics, probiotics

Lactic acid bacteria (LAB) due to their favorable metabolic properties and beneficial effects on human health have long been used in the food industry as probiotics. Therefore, they are generally recognized as safe (GRAS) by the FDA (U.S. Food and Drug Administration) [1]. Antibiotic resistance is one of the crucial factors in assessing the LAB probiotic potential [2]. With some exceptions, antibiotic resistance in these beneficial microbes does not constitute a safety concern in itself, when mutations or intrinsic resistance mechanisms are responsible for the resistance phenotype. In fact, some probiotic strains with intrinsic antibiotic resistance could be useful for restoring the gut microbiota after antibiotic treatment [3]. The increase in antibiotic use has led to deterioration in gut health in humans. Various types of probiotics have been shown to prevent several diseases, such as

cancer and antibiotic-associated diarrhea. They also improve gut health and lactose metabolism, enhance immune responses, and reduce serum cholesterol [4]. Probiotics are considered a promising strategy as an alternative to antibiotics, especially with the emergence of antimicrobial resistance.

Currently, the excessive and improper use of antibiotics leads to an increase in the resistance of pathogenic microbes to antibiotics. This is conditioned by the spread of resistance genes, resulting in a "silent pandemic." By 2050, this factor may overtake other causes of mortality, as well as become another cause for environmental problems [5].

The World Health Organization has established a list of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) as priority pathogens, for which new antibiotics are urgently needed [6]. It is known from the literature that fruits are a reservoir of LAB and yeasts that can be used as microbial consortia to produce probiotic fruit juices for people with lactose intolerance, vegans, and vegetarians [7, 8]. Fruits are considered as functional foods due to their content of vitamins, minerals, dietary fiber, and antioxidants. Their nutritional value and sensory characteristics depend on the fruit species, variety, and cultivation methods [9].

The goal of the research was to study the antibiotic sensitivity of LAB isolated from endemic fruits – prune plums and peaches and to select LAB isolates based on probiotic properties for the fermentation of juices. By developing multi-antimicrobial mechanisms, probiotics induce low risks of resistance to pathogens [10].

Materials and Methods

Peach (*Prunus persica*) and prune plum (*Prunus domestica* subsp. *domestica*) samples (50 units of each) were collected from various regions of Georgia. LAB from the fruits were isolated using MRS and M17 agar, appropriate nutritional media. Individual colonies were obtained by the serial dilution method. The duration of incubation was 48-72 h at 37°C. Primary screening of LAB was carried out by Gram-staining and testing on catalase activity. After being resuscitated in MRS broth, the isolates were plated on MRS agar.

The isolates cell morphology was studied by light microscopy (A12.1029).

The disc diffusion method [11] was used to determine antibiotic susceptibility to 13 antibiotics, including ciprofloxacin (5 μg/ml), erythromycin (15 μg/ml), bacitracin (10 units), tetracycline (30 μg/ml), streptomycin (10 μg/ml and 30 μg/ml), neomycin (10 μg/ml), chloramphenicol (30 μg/ml), and tylosin (30 μg/ml), gentamicin (10 μg/ml), ampicillin (5 μg/ml), vancomycin (5 μg/ml), nalidixic Acid (30 μg/ml), and kanamycin (30 μg/ml). Pure test cultures were streaked over MRS agar, and after 30 minutes, antibiotic discs were placed on them. After 24-48 h of incubation at 37°C, the diameters of isolate inhibition zone were measured. The inoculants tested were classified as resistant (R, zone diameter ≤14 mm), intermediate (I, zone diameter 15-19 mm), or susceptible (S, zone diameter ≥ 20 mm) [12].

Results and Discussion

As a result of repeated purification of 110 prune plum and peach isolates, pure cultures were obtained. For the primary screening of LAB, 20 Gram-positive and catalase-negative rod- and cocci- shaped isolates were selected. Based on different probiotic properties (growth ability at low pH, tolerance to bile salts, antimicrobial activity), 10 isolates were used to study the antibiotic resistance. Cell morphology of these LAB isolates (G – refers to the collection of isolates maintained in glycerol at -80°C) is represented in Table 1.

Selected for study antibiotics are characterized by different mode of action. Erythromycin, tylosin, kanamycin, neomycin, streptomycin, gentamicin, chloramphenicol, and tetracycline are protein synthesis inhibitors; ampicillin and vancomycin are

cell wall synthesis inhibitors; nalidixic acid and ciprofloxacin are DNA gyrase inhibitors; and bacitracin is a cell wall and protein synthesis inhibitor [13]. The 10 LAB isolates susceptibility to studied antibiotics showed a diverse picture (Table 2; Fig. 1).

Table 1. Morphology of LAB isolates from fruits of peaches and prune plums

LAB isolates	Fruit	Morphology
G - 87	Peach	Parallel rod-shaped bacteria
G - 88	Peach	Bead-like chains of cocci
G - 89	Peach	Rod-shaped bacteria
G - 91	Prune plum	Short rod-shaped bacteria
G - 92	Prune plum	Medium size rod-shaped bacteria
G - 95	Prune plum	Prolonged rod-shaped bacteria
G - 99	Prune plum	Rod-shaped bacteria
G - 100	Prune plum	Cocci
G - 101	Prune plum	Medium size rod-shaped bacteria
G - 105	Peach	Cocci

The LAB show susceptibility to protein synthesis inhibitors such as tetracycline, chloramphenicol and erythromycin, and the intermediate susceptibility to gentamicin, streptomycin, and kanamycin, similar to literature data [14].

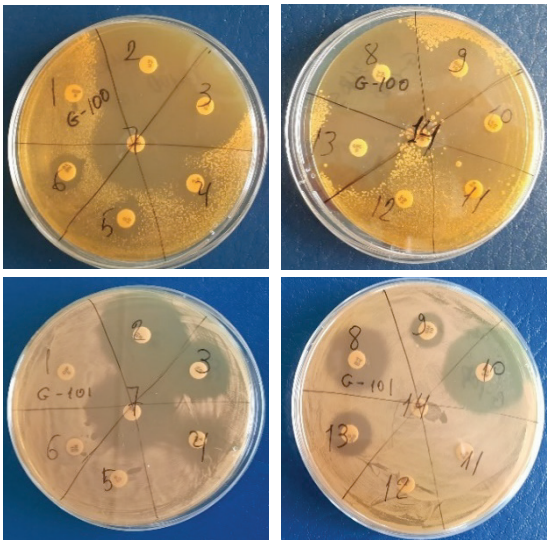

Fig. 1. The resistance and sensitivity of selected LAB isolates (G-100 and G-101) to antibiotics: 1. Ciprofloxacin; 2. Erythromycin; 3. Bacitracin; 4. Tetracycline; 5. Streptomycin 10 µg/ml; 6. Neomycin; 7. Chloramphenicol; 8. Tylosin; 9. Gentamicin; 10. Ampicillin; 11. Vancomycin; 12. Nalidixic acid; 13. Streptomycin (30 µg/ml); 14. Kanamycin.

All indigenous LAB isolates recovered from fruits of peaches and prune plums were found to be resistant to ciprofloxacin, vancomycin, and nalidixic acid. G-99 isolate was distinguished by resistance to about 80% of antibiotics tested. Most of isolates (90%) were resistant to streptomycin, neomycin, and kanamycin similar to reported data [12]. Some isolates were resistant to kanamycin and susceptible to chloramphenicol.

Table 2. Susceptibility of selected LAB isolates to antibiotics

Isolates	Antibiotics													
	Ciprofloxacin, 5 µg/ml	Erythromycin, 15 µg/ml	Bacitracin, 10 units	Tetracycline, 30 µg/ml	Streptomycin, 10 µg/ml	Neomycin, 10 µg/ml	Chloramphenicol, 30 µg/ml	Tylosin, 30 µg/ml	Gentamicin, 10 µg/ml	Ampicillin, 5 µg/ml	Vancomycin, 5 µg/ml	Nalidixic Acid, 30 µg/ml	Streptomycin, 30 µg/ml	Kanamycin, 30 µg/ml
G - 87	0 R	40 S	25 S	18 I	14 R	14 R	30 S	30 S	25 S	30 S	0 R	0 R	30 S	13 R
G - 88	0 R	35 S	27 S	18 I	14 R	11 R	19 I	26 S	28 S	33 S	0 R	0 R	24 S	8 R
G - 89	0 R	33 S	30 S	28 S	13 R	13 R	19 I	32 S	30 S	42 S	0 R	0 R	30 S	11 R
G - 91	0 R	35 S	23 S	17 I	13 R	11 R	40 S	30 S	19 I	41 S	0 R	0 R	29 S	12 R
G - 92	0 R	37 S	25 S	18 I	11 R	10 R	26 S	35 S	35 S	37 S	0 R	0 R	30 S	10 R
G - 95	0 R	32 S	25 S	26 S	17 I	12 R	39 S	26 S	18 I	34 S	0 R	0 R	27 S	12 R
G - 99	0 R	0 R	0 R	0 R	0 R	0 R	0 R	27 S	25 S	0 R	0 R	0 R	27 S	0 R
G- 100	0 R	27 S	27 S	0 R	0 R	22 S	30 S	28 S	28 S	28 S	0 R	0 R	28 S	0 R
G- 101	0 R	30 S	19 I	14 R	0 R	0 R	29 S	19 I	11 R	29 S	0 R	0 R	14 R	0 R

Note: resistant (R, 0-14 mm), intermediate (I, 15-19 mm) and susceptible (S, ≥20 mm).

Conclusions

Ten indigenous LAB isolates from peach and prune plums, characterized by probiotic properties, including resistance to antibiotics: ciprofloxacin, streptomycin, nalidixic acid, vancomycin, and neomycin are relevant candidates for the development of bacterial consortia for the production of functional fruit juices. The potential of probiotics as antibiotic alternatives makes them very important nowadays, as the treatment of infectious diseases becomes increasingly challenging due to antimicrobial resistance.

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მიკრობიოლოგია

ხილიდან გამოყოფილი პრობიოტიკური რძემჟავა ბაქტერიების რეზისტენტობა ანტიბიოტიკების მიმართ

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ხილიდან გამოყოფილი მიკროორგანიზმები ხასიათდება პრობიოტიკური თვისებებით, რომლებიც პერსპექტიულია ფუნქციური წვენების მისაღებად. ასეთი ტიპის სასმელები წარმოადგენს ალტერნატიულ სურსათს ლაქტოზის მოუნელებლობის მქონე ადამიანებისთვის. პრობიოტიკურ მახასიათებლებს შორის ერთ-ერთი მნიშვნელოვანი არის რძემჟავა ბაქტერიების რეზისტენტობა ანტიბიოტიკების მიმართ. ჩვენი სამუშაოს მიზანი იყო საქართველოს სხვადასხვა რეგიონიდან შეგროვილი ხილის ნაყოფებიდან გამოყოფილი ავტოქტონური რძემჟავა ბაქტერიების დამოკიდებულების შესწავლა ანტიბიოტიკების მიმართ. მრავალსაფეხურიანი სკრინინგისა და სხვადასხვა პრობიოტიკური თვისებების (როგორიცაა დაბალ pH-ზე ზრდა, ნაღვლის მარილების მიმართ ტოლერანტობა, ანტიმიკრობული აქტივობა) შესწავლის შედეგად 110 იზოლატიდან შეირჩა 10 იზოლატი და დადგინდა მათი დამოკიდებულება ანტიბიოტიკების მიმართ. ყველა იზოლატი რეზისტენტული აღმოჩნდა ციპროფლოქსაცინის, ვანკომიცინისა და ნალიდიქსის მჟავას მიმართ. G-99 იზოლატი გამოირჩეოდა რეზისტენტობით შესწავლილი ანტიბიოტიკების დაახლოებით 80%-ის მიმართ. იზოლატების უმეტესობა (90%) რეზისტენტული იყო სტრეპტომიცინის, ნეომიცინისა და კანამიცინის მიმართ. მიღებული ანტიბიოტიკურ-რეზისტენტული რძემჟავა ბაქტერიების იზოლატები წარმატებით შეიძლება იქნეს გამოყენებული ბაქტერიული კონსორციუმების შესაქმნელად პრობიოტიკური ხილის წვენების წარმოებისთვის.

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