Wine as a Potential Source of Probiotic Bacteria

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Abstract
Wine has long been under the view of researchers to test it for prospective benefits to human health. Many recent studies have claimed how moderate consumption of wine, can be beneficial for us. The aim of this study was to assess antagonistic activity and probiotic potential of the LAB strains, *Lactobacillus brevis*, *Lactobacillus hilgardii* and *Lactobacillus plantarum*, isolated from wine against pathogenic bacteria. The antimicrobial activity was detected with disc diffusion method against five species of Gram-negative bacteria: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Shigella sonei* CCM 1373, *Yersinia enterocolitica* CCM 5671 and five Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Enterococcus faecalis* CCM 4224, *Listeria monocytogenes* CCM 4699, *Stapylococcus aureus* subsp. *aureus* CCM 2461, *Streptococcus pneumonia* CCM 4501. These results showed antagonistic activity and probiotic potential of pathogenic bacteria isolated from wine. The cell free supernatants (CFS) from *Lactobacillus brevis*, *Lactobacillus hilgardii* and *Lactobacillus plantarum* inhibited the growth of pathogenic bacteria.

Keywords: Lactic acid bacteria, wine, probiotic effect, pathogenic strains

1. Introduction

The conversion from grape must into wine is a complex process that involves the development of various microorganisms, including lactic acid bacteria (LAB). However, wine is considered an unsuitable environment for microbial growth due to its low pH, high concentrations of ethanol and sulfur dioxide (SO_2), and other limiting factors [1]. The LAB capable of overcoming these conditions mainly belong to *Oenococcus*, *Lactobacillus*, *Pediococcus*, and *Leuconostoc* genera [2]. The clear definition of lactic acid bacteria (LAB) is still not well established and this group of bacteria is usually described as lactic acid the producers as a major end product of glucose [3]. LAB are gram-positive cocci or bacilli, non-sporeforming, generally non-motile, catalase negative, aerotolerant, tolerant in acidic environment, chemoorganotrophic, and strictly fermentative organisms. In media with hematin or related compounds some strains of LAB could form catalase or cytochromes [4]. LAB are a group of bacteria typical of non-aerobic habitats. The content and composition of nutrients affect the

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ALB growth. LAB are very tolerant to low pH. These properties allow to ALB to growth in very diverse environments and they were isolated from fermented foods and beverages, fruits and plants, soil and wastewater. The LAB were also described as a part of of the respiratory, intestinal and genital tracts microbiota in man and animals [5,6].

LAB strains show an antagonistic ability. This involves the adhesion to the intestine, altering of pathogens for adhesion to the intestinal epithelium, aggregation, co-aggregation and production of antimicrobial substances, e.g. LAB of products of animal origin and intestinal microbiota of human or animals have been intensively studied previously and their antagonistic ability and probiotic properties were well described. LABs of plant-based fermented foods were studied at the lesser extend [7-9].

Probiotics represent live microorganisms or microbial preparations or metabolites of stabilized microorganisms which confer beneficial effects on host organisms and affect microbial composition with stimulating effects on digestion and immunity of the organisms [10]. They are classified as probiotics as they exert beneficial effects which include reduction of serum cholesterol, aid in lactose digestion, resistance to enteric pathogens, anti-colon cancer effects, small bowel bacterial overgrowth, allergy and mucosal barrier dysfunction including diarrhoea, constipation and immune responses and exerting anti-mutagenic activities [11-15].

The aim of this study were to assess the antimicrobial activity of the LAB strains (*Lactobacillus brevis, Lactobacillus hilgardii*) and *Lactobacillus plantarum* isolated from wine against bacterial pathogens.

2. Materials and methods

2.1. Isolation of lactic acid bacteria

Lactobacillus brevis, Lactobacillus hilgardii and *Lactobacillus plantarum* isolated from red wine Alibernet. Then the material was plated onto de Man, Rogosa and Sharpe agar (MRS, Oxoid), incubated for 48-72h at 30°C and McConkey agar (MCA), incubated for 24-48h at 37°C. Bacterial colonies were identified according to size, color and morphology.

2.2. Sample preparation and MALDI-TOF MS measurement

Prior to identification, the bacterial colonies were subcultured on Trypton Soya agar (Oxoid) at 37°C for 18-24 h. One colony of each bacterial isolate was selected for screening. Subsequently, an analysis of the bacteria identification was performed using the MALDI-TOF MS Biotyper.

2.3. Bacterial strains for testing

The bacterial strains Gram-negative bacteria: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Shigella sonei* CCM 1373, *Yersinia enterocolitica* CCM 5671 and five Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Enterococcus faecalis* CCM 4224, *Listeria monocytogenes* CCM 4699, *Stapylococcus aureus* subsp. *aureus* CCM 2461, *Streptococcus pneumonia* CCM 4501 were obtained from Czech collection of microorganisms (Brno).

2.4. Antibacterial activity of LAB

The antibacterial activity of LAB against following Gram-negative bacteria were tested: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Shigella sonei* CCM 1373, *Yersinia enterocolitica* CCM 5671, *Bacillus thuringiensis* CCM 19, *Enterococcus faecalis* CCM 4224, *Listeria monocytogenes* CCM 4699, *Stapylococcus aureus* subsp. *aureus* CCM 2461, *Streptococcus pneumonia* CCM 4501. The disc well diffusion assay was used. The tested microorganism was spread on the MRS agar, and the LAB strain was added to the well of 6 mm in diameter. The LAB strain was in concentration of 10^8 CFU/mL in broth according to 0.5 McFarland standard. After 48 h incubation at 37°C, the inhibition zone was measured.

2.5. The antimicrobial susceptibility of LAB

Disc diffusion method was applied. The antimicrobial susceptibility of LAB against following antimicrobials were tested: norfloxacin (10 µg), tetracycline (10 µg), streptomycin (10 µg), gentamicin (10 µg), chloramphenicol (10 µg), nalidixic acid (10 µg) and vancomycin (10 µg) (Oxoid, UK). A LAB suspension with McFarland value of 0.5 was spread on MRS agar. The agars were incubated anaerobically at 37°C for 48 h. The inhibition zone diameter was measured and

evaluated as follows: >20; 15-19; and ≤14 mm indicating susceptible, intermediate and resistant [16].

2.6. Statistical analyses

For tested bacteria, the mean and standard deviation of inhibition zones were calculated.

3. Results and discussion

The importance of probiotic bacteria from non-dairy sources were evaluated in the terms of quality, safety and and antibacterial activity.

The mass spectrometry of *Lactobacillus brevis*, *Lactobacillus hilgardii* and *Lactobacillus plantarum* isolated from wine are shown in figure 1. The antagonistic activity of *L. brevis, L. hilgardii* and *L. plantarum* against Gram-positive and Gram-negative pathogen bacteria is shown in table 1. The best antimicrobial activity of *L. brevis* was found against *S. pneumonia* and the lowest antimicrobial activity was found against S. *enterica* subsp. *enterica*. The highest antimicrobial activity of *L. hilgardii* was against *S. pneumonia* and the lowest against *S. sonei*. *L. plantarum* showed the lowest antibacterial activity against *S. sonei* and the highest against *S. aureus*. The strong antimicrobial activity of *L. fermentum* antagonistic activity against Gram-positive was described before. In previous study, also *L. monocytogenes* was significantly inhibited by *L. fermentum* growth. *S. enterica* was significantly tolerant to *L. fermentum* that corresponds to our results [17].

Table 2 shows the antibiotic susceptibility results of *L. brevis* which was resistant to norfloxacin and gentamicin, *L. hilgardii* was resistant to streptomycin and gentamicin and *L. plantarum* to streptomycin, gentamicin and vancomycin. However, the *L. brevis* was sensitive toward streptomycin, chloramphenicol and nalixidic acid. *L. hilgardii* was sensitive to tetracycline and chloramphenicol and *L. plantarum* to norfloxacin and tetracycline.

The aggregation ability of LAB is essential for the antagonistic mechanism to prevent on adhesion and colonization of gut pathogens to intestinal wall [18,19].

It has been reported *Lactobacillus* isolated from pickled cabbage expressed the antimicrobial activity against pathogens (*Bacillus cereus, L. monocytogenes, S. aureus, E. coli*, and *S. enterica*). The weak inhibitory effects was observed against *S. enterica* [20].

It was identified that different LAB strains were resistant to different classes of antibiotics [20].

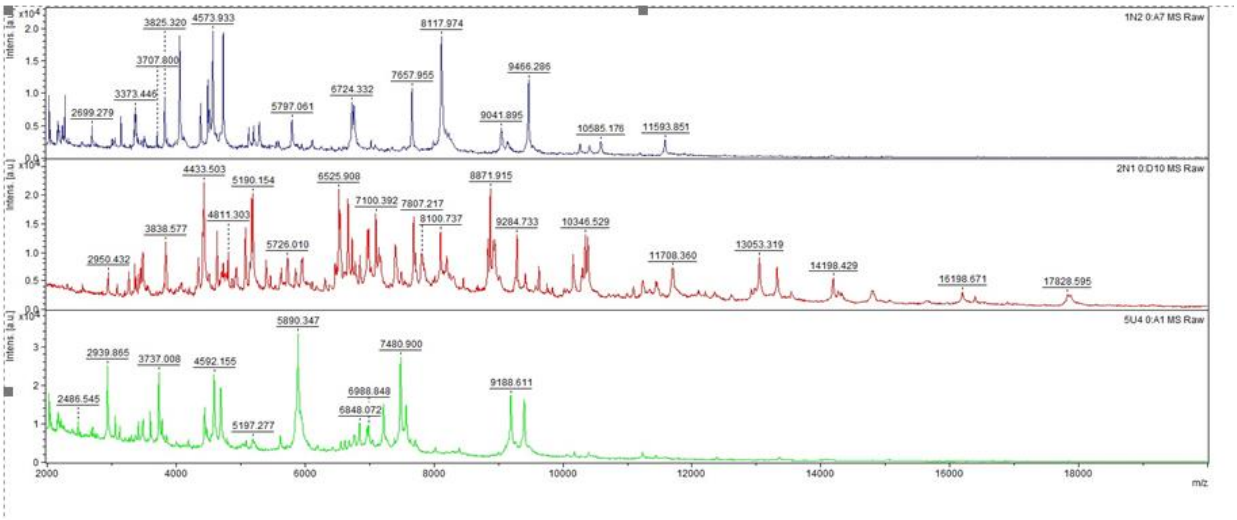

Figure 1. Mass spectrum of *Lactobacillus brevis*, *Lactobacillus hilgardii* and *Lactobacillus plantarum* isolated from wine

Table 1. Antimicrobial activity of *Lactobacillus* spp. against pathogenic bacteria

Bacteria	*L. brevis*	*L. hilgardii*	*L. plantarum*
EC	6.00±1.00	6.67±1.53	7.67±0.58
KP	7.67±0.58	6.67±1.53	7.33±0.58
SE	5.67±0.58	6.00±1.00	6.33±1.53
SS	6.33±1.53	5.33±1.53	4.33±0.58
YE	7.00±1.00	5.67±1.15	5.33±0.58
BT	10.00±1.00	8.33±0.58	9.00±1.00
EF	12.00±1.00	12.67±1.15	13.33±1.53
LM	15.33±0.58	13.67±1.53	16.00±1.73
SA	15.67±1.15	16.67±1.53	17.67±0.58
SP	16.67±1.53	17.00±1.73	14.33±0.58

EC - *Escherichia coli* CCM 3988, KP - *Klebsiella pneumoniae* CCM 2318, SE - *Salmonella enterica* subsp. *enterica* CCM 3807, SS - *Shigella sonei* CCM 1373, YE - *Yersinia enterocolitica* CCM 5671, BT - *Bacillus thuringiensis* CCM 19, EF - *Enterococcus faecalis* CCM 4224, LM - *Listeria monocytogenes* CCM 4699, SA - *Stapylococcus aureus* subsp. *aureus* CCM 2461, SP - *Streptococcus pneumonia* CCM 4501

Table 2. Antibiomicrobial susceptibility of *Lactobacillus*

Antibiotic	*L. brevis*	*L. hligardii*	*L. plantarum*
norfloxacin	R	I	S
tetracycline	I	S	S
streptomycin	S	R	R
gentamicin	R	R	R
chloramphenicol	S	S	I
nalidixic acid	S	I	I
vancomycin	I	I	R

4. Conclusions

Lactobacillus brevis, *Lactobacillus hilgardii* and *Lactobacillus plantarum* isolated from wine exhibited the antibacterial activity against tested bacterial pathogens. The study shows that the non-dairy fermented product could be a source of probiotic bacteria.

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