Selection and biochemical characterization of lactic acid bacteria strains isolated from salad dressings

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Selection and biochemical characterization of lactic acid bacteria strains isolated from salad dressings. *A selection of lactic acid bacteria strains from salad dressings was conducted. 19 strains of lactic acid bacteria were isolated. Four strains were selected on the basis of the results from the performed morphological and cultural studies. The biochemical profiles of the studied strains were determined using the kit system for rapid identification of lactobacilli API 50 CHL and after the subsequent software processing with apiweb® the strains were identified.*

Key words: *lactic acid bacteria, salad dressings*

INTRODUCTION

Probiotics have beneficial effects on human health, protecting it from gastrointestinal infections, stimulating the immune system, antimutagenic and anticancer activities and suppression of harmful bacteria through their inherent antimicrobial activity. A number of species of the genus *Lactobacillus*, that are able to survive in the gastrointestinal tract of humans, are of interest for their possible inclusion in the composition of probiotic preparations [3, 4].

Lactic acid bacteria are found in different ecological niches. Lactobacilli are the most frequently isolated lactic acid bacteria strains. They are a group of lactic acid bacteria characterized by the rod shape of the cells, the rods being different in length and thickness.

Today, consumers' demand for "natural" and "minimally processed" foods is increasing [2]. The popularity of salad dressings among salad consumers requires the production of dressings and sauces, including low-fat dressings and sauces, in order to satisfy users' needs [1].

The purpose of the present study was the selection and biochemical studies of strains of lactic acid bacteria isolated from salad dressings.

MATERIALS AND METHODS

Microorganisms

Four strains of lactic acid bacteria isolated from salad dressings were used in the present work - *Lactobacillus* D2, *Lactobacillus* JH1, *Lactobacillus* DS1, *Lactobacillus* D1.

Media

MRS-broth

Composition (g/dm^3): peptone from casein - 10; yeast extract - 4; meat extract - 8; glucose - 20; K_2HPO_4 - 2; sodium acetate - 5; diammonium citrate - 2; $MgSO_4$ - 0.2; $MnSO_4$ - 0.04; Tween 80 - 1 cm^3/dm^3; pH = 6.5. The medium was sterilized for 15 minutes at 118ºC.

LAPTg10 agar

Composition (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6 - 6.8, and Tween 80 was added - 1cm^3/1dm^3, agar-agar – 15 g. The medium was sterilized for 20 minutes at 121ºC.

Methods

Morphological and culture methods.

The description of the cell morphology and colonial characteristics of the isolated strains of lactic acid bacteria was conducted by microscopic observations of stained microscopic preparations and single colonies developed on LAPTg10 - agar, respectively.

Biochemical methods.

Determination of biochemical profile of the studied cultures.

The system API 50 CHL (BioMerieux SA, France) was used for the identification of the species of the genus *Lactobacillus* based on their ability to utilize 49 carbon sources. Fresh 24-hour culture of the studied strain was centrifuged for 15 min at 5000xg. The obtained sludge, containing biomass, was washed twice with PBS-buffer and resuspended in API 50 CHL medium, an integral part of the used kit. The API strips were placed in the incubation boxes, the microtubules were inoculated with the prepared cell suspension and sealed with sterile liquid paraffin. The results were reported on the 24th and the 48th hour of incubation at 37±1°C. Reporting was done, based on the colour change of each microtubule, compared to the colour of the control microtubule (microtubule 0). Positive results were recorded in the cases of color change from blue to green or bright yellow. The obtained results were processed with apiweb® identification software.

RESULTS AND DISCUSSION

Four lactic acid bacteria strains were isolated from salad dressings. The colonial characteristics of the strains was determined by spread plating on LAPTg10- agar after 48 hours of cultivation and the cell morphology - by dyed microscopic preparation (Table 1).

The ability of the four strains of lactic acid bacteria to absorb the 49 carbon sources included in the kit system for rapid identification of lactobacilli API 50 CHL (BioMerieux SA, France) was examined (Table 2).

After processing of the obtained results with the software apiweb® the strains *Lactobacillus* D2, *Lactobacillus* JH1, *Lactobacillus* DS1 were identified as *Lactobacillus plantarum* strains with high percentage of reliability - between 91.6 and 99.9%. *Lactobacillus* D1 was identified as *Lactobacillus pentosus* with a percentage of reliability of 98.3% (Table 3).

Table 1

Colonial and cellular characterization of the isolated lactic acid bacteria strains

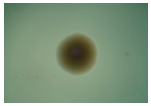
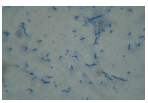
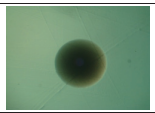
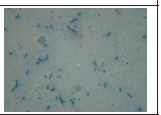
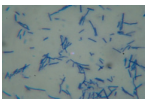
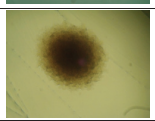
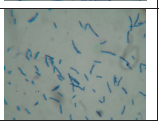
Strain	Colonial characteristics		Cell morphology	
	Description of the colonies	Visualization	Description of the cells	Visualization
D1	Round colonies with smooth edges, protruding, whitish, size - 2-3mm		Short rods with rounded ends, arranged singly or in pairs	
D2	Round colonies with smooth edges, protruding, whitish, size - 2-3mm		Very short rods with rounded ends, arranged singly or in pairs	
JH1	Star-like colonies with jagged edges, protruding, whitish, size - 1-2mm		Long rods with cut corners, arranged singly and in chains	
DS1	Round to oval colonies with jagged edges, protruding, whitish, size - 2-3mm		Short rods with rounded ends, arranged singly or in pairs	

Table 2 Ability of the tested strains to utilize the 49 carbon sources included in the identification system API 50 CHL

#	Carbohydrates	D1	D2	JH1	DS1
1	Glycerol	-	-	-	-
2	Erythriol	-	-	-	-
3	D-arabinose	-	-	-	-
4	L-arabinose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
5	Ribose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
6	D-xylose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
7	L-xylose	-	-	-	-
8	Adonitol	-	-	-	-
9	β-metil-D-xyloside	-	-	-	-
10	Galactose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
11	D-glucose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
12	D-fructose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
13	D-mannose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
14	L-sorbose	-	-	-	-
15	Rhamnose	-	-	-	-
16	Dulcitol	-	-	-	-
17	Inositol	-	-	-	-
18	Manitol	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
19	Sorbitol	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
20	α-methyl-D-mannoside	-	-	-	+ (90%-100%)
21	α-methyl-D-glucoside	-	-	-	-
22	N-acetyl-glucosamine	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
23	Amigdalin	+ (90%-100%)	+ (90%-100%)	-	+ (90%-100%)
24	Arbutin	+ (90%-100%)	+ (90%-100%)	-	+ (90%-100%)
25	Esculin	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
26	Salicin	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
27	Cellobiose	+ (90%-100%)	+ (90%-100%)	-	+ (90%-100%)
28	Maltose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
29	Lactose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
30	Melibiose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
31	Saccharose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
32	Trehalose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
33	Inulin	-	-	-	-
34	Melezitose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
35	D-raffinose	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
36	Amidon	-	-	-	-
37	Glycogen	-	-	-	-
38	Xylitol	-	-	-	-
39	β-gentiobiose	+ (90%-100%)	-	-	-
40	D-turanose	-	-	-	-
41	D-lyxose	-	-	-	-
42	D-tagarose	-	-	-	-
43	D-fuccose	-	-	-	-
44	L-fuccose	-	-	-	-
45	D-arabitol	-	+ (90%-100%)	+ (90%-100%)	+ (90%-100%)
46	L-arabitol	-	-	-	-
47	Gluconate	-	-	+ (90%-100%)	-
48	2-keto-gluconate	-	-	-	-
49	5-keto-gluconate	-	-	-	-

Table 3 Identification of the newly isolated lactobacilli strains after processing of the results from the API 50 CHL kit with apiweb®

Strain	Species	Reliability, %
Lactobacillus D1	Lactobacillus pentosus	98.3
Lactobacillus D2	Lactobacillus plantarum	91.6
Lactobacillus JH1	Lactobacillus plantarum	99.7
Lactobacillus DS1	Lactobacillus plantarum	99.9

CONCLUSION

As a result of the experimental studies the following important conclusions can be resumed:

1. The isolated strains of lactic acid bacteria can be used in the production of salad dressings. It was confirmed that mainly lactic acid bacteria strains are isolated from salad dressings and sauces.

2. The four newly isolated *Lactobacillus* strains were identified by biochemical tests (API 50 CHL) and subsequent processing of the results with apiweb® as *Lactobacillus plantarum* D2, *Lactobacillus plantarum* JH1 and *Lactobacillus plantarum* DS1 and *Lactobacillus pentosus* D1.

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