

GENERIC COMPOSITION OF THE LACTIC ACID BACTERIA ISOLATED FROM THE PHYLLOSHERE OF PLANTS IN THE AZERBAIJAN

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Abstract. Generic composition of different Lactic acid bacteria present on the surface of the plants i.e. *Malus P. Mill* (apple), *Pyrus L.* (pear), *Cydonia Mill* (quince), *Prunus L.* (cherry), *Morus alba L.* (white mulberry), *Morus nigra L.* (black mulberry), *Rubus saxalitis L.* (blackberry) was investigated from different regions of Azerbaijan. It was observed that lactic acid bacteria present on the surface of blackberry, white mulberry and black mulberry were spread more quantitatively as compared to other plants. The number of round shaped bacteria was 3.5 times more than the number of rod shaped bacteria. Out of 412 pure cultured strains, 35% belonged to *Streptococcus* genus, 24% belonged to *Pedicoccus* genus, 17.5% belonged to *Lactobacillus* genus, 13.3% belonged to *Peptococcus* genus and 10% belonged to *Leuconostoc* genus. Bacteria belonging to genus *Lactobacillus* were not found in the samples taken from apple, pear and quince trees. Similarly, bacteria belonging to genus *Leuconostoc* were also not present on samples taken from apple and quince trees. It was concluded that *Streptococcus* genus was dominant genus and *Pedicoccus* was subdominant on the surface of all the investigated plants.

Keywords: Lactic acid bacteria, *Streptococcus*, *Pedicoccus*, *Peptococcus*, *Leuconostoc*.

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1. Introduction

Lactic acid bacteria are heterogenic group of bacteria by ecological point of view and have two morphological forms i.e. round shaped and rod shaped. These bacteria are Gram-positive, non spore forming and non motile. Their characteristic feature is to produce lactic acid as the main product of their metabolism. These bacteria are beneficial to human health by preventing infections of enteropathogenic bacteria (Gad *et al.*, 2014; Soomro *et al.*, 2002; Fernandes *et al.*, 2003).

Lactic acid bacteria are naturally found in the intestine of man and animals and on the surface of the different plants. Their pure cultures are usually obtained from fermented milk products (yogurt, cheese) and intestine of babies (Bardan *et al.*, 2012; Bizarro *et al.*, 2000). The pure cultures of lactic acid bacteria belonging to genera *Lactobacillus* and *Streptococcus* were obtained from Azerbaijan homemade (spontaneous) yogurt, cheese and curd (Ganbarov & Jafarov 2013; Ganbarov *et al.*, 2007; Masumika & Ganbarov, 2017).

Lactic acid bacteria are widely spread on the surface of different plants and these plants can be used as natural sources for obtaining the pure cultures of probiotics (Darsanaki *et al.*, 2012; Nomura *et al.*, 2006). Furthermore, the pure cultures of lactic acid bacteria were isolated from leaves, flowers and stem of five species of *Saliva L.* genus (Teneva & Beshkova, 2006). The pure cultures of lactic acid bacteria belonging

Pedicoccus were isolated from the surface of plants in the Azerbaijan area (Hosseinecat *et al.*, 2018).

The aim of this study was to discover the dominant genera of lactic acid bacteria present on the surface of different plants in the Azerbaijan.

2. Materials and methods

The aboveground organs of cultivated plants i.e. Apple (*Malus P. Mill*), Pear (*Pyrus L.*), Quince (*Cydonia Mill*), Cherry (*Prunus L.*), White mulberry (*Morus Alba L.*), Black mulberry (*Morus nigra L.*) and wild plant Hardseed blackberry (*Rubus saxalitis L.*) from Azerbaijan were used as natural substrate for isolation of lactic acid bacteria. Ten samples of leaves, fruits and green branches each were aseptically taken and put inside the sterile envelopes which were prepared in advance. These samples were kept in the refrigerator initially and microbiologically analyzed during first 24-48 hours. For this purpose, 10 g of the each sample was mixed into 100 ml of sterile normal saline solution (0.9% NaCl). Then 0.1 ml of the suspension was put on the surface of MRS agar medium (Ganbarov *et al.*, 2007) in the Petri plate and spread with steril spreader. The chemical composition of MRS medium was as follows: glucose-2.0%, yeast extract-0.5%, peptone-1.0%, meat extract-0.1%, ammonium citrate-0.2%, sodium acetate-0.5%; KH_2PO_4 - 0.2%, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ - 0.01%, $\text{MnSO}_4 \times 4\text{H}_2\text{O}$ -0.005% (Molskness, 2003).

Planted mediums were placed inside of special airless desicator and incubated for 3-4 days at 30⁰C. The growing colonies of lactic acid bacteria were counted and expressed as colony forming unit per gram (CFU/g) of sample (Netrusov *et al.*, 2005).

The colonies of lactic acid bacteria were defined by Gram staining, production of lactic acid and determination of catalase, oxidase and gelatinase activity (Fernandes *et al.*, 2003). The genera of bacteria were identified as described by the Berjey's manual of systematic bacteriology (2009). All these experiments were carried out four times and obtained data was statistically processed (Plokhinskiy, 1998).

3. Results and discussion

The presence of lactic acid bacteria was studied on the 7 diferent plant species. Among these plants, *Mallus P. Mill.* (apple tree), *Pyrus L.* (pear tree), *Cydonia Mill.* (quince tree), *Prynus L.* (cherry tree), *Morus alba L.* (whitemulberry tree) and *Morus nigra L.* (blackmulberry tree) are cultural fruit trees, while *Rubus saxalitis L.* (hardseed blackberry) is a wild bushy plant.

The higher number of lactic acid bacteria was observed on the surface of blackberry, white mulberry and black mulberry, but lower number of bacteria were present on the surface of cherry, apple, pear and quince. Maximum amount of lactic acid bacteria were present on the surface of blackberry. So, the number of bacteria on the surface of blackberry was 2.3 and 1.8 times more than the number of bacteria on the surface of white and black mulberry, respectively (Table 1). According to obtained results, regarding the number of lactic acid bacteria, blackberry was dominant, but white and black mulberry were subdominant.

Table 1. Number of lactic acid bacteria on the surface of plants

Plants	Number of bacteria (10 ⁴ CFU/g of substrate)		
	Rod shaped	Round shaped	Total
<i>Malus</i> P.Mill. (Apple)	0	1.8±0.07	1.8±0.07
<i>Pyrus</i> L. (Pear)	0	2.2±0.11	2.2±0.11
<i>Morus alba</i> L. (White mulberry)	1.4±0.03	6.7±0.30	8.1±0.30
<i>Morus nigra</i> L. (Black mulberry)	2.2±0.10	7.9±0.45	10.1±0.50
<i>Cydonia</i> Mill. (Quince)	0	1.8±0.08	1.8±0.08
<i>Prunus</i> L. (Cherry)	0.2±0.01	2.2±0.10	2.4±0.10
<i>Rubus saxatilis</i> L. (Blackberry)	6.2±0.30	12.1±0.61	18.3±0.80

Both rod shaped and round shaped (cocci form) lactic acid bacteria were found on the surface of the investigated plants. The amount of cocci form and rod shaped bacteria was 77.6% and 22.4% respectively of total number of lactic acid bacteria. The higher number of cocci form bacteria were observed on the surface of blackberry, white mulberry and blackmulberry, but the higher number of rod shaped bacteria was present on the surface of blackberry. The number of cocci form bacteria on the surface of blackberry was two times more than the amount of rod shaped bacteria. Rod shaped lactic acid bacteria were not observed on the surface of apple, pear and quince trees. In total, the amount of cocci form bacteria was 3.5 times more than the amount of rod shaped bacteria on the surface of investigated plants (Table 1).

Rod shaped lactic acid bacteria belong to *Lactobacillus* genus and cocci form lactic acid bacteria belong to *Streptococcus*, *Pedicoccus*, *Peptococcus* and *Leuconostoc* genera. Out of isolated 412 pure cultures of lactic acid bacteria, 82.5% belonged to the cocci and 17.5% belonged to the rod shaped bacteria. 35% of strains belonged to *Streptococcus*, 24% belonged to *Pedicoccus*, 17.5% belonged to *Lactobacillus*, 13.3% belonged to *Peptococcus* and 10% belonged to *Leuconostoc* (Table 2.). Furthermore, highest number of bacteria belonging to genus *Streptococcus* were observed on the surface of used plants. The number of *Streptococcus* was 2.0, 3.5, 1.4 and 4.1 times more than number of *Lactobacillus*, *Leuconostoc*, *Pedicoccus* and *Peptococcus*, respectively.

Table 2. Genera of lactic acid bacteria isolated from the surface of different plants

Plants	The number of bacterial strains				
	<i>Lactobacillus</i>	<i>Leuconostoc</i>	<i>Pedicoccus</i>	<i>Peptococcus</i>	<i>Streptococcus</i>
<i>Malus</i> P. Mill.	0	0	5	2	14
<i>Pyrus</i> L. (Pear)	0	3	6	3	16
<i>Morus alba</i> L.	19	12	23	13	24
<i>Morus nigra</i> L.	18	10	22	12	26
<i>Cydonia</i> Mill.	0	0	6	4	11
<i>Prunus</i> L.(cherry)	13	6	14	7	21
<i>Rubus saxalitis</i> L.	22	10	24	14	32

It is worth mentioning that 5.1% strains of lactic acid bacteria were isolated from apple trees, 6.7% were isolated from pear trees, 5.1% were isolated from quince trees, 14.8% were isolated from cherry trees, 22.0% were isolated from white mulberry trees, 21.3% were isolated from black mulberry trees and 25.0% were isolated from blackberry bushes. It was observed that *Lactobacillus* and *Leuconostoc* bacteria were not present on the surface of apple and quince trees, and *Lactobacillus* bacteria were not present on the surface of pear trees.

Thus, it was revealed that lactic acid bacteria are quantitatively more present on the surface of blackberry, white mulberry and black mulberry plants. The number of cocci form bacteria is 3.5 times more than rod shaped bacteria. From 412 purely cultured strains, 35.0% belonged to *Streptococcus*, 24.0% belonged to *Pedicoccus*, 17.5% belonged to *Lactobacillus*, 13.3% belonged to *Peptococcus* and 10.0% belonged to *Leuconostoc*. Number of lactic acid bacteria on the surface of blackberry was dominant, but number of lactic acid bacteria on the surface of white mulberry and black mulberry were subdominant. *Streptococcus* genus was found to be dominant while *Pedicoccus* genus was found to be subdominant on the surface of all the investigated plants.

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