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CARBOHYDRATE-SPECIFIC ACTIVITY OF LACTOBACILLI AND LACTIC ACID COCCI

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Introduction

Novel antibacterial agents with the capacity to inhibit bacterial biofilms are important for the treatment of bacterial infections. A promising class of antimicrobial molecules is the family of lectins,since lectin molecules have unique hydrocarbon-recognizing capacities, and pathogens are often decorated with sugars that affect their survival and infectivity [Francois et al., 2012]. Lectins are defined as carbohydrate binding proteins without catalytic activity that are prevalent in all organisms. They often have important functions in cell signaling and cellular interactions [Dias et al., 2015].

An additional bonus from the presence of lectins is the increase due to their action of probiotics. Positive effect from probiotics indirectly due to direct or indirect endogenous flora and the immune system modulation. In this case, the direct contact of the probiotic culture with active epithelial cells terminal is the necessary condition of macroorganism niche. Studies have shown that lactic acid bacteria are in contact with epithelial cells and induces the eRNA of some genes. A clear correlation had shown increased expression between eRNA and extracellular secretion of mucin [Barnett, 2018; Yadav, 2015].

Similar to plants and animals, bacteria can also express lectins on their surface, but reports on their characterization, and especially antipathogenic potential, are very scarce[Dias et al., 2015].

The research aim was to investigate carbohydrate-specific activity of lactobacilli and lactic acid cocci that were isolated from different objects.

Materials and methods

Adhesive activity was studied by the method of determining hemagglutinum activity (HAA) of culture fluid (CF) of all bacterial strains examined.

Lactobacillus and *Lactococcus* strains which were isolated from sea sponges of mussels and sturgeon intestineswere used for the study. The studies were conducted using trypsinized erythrocytes of ram, chicken and sturgeon, fixed by glutaraldehyde. The presence of hemagglutinum or lectin activity was determined by the reaction of hemagglutination (RHAA) by double serial dilutions in sterile 96-well polystyrene microplates with U-shaped wells at room temperature [Луцик и др., 1980]. In all cases, in order to prevent errors associated with autoagglutination of erythrocytes, wasput control of 2% of erythrocyte solution in the physiological solution.

A set of 13 carbohydrates and polyhydric alcohols was used to determine a potential lectin oflactobacilli and lactic acid cocci strains. The degree of inhibition

of GAA by carbohydrates was expressed as the minimum dose of carbon necessary for the complete suppression of RGA with sheep, rabbit and chicken erythrocytes.

The 2% suspension of erythrocytes is added at 0.1 cm^3 each into the U-shaped wells of 96-well polystyrene microplates. Then, the culture fluid with the test compounds is added in the same amounts, and the suspension is gently mixed. The microplatesis covered with a lid and placed in a thermostat at a temperature of 37 ° C for one hour.

After the tablet is incubated in a thermostat, it is removed and preliminary results are taken into account. In the presence of adhesive properties in the studied compounds, red blood cells are distributed evenly over the entire surface of the bottom.

With a negative result, all red blood cells are collected in the form of a point in the middle of the well of the tablet. The final results of the study are taken into account 18 hours after incubation of the tablet at room temperature.

Results

As we see from table 1, the carbohydrate-specific activity of lactobacilli, which is possibly due to the presence of free lectins isolated from sea sponges, has a fairly high variability in terms of the ability to bind to carbohydrates and polyhydric alcohols. According to our data, potential lectins of the strains of *Lactobacillu parabuchneri* ONU10.1, *Lactobacillus vaccinostercus* ONU22 and *Lactobacillus bifermentans* ONU68 are not associated with xylose, and *Lactobacillus parabuchnerus* ONU10. 2a, *Lactobacillus parabuchneri* ONU19.2b and *Lactobacillu parabuchneri* ONU52.1. That is, the only strain that is not able to bind to pentoses is the *Lactobacillu parabuchneri* ONU10.1 strain.

By the level of lectin binding activity, we can separate both the most inactive strain (*Lactobacillus vaccinostercus* ONU2) and the most active strains (*Lactobacillus parabuchneri* ONU39, *Lactobacillus bifermentans* ONU68 and *Lactobacillus bifermentans* ONU53).

Strains of lactic acid bacteria, isolated from sturgeon intestines, showed greater ability to bind carbohydrates and polyhydric alcohols (Table 2) and less variability of this trait.

We also noticed that, in contrast to the data given for strains isolated from sponges, the potentially lectins of strains of lactobacilli isolated from sturgeon are actively associated with sucrose, to which almost no lectins of strains isolated from sponges were bound. The lectins of these strains were least active in relation to fructose and xylose. According to the final results, it was shown that the minimum activity with respect to sugar and polyhydric alcohols demonstrates the strain of *Lactobacillus sp*. ONU2.8 (Tab. 2). The maximum activity is demonstrated by strains of *Lactobacillus sp*. ONU3.6, *Lactobacillus sp*. ONU2.7 and *Lactobacillus sp*. ONU2.2 (Tab. 2). When analyzing the results of the hydrocarbon-specific activity of lectins to hydrocarbons and polyhydric alcohols of strains that were isolated from mussels, we noticed that they are worst associated with fructose, mannose, lactose and glucose. Conversely, they are actively associated with galactose, xylose, maltose, sucrose and mannitol (Tab. 3).

Table 1

Carbohydrate-specific activity of strains of lactobacilli isolated from sea sponges in relation to carbohydrates and polyols

	Rhamnose	Xylose	Dulcite	Galactose	Maltose	Sorbitol	Glucose	Fructose	Mannose	Sucrose	Mannitol	Lactose	Arabinose
Lactobacillus vaccinostercus ONU2	–	+	–	–	+	–	+	–	+	–	+	–	+
Lactobacillu parabuchneri ONU10.1	–	–	–	+	+	+	+	+	+	+	–	–	–
Lactobacillus bifermentans ONU10.2	+	+	–	+	+	+	+	+	+	–	–	–	–
Lactobacillus bifermentans ONU19.2a	+	+	–	+	+	–	+	+	+	+	–	–	–
Lactobacillus parabuchneri ONU19.2b	–	+	–	+	+	+	–	+	+	–	–	–	+
Lactobacillus vaccinostercus ONU22	+	+	+	–	+	–	–	+	+	+	+	–	–
Lactobacillu parabuchneri ONU52.1	+	+	+	+	+	–	+	+	+	–	–	–	–
Lactobacillus bifermentans ONU53	–	–	+	+	+	+	+	–	+	+	+	–	+
Lactobacillus bifermentans ONU68	+	–	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus parabuchneri ONU39	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2

Carbohydrate-specific activity of strains of lactic acid bacteria isolated from sturgeon in relation to carbohydrates and polyols

	Mannose	Dulcite	Arabinoza	Rhamnose	Galactose	Maltose	Glucose	Lactose	Xylose	Sucrose	Sorbitol	Mannitol	Fructose
Lactobacillus sp. ONU1.1	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU1.2	+	+	+	+	+	+	+	+	+	++	+	+	±
Lactobacillus sp. ONU1.3	+	+	+	±	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU1.4	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU2.1	+	±	+	+	+	+	+	+	+	++	+	+	+
Lactobacillus sp. ONU2.2	++	+	+	+	+	+	+	+	+	++	+	+	+
Lactobacillus sp. ONU2.3	+	+	+	+	+	++	+	+	+	+	+	+	±
Lactobacillus sp. ONU2.4	+	+	+	+	+	+	±	+	+	+	+	+	±
Lactobacillus sp. ONU2.5	+	+	+	++	+	+	+	+	+	+	+	+	++
Lactobacillus sp. ONU2.6	+	+	+	+	±	+	+	+	+	++	+	+	+
Lactobacillus sp. ONU2.7	+	+	++	+	+	++	+	+	+	+	+	+	±
Lactobacillus sp. ONU2.8	+	+	+	+	+	+	++	±	+	++	±	+	+
Lactobacillus sp. ONU3.1	+	+	+	++	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU3.2	+	+	+	±	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU3.3	+	+	+	±	+	+	+	+	+	+	++	+	+
Lactobacillus sp. ONU3.4	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU3.6	+	+	+	+	++	+	+	+	+	++	+	+	+

Table 3

Carbohydrate-specific activity of strains of lactic cocci isolated from mussels in relation to carbohydrates and polyhydric alcohols

	Mannose	Dulcite	Arabinoza	Rhamnose	Galactose	Maltose	Glucose	Lactose	Xylose	Sucrose	Sorbitol	Mannitol	Fructose
*Lactococcus sp.*LM7	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactococcus sp. LM8	+	+	+	+	+	+	+	+	+	±	+	+	++
Lactococcus sp. LM10	+	+	+	+	+	+	+	±	+	+	+	±	+
*Lactococcus sp.*LM25	+	+	+	++	++	+	+	+	+	+	+	+	+
Lactococcus sp. LM27	±	+	+	–	++	++	++	++	+	++	++	+	±
Lactococcus sp. LM30	±	+	++	+	++	++	±	++	++	++	+	++	±
Lactococcus sp. LM31	±	+	+	++	++	++	±	±	++	++	+	++	±
*Lactococcus sp.*LM32	±	+	++	++	++	++	±	±	++	++	+	++	±
*Lactococcus sp.*LM33	±	+	+	+	+	++	++	±	++	++	+	++	±

Lectins of the strain *Lactococcus sp*. LM27 were the only ones that did not bind to rhamnose. Lectins of strains of *Lactococcus sp*. LM30, *Lactococcus sp*. LM33, *Lactococcus sp*. LM31 and *Lactococcus sp*. LM32 polyhydric alcohols (Tab. 3).

Conclusion

A study of lactobacilli and lactic acid cocci isolated from sea sponges, mussels and sturgeon intestines showed a significant difference between these strains in their ability to form lectins and their ability to bind to various carbohydrates and polyhydric alcohols.

Thus, we have shown that the hydrocarbon-specific activity of lactic acid bacteria is a fairly variable factor, which depends on the source of isolation and the activity of the strain in the sense of adhesive activity.

Literature

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