Effect of Non-Starch Polysaccharide Calcium Pectate on the Growth and Colonization of Normal and Pathogenic Microflora *In Vitro*

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In vitro experiments showed that calcium pectate added to the culture medium produces a dose-dependent prebiotic effect on lacto-and bifidobacteria cultures and on non-pathogenic strain of *Escherichia coli*. Calcium pectate produced a pronounced bacteriostatic effect on *Candida albicans* strain; the effects was more pronounced in a concentration of 4%.

Key Words: *polysaccharides; calcium pectate; microflora*

It is proved that normal microflora (microbiota) and, especially, intestinal microbiocenose have a significant, and in some cases a decisive influence on vital function of human body [1,4,5]. Local and systemic effects of the normal intestinal microflora are descried: trophic and energetic function, regulation of intestinal peristalsis, participation in regulation of tissue differentiation and regeneration, detoxification and excretion of endo- and exogenous toxic compounds, destruction of mutagens, activation of drug compounds, *etc*. [3,13]. Many systemic diseases and various medications lead to intestinal dysbiosis. In this case, the concentration of obligate microflora decreases, while the number of pathogenic and opportunistic (facultative and transient) bacteria increases [5]. The imbalance of bacteria, in turn, leads to dysfunction of organs and systems (diarrhea, flatulency) and the whole body (allergies, vitamin-deficient states, carcinogenesis, arthritis, Alzheimer's disease, sepsis, *etc*.) [7,12,13].

Nutrition and proper functioning of saprophytic microflora critically depends on the presence of uncleaved carbohydrates (di-, oligo-and polysaccharides) for energy production [2,10]. Apart from their immunomodulatory, antineoplastic, and anti-inflammatory effects polysaccharides suppress multiplication of pathogenic microflora (*St. aureus etc*.) [11]. Our previous experiments demonstrated antiulcer, anti-inflammatory, and antispasmodic effects of non-starch polysaccharide calcium pectate [9].

Here we studied the effect of calcium pectate on the growth and colonization of normal and pathogenic microorganisms *in vitro*.

MATERIALS AND METHODS

Escherichia coli (nonpathogenic) and *Candida albicans* strains obtained from the Museum of Cultures, Department of Microbiology, Siberian State Medical Academy, were used as the test objects. These strains exhibit properties typical of the species. Strains of bifidobacteria and lactobacilli were obtained from dry standardized powder (Virion) by dilution and 3-fold seeding followed by staining after Gram and identification under a microscope. *E. coli* and *C. albicans* (24-h cultures) diluted to a concentration of 500 microbial bodies (m.b.) according to the turbidity standard and controlled microscopically, were used for seeding. Sterile saline (control tubes) or 2 and

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4% solutions of calcium pectate in saline were added to microbial suspension in meat-peptone broth. After 4, 24 and 48 h, 0.05 ml culture was transferred from each test tube to Petri dish and evenly distributed over the culture medium (meat infusion agar for *E. coli* and Sabouraud medium for *C. albicans*) with a sterile glass spatula. The dishes were placed in an incubator at 37°C and the number of colonies per 1 cm^2 was counted after 24 h (4 measurements per plate) and multiplied by 78.5 to obtain the total count per plate. In 24-hour cultures of lactobacilli and bifidobacteria, the following dilutions were prepared according to the turbidity standard: 500,000, 50,000, 5000, 500, 50, and 5 m.b. (for bifidobacteria), and 5000, 500, 50, and 5 m.b. (for lactobacilli). Cultures were placed in test tubes containing 9 ml semisolid bifidum medium and saline or calcium pectate were added to concentrations of 2 and 4%. After 4, 24 and 48 h, the cultures were inoculated into sterile tubes containing bifidum medium by single stabbing of the bacteriological loop from the medium surface to the bottom of the tube. The tubes were placed in an incubator at 37°C and after 24 h the presence/absence and the rate of growth were visually assessed and scored (from 1 to 5). All tubes were duplicated to ensure reliability. Culture purity was routinely monitored under a microscope.

The studied calcium pectate, a low-esterified pectin, was obtained from commercial highly esterified citrus pectin (Copenhagen Pectin A/S, Lille Scensved). It was a dry white powder. Its physical and chemical properties were as follows: anhydrogalacturonic acid content 67.3%, calcium 38 mg/g sample, esterification degree 1.2%, molecular weight 39.3 kDa. Polysaccharide dilutions 2 and 4% were chosen taking into account previous data [11].

Statistical processing of the results was performed using the nonparametric Mann–Whitney test. Differences were considered significant at $P<0.05$ [6].

RESULTS

It was found that the number of *E. coli* colonies increased in Petri dish containing calcium pectate: the number of colonies in the presence of 2 and 4% polysaccharide after 4-h exposure significantly increased by 2.0 and 1.8 times, respectively, after 24 h the this parameter increased by 1.9 and 1.8 times, and after 48 h the number of colonies increased by 3.5 and 1.2 times in comparison with the control values (Table 1).

Calcium pectate stimulated the growth of bifidobacteria colonies (Table 2). Addition of the polysaccharide in a concentration of 2% to the tubes with serial dilutions of bifidus cultures twofold stimulated the growth of microorganisms in concentrations of 50,000 and 5000 m.b. after 24 h and in concentrations of 5000 m.b. and 500 m.b. after 48 h in comparison with the control (no bacterial growth). In the presence of calcium pectate in a concentration of 4% we observed a moderate growth of bacteria in concentrations of 50,000 and 5000 m.b. after 24 hours and in concentrations of 5000 (2 points), 500 (2 points), 50 m.b. (1 point) after 48 h in comparison with the zero reference value.

Addition of 2% polysaccharide solution to the tubes with serial dilutions of *Lactobacillus* strain led to stimulation of the growth of bacteria in concentration of 5 m.b. after 24 h exposure (compared to the absence of bacterial growth in the control, Table 3). The growth of the colonies was also stimulated by addition of 4% calcium pectate in concentrations of 500 and 50 m.b. after 4-h exposure and in concentrations of 5000, 500, 5 m.b. after 24 h. Similar picture was observed 48 h after addition of 4% calcium pectate.

The effect of calcium pectate on the growth of pathogenic intestinal microflora, *C. albicans* culture, was also examined (Table 4). It was shown that polysaccharide has a bacteriostatic effect: the number of colonies in a Petri dish with Sabouraud medium in

TABLE 1. Effect of Calcium Pectate on the Growth of Nonpathogenic *E. coli* Strain ($X\pm m$)

Experimental conditions	Number of colonies of *E. coli* in a Petri dish		
	exposure, h		
	4	24	48
Control (n=12)	1864.38±341.13	3434.38±579.47	57.57±14.26
Calcium pectate 2% (n=12)	3679.69±617.83*	6672.50±177.03**	201.86±19.54**
Calcium pectate 4% (n=12)	3404.94±267.01**	6211.97±287.85**	68.03±18.56

Note. Here and in Table 4: * $P<0.05$ and ** $P<0.01$ in comparison with the control.

TABLE 2. Effect of Calcium Pectate on the Growth of Bifidobacteria Culture

Experimental conditions		500,000 m.b.	50,000 m.b.	5000 m.b.	500 m.b	50 m.b.	5 m.b.
4-h exposure	control	2	-	-	-	-	-
	calcium pectate 2%	2	-	-	-	-	-
	calcium pectate 4%	2	-	-	-	-	-
24-h exposure	control	3	-	-	-	-	-
	calcium pectate 2%	3	2	2	-	-	-
	calcium pectate 4%	3	2	2	-	-	-
48-h exposure	control	3	2	-	-	-	-
	calcium pectate 2%	3	2	2	2	-	-
	calcium pectate 4%	3	2	2	2	1	1

Note. Here and in Table 3: 1-5 is bacterial growth score.

the presence of polysaccharide in a concentration of 2% decreased by 1.3 times after 24-h exposure and by 1.7 and 1.6 times 24 and 48 h after addition of 4% calcium pectate.

Thus, calcium pectate *in vitro* produced a prebiotic effect on lacto-and bifidobacteria cultures and on non-pathogenic strain of *E. coli* and a bacteriostatic effect on *C. albicans* strain. The effect became more pronounced with increasing polysaccharide concentration.

When discussing possible mechanism of the antibacterial effects of calcium pectate, it is logical to assume that the polysaccharide due to its gelling properties is capable of enveloping the bacteria, thereby preventing their adhesion to the substrate and violating the processes of microbial colonization. In addition, administration of the polysaccharide can lead to acidification of the culture medium through the formation of monocarboxylic acids during its cleavage [11]. This leads to damage of bacterial organelles and proteins and inhibits their proliferation. It is possible that calcium salts and trace amounts of methyl alcohol damaging microorganisms are formed in the reaction of saponification of esterified carboxyl groups in pectic substances.

Pronounced prebiotic effect of calcium pectate can be due to the fact that the polysaccharide provides favorable conditions (suitable substrate and weak acidic medium) for proper probiotics [10], improving survival, reproduction and adhesion of obligate bacteria.

The data obtained suggest that calcium pectate can be used as a prebiotic for monotherapy of dysbiosis and in association with probiotics. It is known that enteric dysbiosis is characterized by deficit of aerobes and colonic dysbiosis by deficit of anaerobes. The fact that the polysaccharide stimulates the growth of the main representatives of obligate microflora determines its universal application for various types of dysbiosis, while its antibacterial activity inhibits the growth of pathogenic bacteria.

TABLE 3. Effect of Calcium Pectate on the Growth of Lactobacilli Culture

Experimental conditions		5000 m.b.	500 m.b.	50 m.b.	5 m.b.	<5 m.b.
4-h exposure	control	3	-	-	-	-
	calcium pectate 2%	3	-	-	-	-
	calcium pectate 4%	3	1	1	-	-
24-h exposure	control	3	3	2	1	-
	calcium pectate 2%	3	3	3	1	1
	calcium pectate 4%	4	4	4	3	2
48-h exposure	control	4	3	3	2	1
	calcium pectate 2%	3	2	2	2	2
	calcium pectate 4%	3	3	3	3	2

TABLE 4. Effect of Calcium Pectate on the Growth of *C. albicans* Culture ($X \pm m$)

Experimental conditions	Number of colonies of *C. albicans* in a Petri dish		
	exposure, h		
	4	24	48
Calcium control (n=12)	3738.56±572.44	8811.63±633.19	4474.50±739.23
Calcium pectate 2% (n=12)	3738.56±409.74	6780.44±360.32**	4170.31±454.70
Calcium pectate 4% (n=12)	4101.63±557.99	5308.56±410.30**	2828.56±420.12*

Therefore we can assume that the use of calcium pectate as a prebiotic will help to maintain intestinal biochemical, metabolic, and immunological balance.

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