International Journal of Biomedicine 9(4) (2019) 366-369
http://dx.doi.org/10.21103/Article9(4)_OA18

ORIGINAL ARTICLE

Biotechnology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Study of the Growth of *Escherichia coli* on Pectins

Anatoly A. Shubakov, PhD[1,2]; Elena A. Mikhailova[1*]

[1]*Institute of Physiology of Komi Science Centre of the Ural Branch of the RAS, FRC Komi SC UB RAS*
[2]*Institute of Biology of Komi Science Centre of the Ural Branch of the RAS, FRC Komi SC UB RAS*
Syktyvkar, Komi Republic, the Russian Federation

Abstract

The growth of *E. coli* on pectins isolated from various plant sources was studied. We used commercial apple pectin AU701 and low-methyl esterified pectins (6-22%) from callus cultures of tansy *Tanacetum vulgare* L. (TV, tanacetan), duckweed *Lemna minor* L. (LM, lemnan), and campion *Silene vulgaris* (M.) G. (SV, silenan). Bacterial growth was also tested on the enzymatic degradation products of tanacetan pectin. For comparison, bacterial growth was studied on easily metabolizable carbon sources – glucose and lactose. *E. coli* was cultivated on solid media in Petri dishes and in liquid nutrient media in Erlenmeyer flasks at a temperature of +37°C and at room temperature. It was found that *E. coli* colonies do not form with growth on gels of tanacetan, lemnan and silenan. When growing on a gel of apple pectin, a weak bacterial growth is detected. However, *E. coli* is capable of growth on soluble products of enzymatic hydrolysis of tanacetan pectin – oligogalacturonides. (**International Journal of Biomedicine. 2019;9(4):366-369.**)

Key Words: *Escherichia coli* • liquid and solid nutrient media • pectin • pectin hydrolysis products • glucose • lactose

Abbreviations

AP, apple pectin; **CaPGPs**, calcium-pectic gel particles; **CFU**, colony forming units; **PG-TV**, products of hydrolysis of tanacetan; **LM**, pectin lemnan; **SV**, pectin silenan; **MPA**, meat-peptone agar; **MPB**, meat-peptone broth; **MA**, must agar; **MM**, mineral medium; **SGF**, simulated gastric fluid; **SIF**, simulated intestinal fluid; **SCF**, simulated colonic fluid.

Introduction

Escherichia coli, a gram-negative, non-sporulating facultative anaerobe, is a typical inhabitant of the lower intestines of endothermic animals and humans.(1) Intestinal microbiota consists of more than 500 species of bacteria that make up 10^{10}-10^{11} cells per gram of colon contents. Although anaerobic bacteria in the intestines exceed the number of *E. coli* from 100:1 to 10000:1, *E. coli* is the dominant aerobic organism in the gastrointestinal tract of mammals.(2)

Most strains of *E. coli* are harmless mammalian commensals; however, some strains can cause intestinal or extra-intestinal diseases.(3) The main function of the commensal flora in the intestine, especially in the colon, is the fermentation of indigestible food residues and endogenous mucus produced by the epithelium.(4) In the digestive tract, commensal strains of *E. coli* are localized in the mucus layer covering the epithelial cells of the colon, adapting their metabolism in this ecological niche.(5)

The mucus gel layer of the gastrointestinal tract (mucin), synthesized and secreted by the goblet cells of the host, lubricates and protects the intestinal epithelium from damage caused by food and digestive secrets. The mucus gel layer also acts as a trap for microorganisms, including pathogens, preventing their access to the epithelium.(6) Mucins of the intestinal mucosa are high molecular weight glycoproteins consisting of 80% carbohydrates and 20% protein.(7)

Although the intestines are usually considered anaerobic, the tissues surrounding the lumen are quite rich in oxygen and oxygen diffuses into the intestines and, in addition, oxygen is present in the swallowed air. That is, the intestines are not strictly anaerobic; therefore, the ability to have aerobic respiration provides *E. coli* with a great competitive advantage.(8)

***Corresponding author:** Elena A. Mikhailova. Institute of Physiology of Komi Science Centre of the Ural Branch of the RAS, FRC Komi SC UB RAS. Syktyvkar, Russia. E-mail: elkina@physiol.komisc.ru*

Most carbohydrates in the colon are in the form of mucosal polysaccharides, which degrade with resident anaerobes that dominate the intestinal biota.[9] Monosaccharides or disaccharides released from mucin and other mucosal glycoproteins support the growth of many intestinal bacteria such as *E. coli*, which do not form polysaccharide-degrading enzymes.[10]

In the literature, information on the growth characteristics of indigenous bacteria of the gastrointestinal tract of humans and animals, including *E. coli*, on pectic polysaccharides is quite limited and there are only few reports.[11,12]

The present work is devoted to the study of the growth of the gram-negative bacterium *E. coli* ARCIM B-8208 on pectins and products of their enzymatic hydrolysis.

Materials and Methods

Object of study

The object of the study was the gram-negative bacterium *E. coli* ARCIM B-8208. The culture was maintained on mowed MPA at a temperature of +4°C.

Conditions of cultivation of E. coli and conditions of cultivation of callus cultures

The seed was obtained by deep cultivation of *E. coli* for 2 days in Erlenmeyer flasks with a working volume of MPB of 200ml with stirring (220 rpm) and a temperature of +25°C.

To study the growth characteristics, we studied deep cultivation of *E. coli* under static conditions in Erlenmeyer flasks with a working volume of 50ml of liquid nutrient medium both at a temperature of +37°C and at room temperature.

The ability of *E. coli* to grow on solid media was studied in Petri dishes. The surface of the solid media was seeded with a suitably diluted bacterial inoculum and incubated at a temperature of +37°C.

Solid and liquid nutrient media were used in the experiments. Solid media: MPA, must agar (MA), solid media with different pectins. Liquid media: MPB, mineral medium (MM). The composition of the MPA: meat broth - 1 l, NaCl - 5 g/l, peptone - 10 g/l, agar - 30 g/l. The composition of the MPB: meat broth - 1 l, NaCl - 5 g/l, peptone - 10 g/l. MM composition (g/l): KH_2PO_4 – 0.25, NH_4Cl – 0.5, $MgSO_4 \cdot 7H_2O$ – 0.1, $CaCl_2$ – 0.005, $FeSO_4 \cdot 7H_2O$ – 0.005.

The following pectins were used as difficult metabolizable carbon sources in solid or liquid nutrient media: low-methyl esterified commercial AP AU701 with a degree of methoxylation of 36%-44% and with the molecular weight of 406 kDa (AP, Herbstreith & Fox KG, Germany), low-methyl esterified pectins (6-22%) from callus cultures of tansy *Tanacetum vulgare* L. (TV),[13] duckweed *Lemna minor* L. (LM),[14] and campion *Silene vulgaris* (M.) G. (SV)[15] with a molecular mass of >300 kDa. For comparison, *E. coli* was also cultivated in media with easily metabolizable carbon sources – glucose and lactose.

Callus cultures of tansy, duckweed and campion were grown on modified Murashige and Skoog agar medium.[16] The campion and duckweed callus cultures were cultivated with 1.0 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l of 6-benzylaminopurine (BAP) added to the medium. The tansy callus was cultivated with the addition of 2,4-D(1.5mg/l)+BAP(0.5mg/l). The calluses were subcultured with an interval of 21 days (campion) and 28 days (tansy and duckweed) at the temperature of 26±1°C in the dark. The callus tissue was frozen at the end of the cultivation.[17,18] Pectins from callus cultures were isolated in the group of biotechnology of the Department of Molecular Immunology and Biotechnology of the Institute of Physiology.

Formation of pectic gels and CaPGPs

Pectic gels and spherical CaPGPs were obtained from AP and pectins of callus cultures in the presence of calcium ions by the method of ionotropic gelling.[19]

To obtain CaPGPs, pectins (30 mg) were dissolved in distilled water (1 ml) by slow stirring with an MM-5 magnetic stirrer (Russia) for 2-5 hours at room temperature until complete dissolution.

Gel particles of spherical shape were prepared by drop-by-drop injection of the pectin solution (3%) from a syringe through a needle with a hole diameter of 0.6 mm on a distance of 4-5 cm in the slowly stirred solution of calcium chloride (0.34 M) and further stirring for 20 minutes at room temperature. The resulting gel particles were then washed three times in distilled water with stirring for 5 minutes and dried for 10-14 hours at + 37°C.

Obtaining products of enzymatic degradation of CaPGPs

An artificial gastrointestinal medium was used to obtain degradation products of CaPGPs.

The swelling and degradation of CaPGPs were studied under conditions simulating the gastrointestinal fluid, namely, SGF solution (pH 1.25), SIF solution (pH 7.0) and SCF solution (pH 7.0). The SGF medium was prepared with NaCl (2.0 g/l), KCl (1.12 g/l), KH_2PO_4 (0.4 g/l) and $CaCl_2$ (0.11 g/l). The pH of the solution was adjusted to 1.25 by addition of 0.1N HCl solution. The SIF medium was prepared by addition of 1N $NaHCO_3$ solution to the SGF solution to the pH value of 7.0. The SCF medium was prepared by addition of pectinase (Sigma, 1.18 U/mg, USA) to the SIF solution.[20] The concentration of pectinase in SCF medium was 1.7 mg/ml (2.0 U/ml).

10 mg of dry gel particles of tanacetan pectin were placed in Petri dishes (diameter 3.5 cm) and subsequently incubated in 3 ml of the SGF (2 h), SIF (4 h) and SCF (18 h) solutions with shaking on a shaker (Titramax 1000, Heidolph, Germany) at 100 rpm and at +37 °C. The enzymatic hydrolysis products of tanacetan pectin (PH-TV) obtained in SCF medium were used as a carbon source to study the growth of *E. coli* on them.

Determination of growth parameters of E. coli

The optical density of *E. coli* suspensions during growth in liquid nutrient media was measured on an SF-103 spectrophotometer (Russia) at a wavelength of 660 nm.

The ability to grow *E. coli* in solid media was evaluated by the number of CFU (colony forming unit) by the Koch method. Samples (10 ml) were taken aseptically from bacterial suspensions and a series of 10-fold dilutions were prepared. From the obtained dilutions, 50 µl of aliquots of bacteria were sown on the surface of solid nutrient media. Petri dishes with crops were incubated at a temperature of +37°C. The grown colonies were counted on Days 1, 2, 3, 4, and 5 of incubation.

The number of CFU/ml was determined as the average of three replicates. Each experiment was carried out in three independent experiments.

When bacteria grew in liquid media with soluble carbon sources, the number of CFU in 1 ml of bacterial suspensions was calculated using a calibration graph of the dependence of CFU/ml on the optical density of the bacterial suspension (OD_{660}) (Fig. 1).

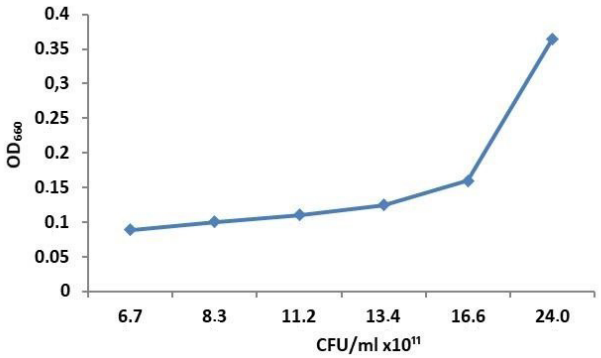

Fig. 1. *Calibration graph of the dependence of CFU/ml on OD_{660} of bacterial suspension.*

The statistical analysis was performed using the statistical software BioStat (version 4.03) and Microsoft Office Excell 2007. The mean (M) and standard deviation (SD) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

We studied the ability of the gram-negative bacterium *E. coli* ARCIM B-8208 to grow on the surface of solid pectin-containing media. Sources of pectins were commercial AP and the pectins tanacetan, lemnan, and silenan, isolated from callus cultures of tansy *Tanacetum vulgare* L., duckweed *Lemna minor* L. and campion *Silene vulgaris* (M.) G., respectively. Low methoxylated pectins, such as AP, tanacetan, lemnan, and silenan, form gels in the presence of calcium ions, and pectin molecules are cross-linked by calcium ions.[21] For comparison, MPA and MA, the standard media for the cultivation of bacteria, were used as solid media. The ability of solid media to modulate the formation of *E. coli* biofilms was evaluated by the number of CFU.

The most active colony formation occurs with the growth of *E. coli* on MA and MPA (Fig.2).

After 5 days of growth on MA and MPA, the number of *E. coli* colonies was $(14.3\pm1.8)\times10^{12}$ and $(13.0\pm2.6)\times10^{12}$ CFU/ml, respectively. With growth on gel of 3% AP, the formation of *E. coli* colonies was observed, like on MPA and MA, also after 2 days of growth, but in a smaller amount, and after 5 days of cultivation on AP, the number of CFU/ml was $(4.6\pm0.9)\times10^{12}$. On the contrary, the formation of *E. coli* colonies did not occur with growth on 3% gels of tanacetan, lemnan and silenan. Thus, gels obtained from callus pectins did not modulate the formation of *E. coli* biofilms.

We studied the effect on the growth of *E. coli* of enzymatic hydrolysis products of CaPGPs obtained under artificial gastrointestinal conditions. CaPGPs were obtained from the pectin tanacetan polysaccharide isolated from callus of tansy *Tanacetum vulgare* L.

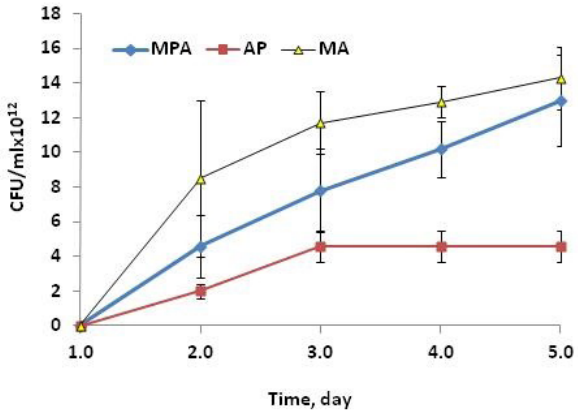

Fig. 2. *Growth of E. coli on solid media.*

The experiments were carried out under conditions of deep periodic cultivation in flasks, with stirring (220 rpm) and without stirring (under static conditions) *E. coli* culture broths at room temperature. It was found that more active bacterial growth occurs under static conditions, without stirring, and the amount of *E. coli* after 6 days of growth in a liquid MM with 1% glucose was $(18.5\pm0.2)\times10^{11}$ CFU/ml against $(13.2\pm0.1)\times10^{11}$ CFU/ml with stirring (Fig.3).

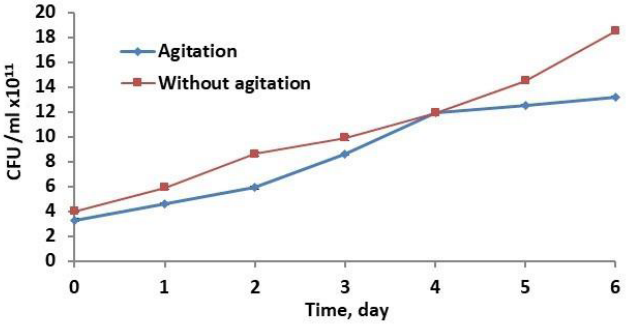

Fig. 3. *Growth of E. coli in liquid mineral medium (MM) with 1% glucose.*

We estimated the growth of *E. coli* in a liquid MM with both easily and hardly metabolizable carbon sources under static conditions at +37°C (Fig.4). The most active growth of bacterium occurred in a mineral medium with easily metabolized carbon sources – lactose and glucose. The products of enzymatic hydrolysis of CaPGPs – oligogalacturonides obtained under conditions of an artificial gastrointestinal environment – contributed more to the growth of *E. coli* than did pectin tanacetan. The number of bacteria after 4 days of growth was $(43.5\pm0.6)\times10^{11}$ and $(25.5\pm0.2)\times10^{11}$ CFU/ml, respectively.

Thus, the gels of tanacetan, lemnan, and silenan do not contribute to the growth of *E. coli* ARCIM B-8208. When growing on a gel of AP, a weak bacterial growth was

detected. However, *E. coli* was capable of growth on soluble products of enzymatic hydrolysis of tanacetan pectin – oligogalacturonides.

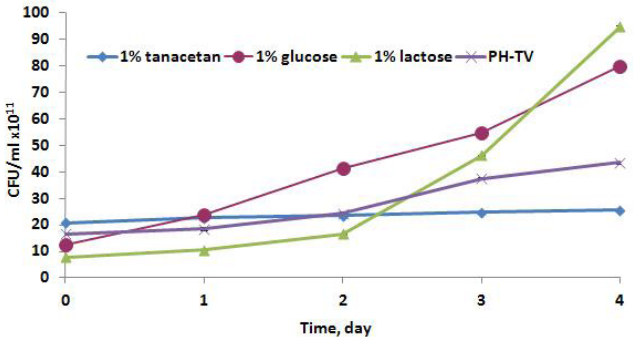

Fig. 4. *Growth of E. coli in a liquid mineral medium with different carbon sources.*

Apparently, in the colon, *E. coli* was also capable of growth on the products of enzymatic hydrolysis of pectins formed under the action of pectinases synthesized by the corresponding symbiotic microflora.

Competing interests

The authors declare that they have no competing interests.

Sources of Funding

The work was performed on the theme of research work (State registration number AAAA-A17-117012310147-8).

Acknowledgments

We express our gratitude to Dr. Elena Günter and Oksana Popeyko for kindly provided samples of pectins from callus cultures.

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