DOI 10.1007/s10517-024-06281-y

IMMUNOLOGY AND MICROBIOLOGY

Evaluation of the Biological Properties of the *Bacteriodaceae* Family Using the Caco-2 Cell Line

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 178, No. 7, pp. 65-68, July, 2024
Original article submitted April 8, 2024

Disorders in the composition of the microbiota are often associated with pathological processes in the body. The use of new biotherapeutic drugs containing bacteria from normal microflora to correct these disorders may contribute to more effective treatment. The *Bacteroidaceae* family dominating in microflora is a candidate for inclusion in bacterial preparations. The adhesion properties of five bacterial strains from the *Bacteroidaceae* family were analyzed using the Caco-2 cell line and their effect on the transepithelial electrical resistance parameters (TEER) was assessed to determine the extent of their impact on the cell monolayer integrity. This approach allowed the selection of strain *B. intestinalis* 181, which was characterized by a moderate adhesion activity combined with a relatively low effect on the TEER parameter.

Key Words: *Bacteroidaceae; adhesion; TEER; Caco-2; new biotherapeutic drugs*

Bacteria of the *Bacteroidaceae* family are the dominant group in the colon microbiota [1,2]. It is thought that one of the reasons for the successful adaptation of *Bacteroidaceae* to inhabit this ecological niche is their ability to utilize indigestible plant polysaccharides that enter the distal digestive tract with food. This is confirmed by the wide distribution of numerous polysaccharide utilization loci in the genomes of members of this family [3,4].

Due to the ability of Bacteroidetes to form and secrete outer membrane vesicles containing a variety of hydrolytic enzymes and metabolites, it has been shown that vesicles are the source of the nutrient substrates for both within their own family and other taxonomic groups [5]. It has been shown that the *Bacteroides thetaiotaomicron* vesicles contain glycosyl hydrolases that are involved in levanes cleavage, the hydrolysis products of which can be utilized by other bacteria [6]. *B. ovatus* vesicles contain glycosyl hydrolases that cleave inulin to metabolites utilized by *B. vulgatus* [7]. In addition, *B. thetaiotaomicron* can alter flavonoid fermentation (*e.g.*, quercetin) and butyrate production by *Eubacterium ramulus* [8], providing antiproliferative activity of the microbiota against colon tumor cells [9]. Thus, *Bacteroidaceae* family may not only contribute to the microbial ecosystem formation and participate in digestive processes, but they also play a beneficial role in human health [10].

On the other hand, when developing new biotherapeutic drugs, it is important to consider the colonization activity of microorganisms included in them, particularly their ability to adhere to intestinal epithelium. Additionally, the study of promising bacteria for probiotic drug development should include assessing

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their ability to damage intestinal epithelium and invade, which could indicate pathobiotic properties.

The aim of this research was to investigate the biological properties of four *Bacteroides* and one *Phocaeicola* strains *in vitro*, including its adhesion activity and effect on cell monolayer integrity using the Caco-2 cell line.

MATERIAL AND METHODS

Bacterial strains of the genus *Bacteroides* (*B. thetaiotaomicron* 6-237, *B. stercoris* 5888, *B. xylanisolvens* Pik, and *B. intestinalis* 181) and *Phocaeicola coprocola* strain EBA 6-21 were used in this study. These strains were isolated and stored in the Department of Microbiology and Virology, Pirogov Russian National Research Medical University [5,11]. *Lacticaseibacillus rhamnosus* DSM 8743 and *E. coli* ATCC 8739 were used as a positive control for the determination of adhesion activity and for the determination of transepithelial electrical resistance (TEER), respectively.

Test tubes containing lyophilized microbial strains were stored at -80°C. Prior to testing, strains were plated on Anaerobe Basal Agar (Oxoid) supplemented with 5% sheep blood (v/v). After incubation at 37°C for 48 h under anaerobic conditions, the strains were subcultured in Schaedler Anaerobe Broth (Oxoid). *L. rhamnosus* and *E. coli* strains were seeded on MRS agar (Himedia Labs, Inc.) and Columbia agar (Oxoid), respectively, and incubated at 37°C and 5% CO_2 for 24 h (*L. rhamnosus*) and in aerobic conditions (*E. coli*). These strains were further subcultured in MRS broth (Himedia Labs, Inc.) or Schaedler anaerobic broth for 24 h under the same conditions.

The human intestinal cell line Caco-2 was kindly provided by Dr. I. V. Arutyunyan, Head of the Laboratory of Cell Technologies and Tissue Engineering, Research Institute of Molecular and Cellular Medicine, Peoples' Friendship University of Russia named after Patrice Lumumba. Cells were cultured in high glucose DMEM medium (HG-DMEM, Capricorn Scientific) supplemented with 10% (v/v) fetal bovine serum (Capricorn Scientific), 100 IU/m penicillin, 100 μg/ml streptomycin, and 1% L-glutamine (Corning). For analysis of bacterial adhesion to the epithelium, 6×10^4 cells/well were seeded into 12-well microplates (Nest) and cultured for 21 days to obtain a homogeneous and polarized cell monolayer. After 21 days, the cells were washed twice with phosphate buffer (Amresco), and 1 ml of bacterial suspension (in HG-DMEM medium without antibiotics) was added at 1.5×10^9 CFU/ml (5 McFarland units). The microplates were then incubated for 3 h at 37°C in a 5% CO_2 atmosphere and washed 3 times with phosphate buffer to remove non-adherent bacteria. Caco-2 cells and adherent bacteria were resuspended in 1 ml phosphate buffer using a cell scraper. After that DNA was extracted (HiPure Universal DNA Kit) and the specific 16S rRNA region was amplified using the general bacterial primers 27F (5-AGAGAGTTTGATCCTGGCTCAG-3) and 1492R (5-ACGGGGYTACCTTGTTACGACTT-3) (Eurogen). The following formula was used to calculate the initial concentration of matrix in the sample:

$$N=2(40-n),$$

where n is the threshold cycle, N is the amount of matrix in the PCR sample at the start of the reaction [12].

TEER of the Caco-2 monolayer cell line were assessed using the Millicell ERS-2 system (Millipore). Caco-2 cells were seeded in 24-well Transwell filter (on the apical side of a 0.4-μm polycarbonate membrane; Nest) at a density of $10^5/cm^2$ in culture medium as described previously. After 21 days, the cells were washed twice with phosphate buffer and bacterial suspensions were prepared in HG-DMEM medium and applied to the surface of the cell monolayer in a volume of 200 μl per Transwell with a bacteria/cell ratio of 10:1. TEER were measured before bacterial inoculation (T0) and after 24 h (T24) incubation at 37°C, 5% CO_2. The change in TEER was calculated by comparing the electrical resistance obtained after 24 h of incubation with cells and bacteria deposited on them with initial resistance [12,13].

Statistical processing of the results obtained was performed in Microsoft Excel 16.79.1). The significance of the differences in the obtained data was evaluated using the two-sample Student's *t* test for independent samples. The data are presented as the mean (at least 3 experiments) and standard deviation ($M\pm SD$). The differences were considered statistically significant at $p<0.05$.

RESULTS

The data obtained from the study of the adhesion of bacteria from the genus *Bacteroides* and *Phocaeicola* indicates that all strains tested adhere to the Caco-2 cell monolayer. The adhesion activity of the strains ranged from $8.5\pm1.0\times10^7$ bacteria/cm^2 (*B. thetaiotaomicron* 6-237) to $4.4\pm0.3\times10^8$ bacteria/cm^2 (*B. xylanisolvens* Pik). At the same time, *B. intestinalis* 181 and *P. coprocola* EBA 6-21 showed adhesion activity relatively close to that of the positive control, *L. rhamnosus* DSM 8743. The strains *B. stercoris* 5888 ($p=0.007$) and *B. xylanisolvens* Pik ($p=0.001$) had the most pronounced and statistically significant adhesion activity, whereas *B. thetaiotaomicron* 6-237 ($p=0.02$) had the lowest adhesion activity (Fig. 1).

Compared to the negative control, all five strains tested significantly reduced the TEER of the Caco-2 monolayer in the range of 9-12%. *B. intestinalis* 181

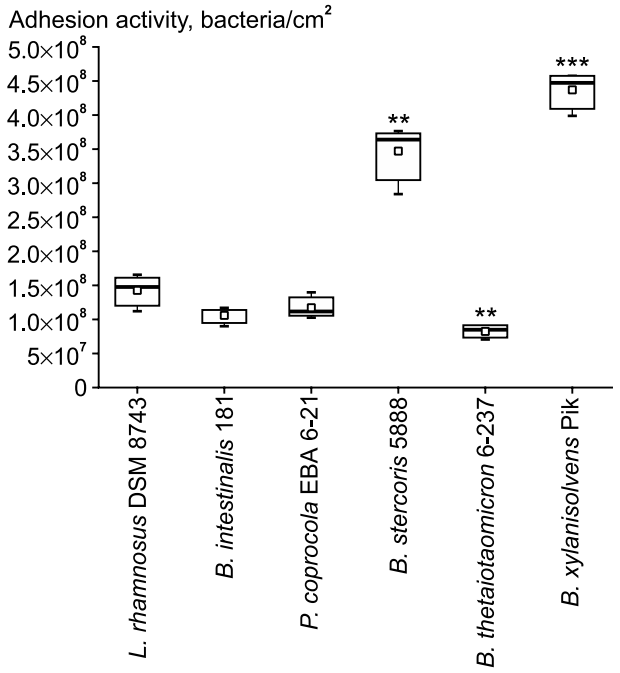

Fig. 1. Adhesion properties of four *Bacteroides* strains, one *Phocaeicola* strain, and *L. rhamnosus* DSM 8743 (positive control) on Caco-2 cells after 3 h incubation at 37°C and 5% CO_2. ***p<0.001, **0.001<p<0.01 in comparison with positive control (two-sample Student's t test).

caused a less pronounced change in the level of contacts in the Caco-2 monolayer compared to the other strains, reducing TEER by only 9% compared to the control. Furthermore, the effect of *B. intestinalis* 181 on TEER was significantly less pronounced than that of *B. thetaiotaomicron* 6-237 (p=0.04) and *B. stercoris* 5888 (p=0.04) (Fig. 2).

Thus, among the strains studied, only *B. intestinalis* 181 reduced the density of intercellular contacts in the monolayer of Caco-2 cells to a lesser extent, while also exhibiting moderate adhesive activity. The approaches we have used to evaluate the biological properties of bacterial commensals in the gastrointestinal microbiota can be used as additional steps in selecting strains for the production of new biotherapeutics based on them.

The research was supported by Russian Science Foundation (grant No. 21-75-10172).

Conflict of interest. The authors have no conflicts of interest to declare.

REFERENCES

1. Kulagina EV, Efimov BA, Maximov PY, Kafarskaia LI, Chaplin AV, Shkoporov AN. Species composition of *Bacteroidales* order bacteria in the feces of healthy people of various ages. Biosci. Biotechnol. Biochem. 2012;76(1):169-171. doi: 10.1271/bbb.110434
2. Kim S, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. Immunol. Rev. 2017;279(1):90-105. doi: 10.1111/imr.12563
3. Grondin JM, Tamura K, Déjean G, Abbott DW, Brumer H. Polysaccharide utilization loci: fueling microbial communities. J. Bacteriol. 2017;199(15):e00860-16. doi: 10.1128/JB.00860-16
4. Foley MH, Cockburn DW, Koropatkin NM. The Sus operon: a model system for starch uptake by the human gut Bacteroidetes. Cell Mol. Life Sci. 2016;73(14):2603-2617. doi: 10.1007/s00018-016-2242-x
5. Shagaleeva OY, Kashatnikova DA, Kardonsky DA, Konanov DN, Efimov BA, Bagrov DV, Evtushenko EG, Chaplin AV, Silantiev AS, Filatova JV, Kolesnikova IV, Vanyushkina AA, Stimpson J, Zakharzhevskaya NB. Investigating volatile compounds in the *Bacteroides* secretome. Front. Microbiol. 2023;14:1164877. doi: 10.3389/fmicb.2023.1164877
6. Poeker SA, Geirnaert A, Berchtold L, Greppi A, Krych L, Steinert RE, de Wouters T, Lacroix C. Understanding the prebiotic potential of different dietary fibers using an in vitro continuous adult fermentation model (PolyFermS). Sci. Rep. 2018;8(1):4318. doi: 10.1038/s41598-018-22438-y

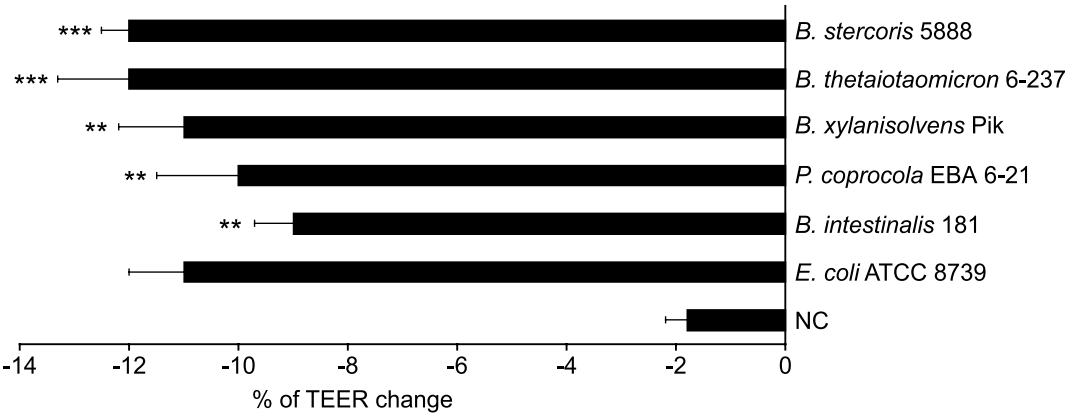

Fig. 2. Effect of four *Bacteroides* strains, one *Phocaeicola* strain, and *E. coli* ATCC 8739 (positive control) on TEER of Caco-2 cell monolayer after 24-h incubation at 37°C and 5% CO_2. NC: Caco-2 cells treated with HG-DMEM medium (negative control). ***p<0.001, **p<0.01 in comparison with negative and positive controls (two-sample Student's t test).

7. Rakoff-Nahoum S, Foster KR, Comstock LE. The evolution of cooperation within the gut microbiota. Nature. 2016;533:255-229. doi: 10.1038/nature17626
8. Rodriguez-Castaño GP, Dorris MR, Liu X, Bolling BW, Acosta-Gonzalez A, Rey FE. Bacteroides thetaiotaomicron starch utilization promotes quercetin degradation and butyrate production by Eubacterium ramulus. Front. Microbiol. 2019;10:1145. doi: 10.3389/fmicb.2019.01145
9. Gao K, Xu A, Krul C, Venema K, Liu Y, Niu Y, Lu J, Bensoussan L, Seeram NP, Heber D, Henning SM. Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity. J. Nutr. 2006;136(1):52-57. doi: 10.1093/jn/136.1.52
10. Zafar H, Saier MH Jr. Gut Bacteroides species in health and disease. Gut Microbes. 2021;13(1):1-20. doi: 10.1080/19490976.2020.1848158
11. Efimov BA, Chaplin AV, Sokolova SR, Chernaia ZA, Pikina AP, Savilova AM, Kafarskaya LI. Application of culture-based, mass spectrometry and molecular methods to the study of gut microbiota in children. Bull. RSMU. 2019;4:54-65. doi: 10.24075/brsmu.2019.048
12. Chamarande J, Cunat L, Pavlov N, Alauzet C, Cailliez-Grimal C. *Parabacteroides distasonis* properties linked to the selection of new biotherapeutics. Nutrients. 2022;14(19):4176. doi: 10.3390/nu14194176
13. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. TEER measurement techniques for in vitro barrier model systems. J. Lab. Autom. 2015;20(2):107-126. doi: 10.1177/2211068214561025