CHARACTERIZATION OF THE ADHESIVE PROPERTIES OF *BACILLUS INTESTINALIS* GM2

A.A. Nikolaeva, G.F. Lutfullina, A.M. Mardanova*

Kazan Federal University, 18 Kremliovskaya St., Kazan, 420008, Russia.

* Corresponding author: azazel1212@rambler.ru

Abstract. In this paper, we present the data on the properties of the probiotic strain *Bacillus intestinalis* GM2 related to adhesion, auto-, and coaggregation. GM2 exhibits a strong autoaggregation phenotype. The autoaggregation ability of GM2 did not change after trypsin treatment but was reduced under the influence of extracellular culture metabolites. Coaggregation of probiotic strains with pathogens is one of the mechanisms of displacement of pathogens from the intestinal microbiota. It was shown that the GM2 strain exhibits the ability to coaggregate with tested strains of Escherichia coli, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*. The studied properties of bacilli can be useful for the creation of new probiotics for poultry farming.

Keywords: probiotics, *Bacillus* subtilis GM2, adhesion, autoaggregation, coaggregation, biosurfactants.

List of Abbreviations

PBS – phosphate-buffered saline
LB – lysogeny broth
SMN – soybean medium nutrition

Introduction

To improve the performance of farm animals, nutritional measures are developed, primarily aimed at changing the functions of the gastrointestinal tract. Probiotics and prebiotics are potential alternatives to antibiotics in livestock production. Probiotic strains limit direct contact of pathogenic bacteria with the gut epithelium by competitively excluding nutrients and surfaces, as well as by creating a hostile environment and producing bioactive compounds acting selectively on pathogens (Binns *et al*., 2013; Celi *et al*., 2019; Peralta-Sánchez *et al*., 2019).

To exhibit beneficial effects, it is vital that probiotic bacteria achieve a specific cellular concentration through aggregation (Collado *et al*., 2008). Autoaggregation, or autoagglutination, is one of the means, by which cells adapt to environmental conditions. By binding bacteria to each other, it increases the relative adaptability of aggregates compared to single cells in the space and resource-constrained environment that is the gut environment (Kragh *et al*., 2016; Isenring *et al*., 2021; Nwoko & Okeke, 2021). In addition, autoaggregation facilitates biofilm formation, which also enhances adaptability to the environment through increased cell density and cell-to-cell interaction (Giaouris *et al*., 2015; Laganenka *et al*., 2016; Kragh *et al*., 2016). Nonetheless, it has been reported that coaggregation does not always lead to biofilm formation (Hiramatsu *et al*., 2016).

The genus *Bacillus* includes rod-shaped, endospore-forming, saprophytic soil aerobes. Several strains of the *Bacillus* genus have been isolated from the feces of broiler chickens, which suggests their presence in the intestines of birds (Barbosa *et al*., 2005). The ability of these microorganisms to proliferate and sporulate in the gut suggests a high potential for transit between the soil (natural reservoir) and the gut. It has been confirmed that *Bacillus* spores are able to germinate in the small intestine and interact with enterocytes, immunocompetent cells, and/or the intestinal microbiota of the host (Barbosa *et al*., 2005; Menconi *et al*., 2013).

Biosurfactants have both hydrophilic and hydrophobic moieties, causing aggregation at interfaces between liquids with different polarities, such as hydrocarbons and water. Thus, they reduce surface and interfacial tension as well as form emulsions (Sarwar *et al*., 2018). Compared to chemical or synthetic surfactants, biosurfactants offer several advantages, including high biodegradability, low toxicity, and effectiveness at extreme temperatures or over a

wide pH range. Lipopeptides rank first among the numerous classes of biosurfactants. The interest in lipopeptides is mainly due to their high surface activity and antimicrobial potential (Vaz *et al*., 2012). *Bacillus* strains are known to produce a wide range of lipopeptides: fengicins, iturins, mycosubtilins, bacillomicins, and surfactins with great potential for biotechnology and biopharmaceutical industries (Perez *et al*., 2017). Biosurfactants change the hydrophobicity of the bacterial surface and therefore are able to regulate the adhesion of microorganisms to solid surfaces. The involvement of bacterial biosurfactants in microbial adhesion and desorption has been extensively described by Rodrigues *et al*. (Rodrigues *et al*., 2011). The preadsorption of biosurfactants on solid surfaces can be an effective strategy to reduce microbial adhesion and prevent colonization by pathogens (Gudina *et al*., 2010).

We have previously shown that the *Bacillus* intestinalis GM2 strain exhibits high antagonistic activity against pathogenic and opportunistic enterobacteria, resistance to bile and a wide range of pH environments, as well as the ability to synthesize proteolytic and phytate hydrolyzing enzymes (Hadieva *et al*., 2018). In addition, in vivo studies have shown a positive effect of a feed additive based on *B. intestinalis* GM2 spores in the diet of Cobb-500 broiler chickens on growth performance and nutrient digestibility (Hadieva *et al*., 2019).

This study aimed to evaluate the adhesive properties of the *B. intestinalis* GM2 strain and its ability to produce biosurfactants and to coaggregate with strains of opportunistic bacteria.

Materials and Methods

Bacterial strains and growth conditions

Strains of *B. intestinalis* GM2 and *B. subtilis* GM5 with high antagonistic activity were isolated from the potato rhizosphere (Mardanova *et al*., 2017). *B. subtilis* strain VKPM B-10641, which was isolated from the commercial probiotic Vetom 1.1, was used for comparative assays. For comparative analysis of coaggregation, the following strains were used: *Escherichia coli* isolated from the urethra and provided by the clinical and diagnostic laboratory BIOMED of Kazan, as well as strains of *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus* obtained from the culture collection of the Department of Microbiology of KFU.

Bacteria were cultured in LB (Lysogeny broth) medium (g/l): tryptone – 10.0, yeast extract – 5.0, NaCl – 5.0; LA medium (g/l): tryptone – 10.0, yeast extract – 5.0, NaCl – 5.0, agar – 20.0. To study the synthesis of biosurfactants by *B. intestinalis* GM2 strain, soybean medium nutrition (SMN) (g/l): mannitol – 26.2, soybean meal – 21.9, $NaNO_3$ – 3.1, $MnSO_4 \times 4H_2O$ – 0.2 (pH 7.5) was used. Bacteria were cultured at 37 °C. When necessary, cultures were maintained in a shaker thermostat at 37 ºC and aerated at 200 rpm.

Autoaggregation and coaggregation assays

The study was performed as described in (Kos *et al*., 2003) with certain modifications. Night culture cells were harvested by centrifugation, washed twice, and resuspended in phosphate-buffered saline (PBS) or in culture supernatant fluid to obtain $OD_{600} = 0.3 \pm 0.05$ (10^7–10^8 CFU/ml). The cell suspension (3 ml) was mixed for 10 s and then incubated at room temperature for 24 h without aeration. The optical density (OD_{600}) of the top of the suspension was measured after 2, 4, 6, and 24 hours of incubation on a spectrophotometer (Bio-Rad xMark, U.S.A.) The percentage of autoaggregation was expressed as:

$$A\ (\%) = 1 - (A_t / A_0) \times 100,$$

where A_t is the optical density at time t = 2, 4, 6, and 24 h, and A_0 is the optical density at 0 h.

The method of preparing the bacterial suspension for the coaggregation assay was the same as in the autoaggregation assay. Equal volumes (1.5 ml) of each bacterial cell suspension for coaggregation were mixed in pairs by shaking for 10 s. The control variants each contained 3 ml of a suspension of axenic bacterial cultures. The absorbance (A) at 600 nm was measured immediately after mixing, at 4 and 24 h of incubation at room temperature. The percentage of coaggregation was calculated as follows:

$$A_o\ (\%) = [(A_{pat} + A_{probio})/2 - \\ - (A_{mix})/(A_{pat} + A_{probio})/2] \times 100,$$

where A_{pat} and A_{probio} represent A 600 of the separate bacterial suspensions in control tubes at 0 h, and A_{mix} represents the absorbance of the mixed bacterial suspension (Collado *et al*., 2008).

Trypsin treatment of bacterial cells

For trypsin treatment, overnight culture cells were washed in PBS, then 100 µl of 10x trypsin was added to 900 µl (OD_{600} = 1) of the suspension, cultured at 37 °C for 1 hour, then treated as described above.

Adhesion ability to polystyrene

To study the adhesive properties, overnight culture cells were diluted with fresh sterile medium to OD_{600} = 0.5, then 1.0 ml of diluted cultures were introduced into polystyrene Petri dishes (4 cm diameter), which were incubated for 4 h at 30 °C. Next, non-adherent cells were drained and washed with sterile saline. Adherent bacteria were stained with gentian violet solution for 2 min and then the non-adherent dye was washed off. E. coli and S. aureus strains were used to study coadhesion and coaggregation of *B. intestinalis* GM2 in mixed culture. Equal volumes (0.5 ml) of each cell suspension were mixed by suspension and introduced into polystyrene Petri dishes. After incubation and staining, they were microscopically examined using the MICROS AUSTRIA MC 300 (Austria) equipment at a magnification of 100x.

Measurement of surface tension

The dynamics of biosurfactant synthesis by *B. intestinalis* GM2 strain were tested by reducing the surface tension of the culture medium. The change in surface tension was determined by the Dubois Ring method, as described earlier (Rodriguez *et al*., 2006). The surface tension of the medium was measured on a BT-500 torsion scale (Russia) equipped with a platinum ring with a diameter of 1.9 cm. The surface tension values represent the average value.

Statistical Analysis

All experiments were performed in triplicate. Data were evaluated by ANOVA with repeated measures with a significance level of $P < 0.05$. Results are presented as mean ± standard deviation.

Results

Comparative characterization of the sedimentation rate of the three *Bacillus* strains showed that strain GM2 exhibited the strongest autoaggregation ability within 24 hours as compared with the probiotic strains *B. subtilis* GM5 and *B. subtilis* VKPM B-10641 (Fig. 1). The GM2 strain also showed a visually more transparent supernatant even at 2 h of incubation relative to the other strains.

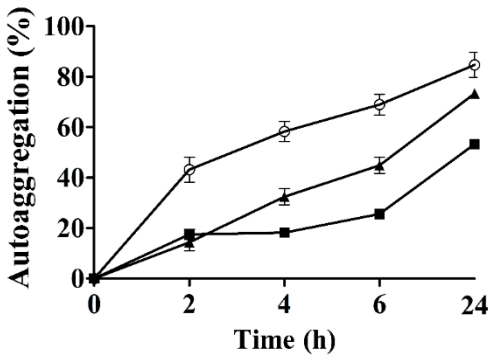

Fig. 1. Comparison of the autoaggregation ability of different probiotic *Bacillus* strains: *Bacillus* intestinalis GM2 (○), *Bacillus* subtilis GM5 (■) *Bacillus* subtilis VKPM B-1064 (▲)

The observed autoaggregation was not lost after washing and suspending the cells GM2 in PBS. At the same time, extracellular metabolites reduced the ability of the strain to form aggregates (Fig. 2), since the autoaggregation activity was significantly reduced ($p < 0.05$) when the cells were resuspended in the culture fluid in which they were initially growing. There was no significant difference in the ability to autoaggregate after treatment of the cells with trypsin.

Autoaggregation and coaggregation are important for biofilm formation by probiotic strains to protect the host from colonization by pathogens (Botes *et al*., 2008).

It was previously shown that in contrast to 37 °C, 20 °C was a more suitable temperature

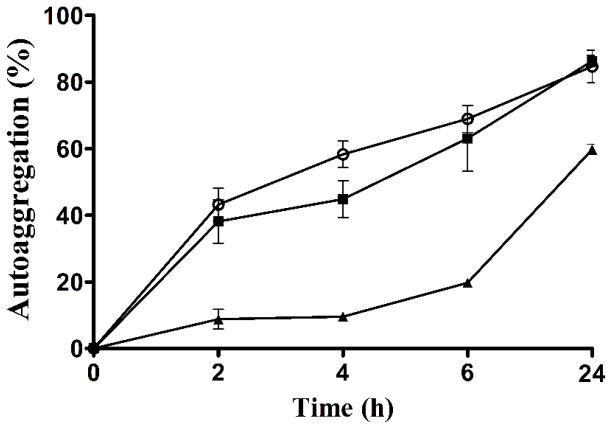

Fig. 2. Comparative analysis of the autoaggregation ability of *Bacillus* intestinalis cells resuspended in PBS (pH 7.2) (○) after trypsin treatment (■) or resuspended in their own culture supernatant fluid (▲)

Table 1

Coaggregation capacity of *Bacillus intestinalis* GM2 with potential pathogenic bacteria at room temperature in PBS (pH 7.2)

	Coaggregation with *B. intestinalis* GM2 (%)			
	4 hour		24 hour	
	Mean	SD	Mean	SD
E. coli	35,46	2,32	42,96	3,15
S. aureus	20,81	3,62	48,76	4,44
B. cereus	26,05	0,76	73,3	1,49
E. faecalis	12,33	1,57	49,2	3,89

when analyzing the ability of bacteria to coaggregate (Collado *et al*., 2008). Therefore, the coaggregation ability of the GM2 strain was tested at room temperature (Table 1). The GM2 strain showed the ability to coaggregate with the tested strains of potential pathogens, and the percentage of coaggregation depended on the incubation time. The highest percentage of bacterial coaggregation at 4 hours of incubation was with cells with E. coli (35.5%) and the lowest with E. faecalis (12.3%). At 24 hours the percentage of co-aggregation with B. cereus reached 73%, while with E. coli it did not exceed 43%. Thus, the GM2 strain showed the ability to coaggregate with all tested pathogens, but the efficiency of coaggregation depended on the test culture strain and the time of co-incubation.

The adhesive ability of the GM2 strain on the polystyrene surface was studied (Fig. 3). GM2 cells in the culture medium (LB) were attached in moderate numbers, with adhesion to the surface occurring in small groups with close contact between individual cells (Fig. 3a). A large percentage of the surface was covered with culture at 4 hours of incubation, and cell morphology was well traceable (Fig. 3b). Trypsin treatment did not reduce the adhesive ability of GM2. On the contrary, cell adhesion was observed in large aggregates (Fig. 3c, d) in contrast to GM2 cells in nutrient broth, where adhesion to the surface was observed more often in small groups of cells. The inhibition of adhesive ability for cells suspended in PBS was observed in the early hours of cultivation (Fig. 3e). GM2 cells in PBS formed large aggregates at

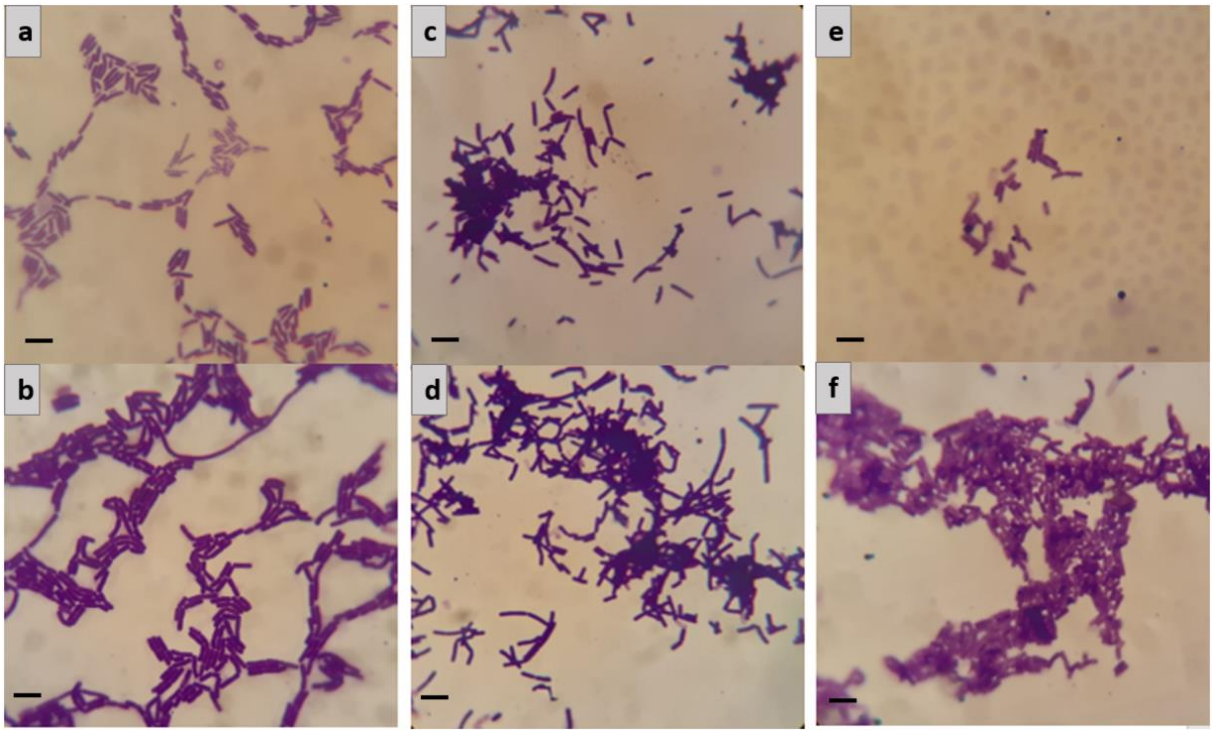

Fig. 3. Adhesion of *B. intestinalis* GM2 to the surface of polystyrene petri dishes after 2h (a, c, e) and 4h (b, d, f) incubation. *B. intestinalis* GM2 cells in LB medium (a, b), after trypsin treatment (c, d) or resuspended in PBS (pH 7.2) (e, f). Staining with gentian violet, magnification x1600. The bar corresponds to 5 μm

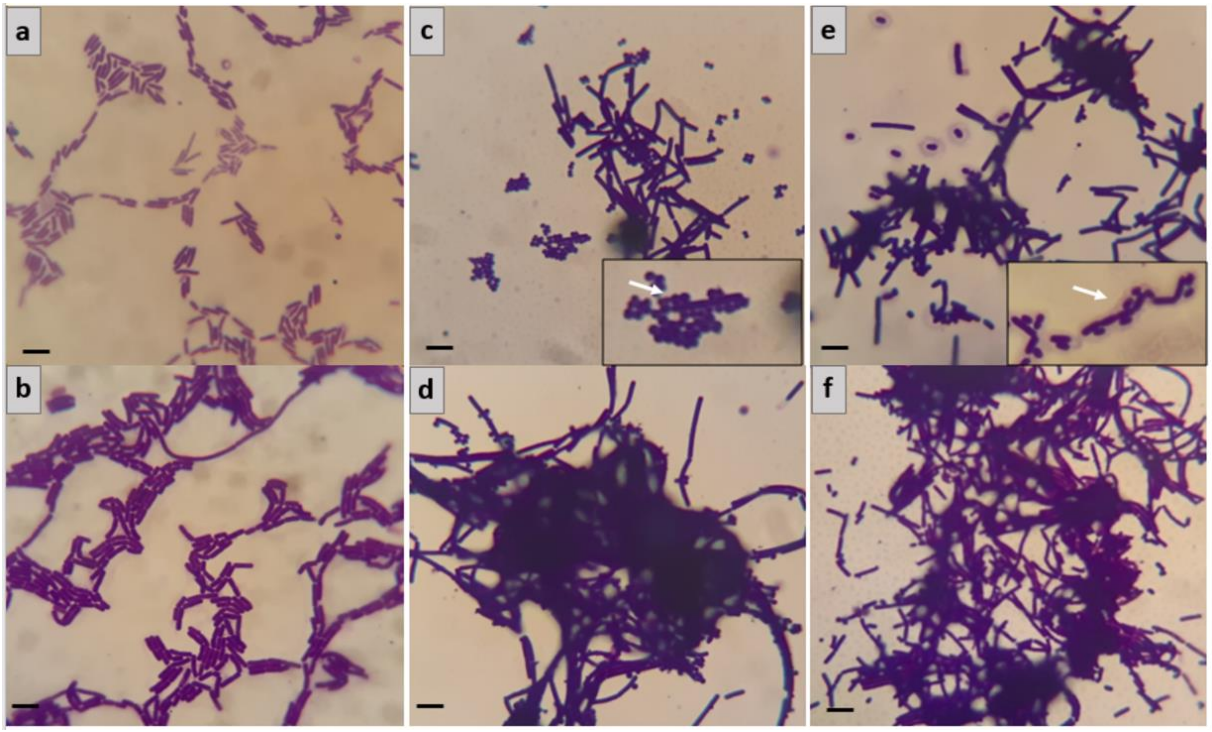

Fig. 4. Coadhesion of *B. intestinalis* GM2 to the surface of polystyrene petri dishes after 2h (a, c, e) and 4h (b, d, f) incubation. *B. intestinalis* GM2 cells in LB medium (a, b), GM2 and *Staphylococcus aureus* (c, d) or GM2 and *Escherichia coli* (e, f). Staining with gentian violet, magnification x1600. The bar corresponds to 5 μm

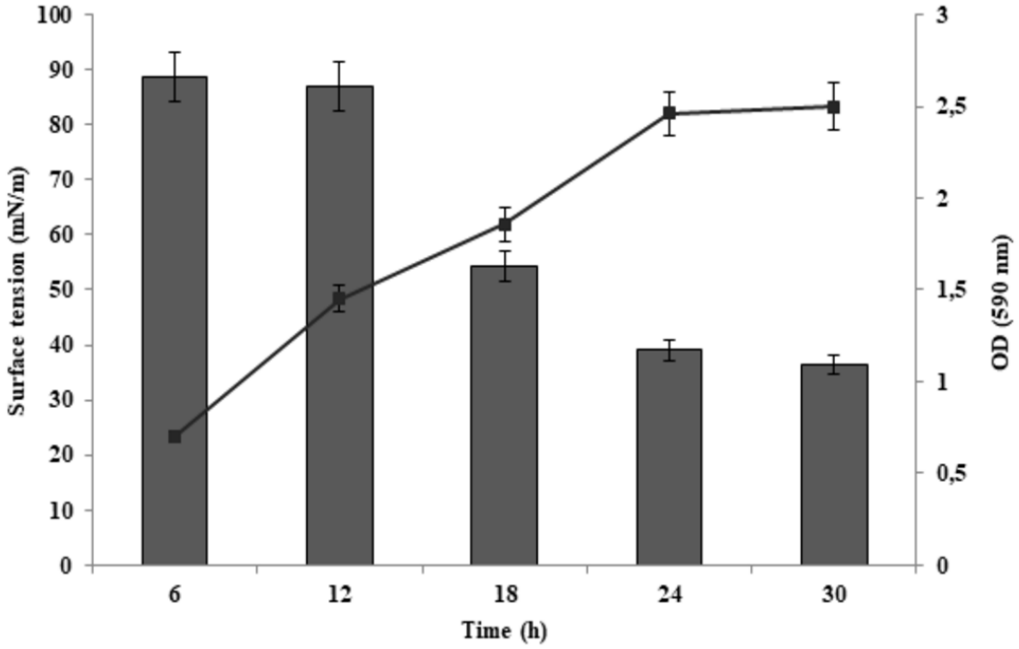

Fig. 5. Growth dynamics and changes in surface tension of culture fluid of *B. intestinalis* GM2 on SMN medium (■)

4 h, in which they adhered to the polystyrene surface (Fig. 3f). The presence of a large number of multilayer aggregates was noted. At the same time, GM2 cells were smaller in size, which may be due to nutrient deficiencies.

In the case of mixed GM2 and S. aureus cultures, individual clusters of cells were found on the polystyrene surface during microscopy at 2 hours of incubation, but at 4 hours, large multilayer bacterial aggregates were noticed (Fig. 4c, d). Moreover, the formation of these aggregates enhanced cell adhesion. Also during coincubation of GM2 and E. coli cultures, the formation and adhesion of coaggregates on the polystyrene surface were observed as early as after 2 h, which significantly increased in number after 4 h (Fig. 4e, d). Thus, (Fig. 4a, b), the coincubation of *Bacillus* cells with both test bacteria significantly increased the ability of bacteria to aggregate, coaggregate, and consequently, adhere to a solid surface in comparison with GM2 monoculture.

We studied the dynamics of bacterial growth and accumulation of biosurfactants in SMN medium exploring extracellular metabolites of *B. intestinalis* GM2 exhibiting anti-adhesion activity. When GM2 was cultured at 37ºC on SMN medium, a stationary growth phase was observed after 24 hours of growth (Fig. 5). The late shift to the stationary phase was due to the long period of the log phase during which the bacteria adapted to the conditions of the cultivation. The rationale for the inclusion of soybean meal in the GM2 strain cultivation medium was based on the use of this substrate in the industrial production of surfactants as a nitrogen-containing raw material. Soybean meal contains a balanced composition of proteins and carbohydrates that are necessary for the growth and synthesis of secondary metabolites (Zhang *et al*., 2016). Accumulation of biosurfactants by GM2 strain in the culture medium was determined by a decrease in surface tension. At the initial point, the surface tension index was at 88–90 mN/m. The surface tension was reduced after 12 h of cultivation (Fig. 5) and remained at 36 mN/m at 30 h, indicating a high level of biosurfactant synthesis by the GM2 strain. It was shown that the synthesis of biosurfactants in the cultivation medium did not negatively affect the growth of the *B. intestinalis* GM2 strain, since the proliferation and accumulation of bacterial biomass in the culture continued for 48 h. Thus, a decrease in the sur-

face tension of the cultivation medium indicates efficient synthesis of biosurfactants by *B. intestinalis* GM2 culture.

Discussion

The ability to adhere to epithelial cells and mucosal surfaces is considered an important property of many bacterial strains used as probiotics. In most cases, the ability to aggregate is related to the adhesive properties of the cells (Kos *et al*., 2003). The relationship between autoaggregation and adhesion ability has been reported for some bifidobacterial species (Del Re *et al*., 2000). It has been shown on lactobacilli how surface-mediated properties of bacteria, such as aggregation, can have a role in the adhesion and colonization of the gastrointestinal tract (Cesena *et al*., 2001). On the other hand, other studies have found no correlation between aggregation and adhesion to Caco-2 cells (Botes *et al*., 2008). These discrepancies can be explained by differences in methods. The GM2 strain exhibited a strong auto-aggregation capacity that was not lost after washing and suspending the cells in PBS and reached more than 80% at 24 h, which is consistent with studies by other authors. For instance, it has been reported that the percentages of 93.42 ± 0.86%; 86.03 ± 2.46% and 91.32 ± 0.74% were recorded for B. clausii ATCC 700160, *B. subtilis* P223, and *B. subtilis* MKHJ 1-1 after 24 hours of incubation, respectively (Jeon *et al*., 2017; Lim *et al*., 2021). In another study, the autoaggregation activity of several probiotic strains of Bifidobacterium and Lacto*Bacillus* was examined; the percentage of autoaggregation at 24 hours ranged from 31.2 ± 2.2% (B. breve 99) to 76.4 ± 8.3% (L. plantarum Lp-115) (Collado *et al*., 2008).

Autoaggregation and coaggregation are known to have an important role in biofilm formation to protect the host from colonization by pathogens (Botes *et al*., 2008). The relationship between the coaggregation abilities of bacteria and autoaggregation properties has been shown previously (Collado *et al*., 2008). It has been suggested that probiotic bacteria, by coaggregating with pathogens, produce antimicrobial compounds, or remove pathogens from the gut environment as part of coaggregates, which depicts an important mechanism for protecting the gut from infection (Spencer *et al*., 1994; Botes *et al*., 2008). The GM2 strain has been shown to be capable of coaggregating with potential pathogens, which may be useful when used as probiotic cultures.

Bacterial surface adhesion is influenced by various factors including microbial type, hydrophobicity, surface electrical charges, environmental conditions, and the ability of microorganisms to produce extracellular polymers that help cells attach to surfaces (Vijayakumar & Saravanan, 2015). Bacterial biosurfactants can be used to change the hydrophobicity of the surface, which affects bacterial adhesion. Chakrabarti *et al*., showed that a biosurfactant isolated from the culture fluid of Streptococcus thermophilus inhibits the colonization of other thermophilic Streptococcus strains. Biosurfactant produced by the bacterium Pseudomonas fluorescens inhibited the attachment of Listeria monocytogenes to the steel surface (Chakrabarti, 2012). A biosurfactant isolated from the supernatant of *B. subtilis* EG1 exhibits anti-adhesive activity against S. aureus and E. coli. The highest anti-adhesion percentage was obtained for S. aureus (28.6%) at a biosurfactant concentration of 3000 mg/L. Similar studies of the antiadhesive properties of surfactin on polystyrene surfaces were carried out (Zeraik & Nitschke, 2010). The authors showed that S. aureus attachment to polystyrene could be reduced by 42.2% by surface conditioning with surfactin. E. coli CFT073 and S. aureus ATCC 29213 biofilm formation was also shown to be reduced by 97% and 90%, respectively, by the presence of surfactin in the medium (Rivardo *et al*., 2009). Thus, the decrease in autoaggregation activity of the GM2 strain, as shown in Fig. 2, may be due to the synthesis of biosurfactants in the culture medium.

The GM2 strain showed a high ability to adhere to polystyrene surfaces. Adhesive and aggregation properties have been shown to depend on environmental conditions. Apparently, microbial aggregation ability is enhanced under stress conditions, as the largest bacterial aggregates were noted during nutrient deficiency (in

phosphate-buffered saline), which enhanced cell adaptation due to higher spatial positioning (Kragh *et al*., 2016).

Adhesion assays of axenic GM2 culture and mixed cultures of GM2 with other gram-positive and gram-negative bacteria showed that co-incubation of different species affects the ability of bacteria to form aggregates and exhibit adhesive properties (Table 1 and Fig. 4). Thus, the presence of E. coli and S. aureus has a positive effect on GM2 adhesion by promoting the formation of multilayer aggregates (Fig. 4). It can be noted that a comparative assay of bacterial coaggregation at 4 h of incubation showed a greater ability of GM2 cells to interact most strongly with E. coli. The effect of one bacterial species on the adhesion of another bacterial species has been shown previously, with both positive and negative effects on adhesion being observed. The ability of bacterial cells to adhere to the surface and each other can be affected by the secretion of polymers, proteins, surfactants, and metabolites by microorganisms present in suspension or on the surface (Cutter *et al*., 2003). Also, despite similar physicochemical mechanisms of coadhesion and coaggregation, coadhesion of coaggregating bacterial pairs does not always occur (Rolf *et al*., 1999).

In conclusion, the *B. intestinalis* GM2 strain showed high adhesive properties, which could potentially be useful in the gastrointestinal environment. Additional studies of the bacteria's ability to aggregate under conditions simulating the avian gastrointestinal tract conditions are needed.

Acknowledgments

This study was supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030). This work was funded by the grant RFBR-20-34-90130. The authors thank Y.A. Akosah for helping with the language editing of the manuscript.

References

BARBOSA T.M., SERRA C.R., LA RAGIONE R.M., WOODWARD M.J. & HENRIQUES A.O. (2005): Screening for bacillus isolates in the broiler gastrointestinal tract. *Applied and environmental microbiology* **71**(2), 968–978.

BINNS N. (2013): Probiotics, prebiotics and the gut microbiota. *ILSI Europe Concise Monograph*. Belgium, 1–32.

BOS R., VAN DER MEI H.C. & BUSSCHER H.J. (1999): Physico-chemistry of initial microbial adhesive interactions--its mechanisms and methods for study. *FEMS microbiology reviews* **23** (2), 179–230.

BOTES M., LOOS B., VAN REENEN C.A. & DICKS L.M. (2008): Adhesion of the probiotic strains *Enterococcus mundtii* ST4SA and *Lactobacillus plantarum* 423 to Caco-2 cells under conditions simulating the intestinal tract, and in the presence of antibiotics and anti-inflammatory medicaments. *Archives of microbiology* **190**(5), 573–584.

CELI P., VERLHAC V., PÉREZ CALVO E., SCHMEISSER J. & KLUENTER A. (2019): Biomarkers of gastrointestinal functionality in animal nutrition and health. *Animal Feed Science and Technology* **250**, 9–31.

CHAKRABARTI, S. (2012): Bacterial biosurfactant: Characterization, antimicrobial and metal remediation properties. *Ph.D. Thesis, National Institute of Technology*.

COLLADO M.C., MERILUOTO J. & SALMINEN S. (2008): Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology* **226,** 1065–1073.

CUTTER L.A., VAN SCHIE P.M. & FLETCHER M. (2003): Adhesion of anaerobic microorganisms to solid surfaces and the effect of sequential attachment on adhesion characteristics. *Biofouling* **19,** 18–9.

GIAOURIS E., HEIR E., DESVAUX M., HÉBRAUD M., MØRETRØ T., LANGSRUD S., DOULGERAKI A.I., NYCHAS G.E., KAČÁNIOVÁ M., CZACZYK K., ÖLMEZ H. & SIMÕES M. (2015): Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. *Frontiers in Microbiology* **6**.

GUDIÑA E.J., ROCHA V., TEIXEIRA J.A. & RODRIGUES L.R. (2010): Antimicrobial and antiadhesive properties of a biosurfactant isolated from *Lactobacillus paracasei* ssp. paracasei A20. *Letters in applied microbiology* **50**(4), 419–424.

HADIEVA G.F., LUTFULLIN M.T., MOCHALOVA N.K., SHARIPOVA M.R., MARDANOVA A.M. & LENINA O.A. (2018): New *Bacillus subtilis* strains as promising probiotics. *Microbiology* **87** (4), 463–471.

HADIEVA G.F., LUTFULLIN M.T., NIKOLAEVA A.A., MOCHALOVA N.K., SMOLENCEV S.Y., MARDANOVA A.M. & SHARIPOVA M.R. (2019): The effect of *Bacillus subtilis* GM2 and GM5 probiotics on the growth and fodder digestibility of broiler chickens. *Uchenye Zapiski Kazanskogo Universiteta. Seriya Estestvennye Nauki* **161** (3), 472–489.

HIRAMATSU Y., SAITO M., OTSUKA N., SUZUKI E., WATANABE M., SHIBAYAMA K., & KAMACHI K. (2016): BipA Is Associated with Preventing Autoagglutination and Promoting Biofilm Formation in Bordetella holmesii. *PLoS ONE* **11**.

ISENRING J., GEIRNAERT A., LACROIX C., & STEVENS M.J. (2021): Bistable auto-aggregation phenotype in *Lactiplantibacillus plantarum* emerges after cultivation in in vitro colonic microbiota. *BMC Microbiology* **21**.

KOS B., ŠUŠKOVIĆ J., VUKOVIĆ S., ŠIMPRAGA M., FRECE J. & MATOŠIĆ S. (2003): Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92. *Journal of Applied Microbiology* **94**.

KRAGH K.N., HUTCHISON J.B., MELAUGH G., RODESNEY C.A., ROBERTS A.E., IRIE Y., JENSEN P.Ø., DIGGLE S.P., ALLEN R.J., GORDON V.D. & BJARNSHOLT T. (2016): Role of Multicellular Aggregates in Biofilm Formation. *mBio* **7.**

LAGANENKA L., COLIN R. & SOURJIK V. (2016): Chemotaxis towards autoinducer 2 mediates autoaggregation in Escherichia coli. *Nature Communications* **7**.

MARDANOVA A., HADIEVA G., LUTFULLIN M., KHILYAS I.V., MINNULLINA L., GILYAZEVA A.G., BOGOMOLNAYA L.M. & SHARIPOVA M.R. (2017): Bacillus subtilis Strains with Antifungal Activity against the Phytopathogenic Fungi. *Agricultural sciences* **08**, 1–20.

MENCONI A., MORGAN M.J., PUMFORD N.R., HARGIS B. & TELLEZ G. (2013): Physiological Properties and Salmonella Growth Inhibition of Probiotic *Bacillus* Strains Isolated from Environmental and Poultry Sources. *International Journal of Bacteriology* **2013**.

NWOKO E.Q. & OKEKE I.N. (2021): Bacteria autoaggregation: how and why bacteria stick together. *Biochemical Society Transactions* **49**, 1147–1157.

PERALTA-SÁNCHEZ J.M., MARTÍN-PLATERO A.M., ARIZA-ROMERO J.J., RABELO-RUIZ M., ZURITA-GONZÁLEZ M.J., BAÑOS A., RODRÍGUEZ-RUANO S.M., MAQUEDA M., VALDIVIA E. & MARTÍNEZ-BUENO M. (2019): Egg Production in Poultry Farming Is Improved by Probiotic Bacteria. *Frontiers in Microbiology* **10**.

PEREZ K. J., SANTOS VIANA J. DOS, LOPES F.C., PEREIRA J.Q., SANTOS D.M. DOS, OLIVEIRA J.S., VELHO R.V., CRISPIM S.M., NICOLI J. R., BRANDELLI A. & NARDI, R.M.D. (2017): *Bacillus* spp. Isolated from Puba as a Source of Biosurfactants and Antimicrobial Lipopeptides. *Front Microbiol*. **8**.

RIVARDO F., TURNER R.J., ALLEGRONE G., CERI H. & MARTINOTTI M.G. (2009): Anti-adhesion activity of two biosurfactants produced by *Bacillus* spp. prevents biofilm formation of human bacterial pathogens. *Appl. Microbiol. Biotechnol.* **83** (541).

RODRIGUES L.R., IN: LINE D., GOLDMAN A. (EDS.). (2011): Bacterial Adhesion: Biology, Chemistry, and Physics, Book Series "Advances in Experimental Medicine and Biology". *Springer*.

RODRIGUES L.R., TEIXEIRA J.A., VAN DER MEI H.C. & OLIVEIRA R. (2006): Isolation and partial characterization of a biosurfactant produced by *Streptococcus thermophilus* A.*Colloids Surf. B Biointerfaces* **49** (78).

SARWAR A., BRADER G., CORRETTO E., ALETI G., ULLAH M. A., SESSITSCH A. & HAFEEZ F.Y. (2018): Qualitative Analysis of Biosurfactants from *Bacillus* Species Exhibiting Antifungal Activity. *PLoS One* **13** (6).

VAZ D.A., GUDINA E.J., ALAMEDAA E.J., TEIXEIRA J.A. & RODRIGUES L.R. (2012): Performance of a biosurfactant produced by a *Bacillus subtilis* strain isolated from crude oil samples as compared to commercial chemical surfactants. *Colloids and Surfaces B: Biointerfaces* **89**, 167–174.

VIJAYAKUMAR S. & SARAVANAN V. (2015): Biosurfactants-Types, Sources and Applications. *Research Journal of Microbiology* **10**(5), 181–192.

ZERAIK A.E. & NITSCHKE M. (2010): Biosurfactants as Agents to Reduce Adhesion of Pathogenic Bacteria to Polystyrene Surfaces: Effect of Temperature and Hydrophobicity. *Curr. Microbiol.* **61** (554).

ZHANG J., XUE Q., GAO H., LAI H. & WANG P. (2016): Production of lipopeptide biosurfactants by *Bacillus atrophaeus* 5-2a and their potential use in microbial enhanced oil recovery. *Microb Cell Fact*. **15**.