JRIFST

https://journals.rifst.ac.ir
ISSN: 2252-0937(print), 2538-2357(online)
Journal of Research and Innovation in
Food Science and Technology

Research Institute of
Food Science and Technology

Volume 11, Issue 1, June 2022, Pages 29-42
Document Type: Extended Abstract
https://doi.org/10.22101/JRIFST.2021.266560.1216

Extended Abstract

Effect of Heat Stress on the Resistance of two Spore Forming Bacillus Species in the Gastrointestinal Tract Simulation Model and their Probiotic Properties

Nasim Adibpour[1], Marzieh Hosseininezhad[1*], Abolfazl Pahlevanloo[1]

1- Department of Food Biotechnology, Research Institute of Food Science and Technology, Mashhad, Iran
* Corresponding author (m.hosseininezhad@rifst.ac.ir)

Recieved: 2021.01.14; **Accepted:** 2021.05.16

Abstract

In recent years, the application of spore-forming bacteria in probiotic food supplements and medicine have become more interesting due to their stability in stressful condition of production line and gut environment. In the current study, the resistance of *Bacillus coagulans* and *Bacillus subtilis* in response to heat stress and simulated gastrointestinal tract was investigated. Moreover, the aggregation and hydrophobicity of cell surface of these strains were evaluated. The results showed a survival rate of more than 80% for both species after enduring heat stress and undergoing simulated gastrointestinal conditions. In addition, *Bacillus coagulans* showed a higher autoaggregation and coaggregation ability compared to *Bacillus subtilis*. In addition, both probiotic species presented a high tendency toward adhering to the hydrocarbon solvents like chloroform and ethyl acetate. This study was a continuation of previous studies conducted with the aim of developing and optimizing functional edible coating for the production of probiotic rock candy (Nabat) using spore-forming probiotic bacillus, to ensure the survival and effectiveness of the strains.

Keywords: *Bacillus coagulans*, *Bacillus subtilis*, Heat stress, Probiotic, Simulated gastrointestinal tract

Introduction

Spore-forming probiotics have received more attention in recent years due to their stability under stressful food processing conditions such as high temperature and pressure (Cutting, 2011). Studies show that these bacteria have better survival in gastrointestinal conditions and, in addition, can survive long periods at room temperature, refrigerator, freezer, and dry conditions (Cutting, 2011; Haldar & Gandhi, 2016). Unlike Lactobacillus strains, the spores of some Bacillus strains used as probiotics are not vegetatively active but dormant (Adibpour, Hosseininezhad, Pahlevanlo, *et al.*, 2019). Among the hundreds of Bacillus spp. only *Bacillus coagulans* and *Bacillus subtilis* have been accepted as human-friendly probiotics (Adibpour, Hosseininezhad, & Pahlevanlo, 2019). In previous studies, the optimization of edible coating formulation of probiotic rock candy (Nabat) as a functional sweetener, using two strains of spore-forming probiotic bacilli besides rheological and textural properties were investigated. Moreover, survival of the strains in a shelf life of six months were evaluated (Adibpour, Hosseininezhad, & Pahlevanlo, 2019; Adibpour *et al.*, 2020). Nabat is typically consumed as a traditional and medicinal sweetener with water, tea, and hot beverages; hence the probiotic strains would be exposed to heat stress when consumed. Thermal stress increases the risk of damage to the bacterial spore wall. It also makes it possible to convert spores to a vegetative form before taking probiotics and reduce the survival of bacteria exposed to acidic stomach conditions. Therefore, it is necessary to investigate the effect of these stresses on the probiotic strains. In the present study, the survival of *Bacillus coagulans* and *Bacillus subtilis* strains used in coated probiotic rock candy after tolerating heat stress of boiling water and simulated conditions of the gastrointestinal tract was investigated. In the following, the property of accumulation and hydrophobicity of the cell surface of these strains has been evaluated.

Materials and methods

Culture and spore counting

The probiotic strains of *Bacillus coagulans* Unique IS-2 and *Bacillus subtilis* UBBS-14 were purchased from Unique Biotech, India. All culture media and chemicals used were provided from Merck, Germany. In order to initial count and determine the survival of the strains after each test, the method presented by Pourmantazer et al. was used (Poormontaseri *et al.*, 2017). *Bacillus coagulans* and *Bacillus subtilis* were cultured on nutrient agar and tryptic soy agar medium, respectively.

Evaluation of bacterial resistance to acid and bile in a simulated model of gastrointestinal tract exposed to heat stress

Probiotic spores were exposed to simulated gastrointestinal conditions after exposure to boiling water according to the method of Ozdemir & Floros (2001). Bacterial strain viability was assessed at zero, 10, 30, 60, 90, and 120 min intervals.

Evaluation of the ability of accumulation of probiotic strains

The vegetative form of two strains of spore-forming probiotic plus two pathogenic strains, including *Escherichia coli* 1330 and *Salmonella enterica* (ATCC 10708), was used to measure bacterial aggregation. The autoaggregation and coaggregation potential of bacteria were evaluated based on the method of Pandey *et al.* (2015).

Measurement of bacterial surface hydrophobicity

The spores of *B. coagulans* and *B. subtilis*, and two pathogenic strains were first activated. Then, determination of bacterial surface hydrophobicity was done according to the method presented by Meidong *et al.* (2018).

Statistical analysis

Data analysis based on a completely randomized design was performed using SAS software version 9.4, and charts were performed using Microsoft Excel software version 2013.

Results and discussion

According to the results of the present study, *B. coagulans* Unique IS-2 and *B. subtilis* UBBS-14 showed over 85 and 82% survival after exposure to boiling water and in gastrointestinal simulation conditions, respectively (Table 1). Similarly, various studies have shown acceptable survival of spore-forming strains in the animal gastrointestinal tract and simulated conditions of the human gastrointestinal tract (Cartman *et al.*, 2008; Hoa *et al.*, 2001; Keller *et al.*, 2019; Setlow, 2003). In another study, monitoring for the ftsH-lacZ gene in bacteria showed that probiotic spores could germinate and grow in different parts of the gut (Casula & Cutting, 2002).

Table 1. Survival rate of *Bacillus coagulans* and *Bacillus subtilis* strains in gastric, intestinal and gastrointestinal simulation condition

Treatment	*Bacillus subtilis* (control)	*Bacillus subtilis* (Boiling water)	*Bacillus coagulans* (control)	*Bacillus coagulans* (Boiling water)
A	104.19±0.47^{a}	87.67±1.25^{a}	94.16±1.12^{b}	86.01±2.3^{a}
B	93.96±0.79^{b}	86.7±1.46^{a}	95.48±0.02^{a}	86.92±1.33^{a}
C	103.89±0.1^{a}	82.7± 1^{b}	92.12±1.8^{c}	87.07±1.76^{a}

(A) Viability after 120 minutes in gastric condition (%), (B) Viability after 120 minutes in intestinal condition (%) and (C) Viability after 240 minutes in gastrointestinal condition (%).
Mean ± Std. Different letters indicate significant difference in each column ($P<0.05$).

The autoaggregation results of probiotic and pathogenic strains indicated that *B. coagulants* and *E. coli* had the highest, and *S. enterica* the lowest autoaggregation ability after 24 h (Fig. 1). Based on the results of Fig. (2), it can be seen that the co-aggregation potential of *B. coagulans* with each of the pathogenic strains (*E. coli* and *S. enterica*) is higher than that of *B. subtilis* for 24 h. Although both bacteria have higher aggregation potential with *E. coli* compared to salmonella showed.

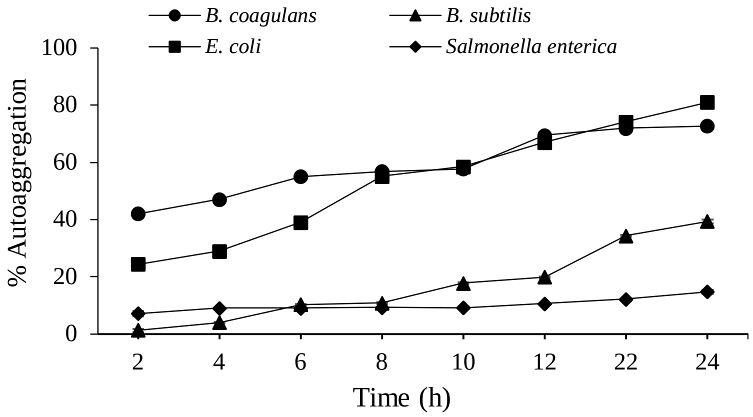

Fig. 1. Autoaggregation ability of Bacillus strains and pathogenic strains

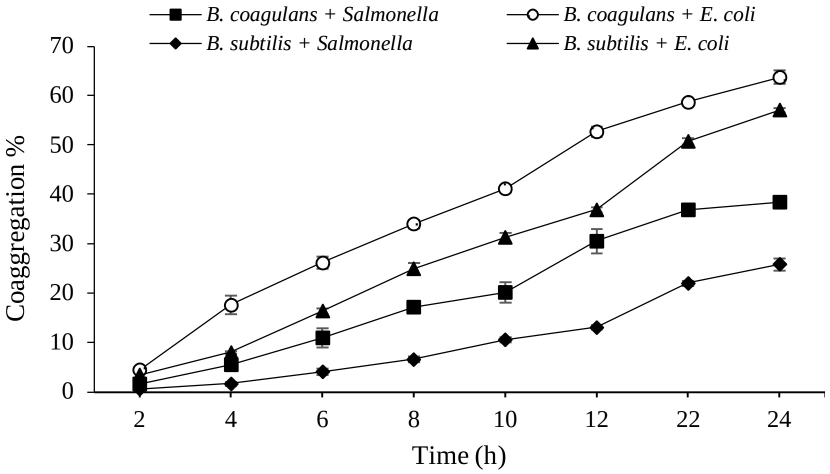

Fig. 2. Coaggregation ability of Bacillus strains with pathogenic strains

The results of bacterial aggregation (auto and coaggregation efficiency) of probiotic culture indicates the ability of these cells to prevent pathogenic strains from adhering to intestinal epithelial cells, increasing the chance of colony formation and accumulation in the intestine (Jeon *et al.*, 2018; Pandey *et al.*, 2015). According to research, a group of proteins and cell surface exopolysaccharides, termed self-agglutination compounds, are involved in the autoaggregation of bacteria (Trunk *et al.*, 2018).

Assessment of surface hydrophobicity of probiotic and pathogenic strains showed that *B. coagulans* 45-95%, *B. subtilis* 36-60%, *E. coli* 1-69%, and *S. enterica* 0-38% could bind to hydrocarbon solvents (Fig. 3). The tendency of bacteria to bind to hydrocarbon solvents is related to their cell surface's electron-donor/electron-acceptor properties (Thankappan *et al.*, 2015). Cell surface hydrophobicity is, in fact, a nonspecific interaction between microbial and host cells in which proteins, membrane lipids, and lipoteichoic acids are involved (Krasowska & Sigler, 2014; Vaidya *et al.*, 2018).

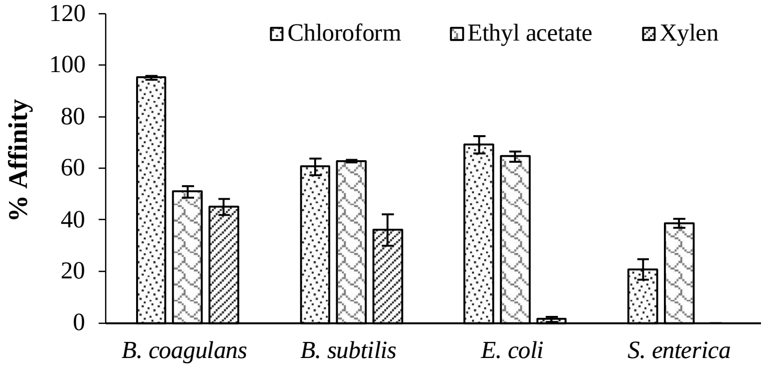

Fig. 3. Cell surface hydrophobicity of probiotic and pathogenic strains against various solvents.

Conclusions

The remarkable thermal stability and viability of spore-forming bacilli under stressful gastrointestinal tract conditions and food processes have made the use of these bacteria in the formulation of probiotic products increasingly successful compared to lactobacilli. This study showed that the spores of *Bacillus coagulans* and *Bacillus subtilis* could tolerate the stress after undergoing thermal processes and during consumption of this product with boiling water. Besides, it tolerates the passage conditions of the host gastrointestinal tract well. Also, the success of this family of probiotics in cell aggregation and the ability of the cell surface hydrophobicity, as the most critical determinants of probiotic properties, were high. This makes it possible to use bacilli compared to lactobacilli in a wide range of food and medicine products.

References

Adibpour, N., Hosseininezhad, M., & Pahlevanlo, A. (2019). Application of spore-forming probiotic Bacillus in the production of Nabat-A new functional sweetener. *LWT, 113,* 108277. https://doi.org/10.1016/j.lwt.2019.108277

Adibpour, N., Hosseininezhad, M., & Pahlevanlo, A. (2020). Optimization of probiotic edible coating formulation and evaluation of physical and textural properties for rock candy coating. *Food Science and Technology*, *17*(100), 103-115 . (in Persian)

Adibpour, N., Hosseininezhad, M., Pahlevanlo, A., & Hussain, M. A. (2019). A review on Bacillus coagulans as a Spore-Forming Probiotic. *Applied Food Biotechnology*, *6*(2), 91-100. https://doi.org/10.22037/afb.v6i2.23958

Cartman, S. T., La Ragione, R. M., & Woodward, M. J. (2008). Bacillus subtilis spores germinate in the chicken gastrointestinal tract. *Applied and Environmental Microbiology*, *74*(16), 5254-5258. https://doi.org/10.1128/AEM.00580-08

Casula, G., & Cutting, S. M. (2002). Bacillus probiotics: spore germination in the gastrointestinal tract. *Appl. Environ. Microbiol.*, *68*(5), 2344-2352. https://doi.org/10.1128/AEM.68.5.2344-2352.2002

Cutting, S. M. (2011). Bacillus probiotics. *Food microbiology*, *28*(2), 214-220. https://doi.org/10.1016/j.fm.2010.03.007

Haldar, L., & Gandhi, D. N. (2016). Effect of oral administration of Bacillus coagulans B37 and Bacillus pumilus B9 strains on fecal coliforms, Lactobacillus and Bacillus spp. in rat animal model. *Veterinary World*, *9*(7), 766-772. https://doi.org/10.14202/vetworld.2016.766-772

Hoa, T. T., Isticato, R., Baccigalupi, L., Ricca, E., Van, P. H., & Cutting, S. M. (2001). Fate and dissemination of Bacillus subtilis spores in a murine model. *Appl. Environ. Microbiol.*, *67*(9), 3819-3823. https://doi.org/10.1128/AEM.67.9.3819-3823.2001

Jeon, H.-L., Yang, S.-J., Son, S.-H., Kim, W.-S., Lee, N.-K., & Paik, H.-D. (2018). Evaluation of probiotic Bacillus subtilis P229 isolated from cheonggukjang and its application in soybean fermentation. *LWT*, *97*, 94-99. https://doi.org/10.1016/j.lwt.2018.06.054

Keller, D., Verbruggen, S., Cash, H., Farmer, S., & Venema, K. (2019). Spores of Bacillus coagulans GBI-30, 6086 show high germination, survival and enzyme activity in a dynamic, computer-controlled in vitro model of the gastrointestinal tract. *Beneficial microbes*, *10*(1), 77-87. https://doi.org/10.3920/BM2018.0037

Krasowska, A., & Sigler, K. (2014). How microorganisms use hydrophobicity and what does this mean for human needs? *Frontiers in cellular and infection microbiology*, *4*, 112. https://doi.org/10.3389/fcimb.2014.00112

Meidong, R., Khotchanalekha, K., Doolgindachbaporn, S., Nagasawa, T., Nakao, M., Sakai, K., & Tongpim, S. (2018). Evaluation of probiotic Bacillus aerius B81e isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of Pla-mong Pangasius bocourti. *Fish & shellfish immunology*, *73*, 1-10. https://doi.org/10.1016/j.fsi.2017.11.032

Ozdemir, M., & Floros, J. (2001). Analysis and modeling of potassium sorbate diffusion through edible whey protein films. *Journal of Food Engineering*, *47*(2), 149-155. https://doi.org/10.1016/S0260-8774(00)00113-8

Pandey, K. R., Shinde, P. S., & Vakil, B. V. (2015). Evaluation of molecular variations in probiotic Bacillus coagulans and its bacteriophage resistant mutants. *Int J Curr Microbiol Appl Sci*, *4*(4), 343-355 .

Poormontaseri, M., Hosseinzadeh, S ,.Shekarforoush, S. S., & Kalantari, T. (2017). The effects of probiotic Bacillus subtilis on the cytotoxicity of Clostridium perfringens type a in Caco-2 cell culture. *BMC microbiology*, *17*(1), 150. https://doi.org/10.1186/s12866-017-1051-1

Setlow, P. (2003). Spore germination. *Current opinion in microbiology*, *6*(6), 550-556. https://doi.org/10.1016/j.mib.2003.10.001

Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K., & Anbarasu, K. (2015). Characterization of Bacillus spp. from the gastrointestinal tract of Labeo rohita—towards to identify novel probiotics against fish pathogens. *Applied biochemistry and biotechnology*, *175*(1), 340-353. https://doi.org/10.1007/s12010-014-1270-y

Trunk, T., Khalil, H. S., & Leo, J. C. (2018). Bacterial autoaggregation. *AIMS microbiology*, *4*(1), 140. https://doi.org/10.3934/microbiol.2018.1.140

Vaidya, Y., Patel, S., Kunjadiya, P., Joshi, C., & Kunjadiya, A. (2018). The effect of prebiotics on bacteriocin production and gut adhesion potential of Lysinibacillus sphaericus DY13 and Bacillus clausii DY14. *Journal of Microbial World*, *10*(4), 369-385 .