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Crescimento e esporulação de *Alicyclobacillus Acidoterrestris* em meio de cultura e em suco de laranja industrializado

Determination of the growth and sporulation of *Alicyclobacillus acidoterrestris* in the culture medium and industrialized orange juice

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RESUMO

Alicyclobacillus acidoterrestris é microrganismo aeróbio, Gram-positivo, ácido-termorresistente, não patogênico e formador de esporos. Devido a sua capacidade de esporulação, esse microrganismo é frequentemente encontrado em sucos cítricos industrializados, pois sobrevivem a etapas térmicas do processamento, multiplica-se e acarreta a deterioração do produto, devido ao seu metabolismo. Desta maneira, leva ao desenvolvimento de atributos sensoriais indesejáveis no alimento, como a produção de 2,4-dibromofenol e 2-metoxifenol (guaiacol). Dessa forma, representam sério problema para o setor citrícola brasileiro, uma vez que o Brasil é responsável por mais da metade da produção mundial e exportação de suco de laranja. Assim, este trabalho teve como objetivo avaliar o crescimento, enumeração, esporulação de *A. acidoterrestris*. Foram utilizados no presente estudo o meio de cultura específico (BAT) e o suco de laranja reconstituído (11 °Brix) como meio de cultivo. Realizou-se a curva de crescimento para determinação e enumeração das células vegetativas e da esporulação, foram utilizados o meio BAT, pH 4,0 e suco de laranja reconstituído incubados a 45 ºC nos tempos 0, 3, 6, 9 e 12 horas. Ambos os meios de cultivos avaliados resultaram em valores de contagem de células vegetativa sem função do tempo de incubação. No tempo 0 a contagem foi de 2,301 e 1,699 log UFC/mL no meio BAT e no suco de laranja, respectivamente. Após 12h de incubação a contagem obtida no meio BAT foi de 8 log UFC/mL e 6,279 log UFC/mL no suco de laranja. Nos ensaios efetuados para a quantificação de esporos, onde se utilizou o choque térmico (80 °C/10min), apenas após 9 horas de incubação foi possível obter contagem de células, 4,477 log UFC/mL, esses resultados indicam a germinação de esporos ocorrida devido ao choque. Este mapeamento detalhado auxilia na busca de alternativas para o monitoramento e controle rápido de *A. acidoterrestris* no processamento industrial, visto que esta espécie está sendo utilizada como indicador de qualidade do suco de laranja.

Palavra-Chave: *Alicyclobacillus acidoterrestris*; suco de laranja; esporulação

ABSTRACT

Alicyclobacillus acidoterrestris is an aerobic, Gram-positive, acid-resistant, non-pathogenic and spore-forming microorganism. Due to its sporulation capacity, this microorganism often found in industrialized citrus juices because they survive thermal processing, multiply and deteriorate the product. In this way, it leads to the development of undesirable sensory

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attributes in the food, such as the production of 2,4-dibromophenol and 2-methoxyphenol (guaiacol). In this way, it represents a serious problem for the Brazilian citrus sector, since Brazil is responsible for more than half of the world production and export of orange juice. Thus, this work had as objective to evaluate the growth, enumeration and sporulation of *A. acidoterrestris*. The specific culture medium (BAT) and the reconstituted orange juice (11 °Brix) were used as the culture medium in the present study. The growth curve was determined for the determination and enumeration of vegetative cells and sporulation, BAT medium, pH 4.0 and reconstituted orange juice were incubated at 45 °C at 0, 3, 6, 9 and 12 hours, with initial bacterial inoculum of 10^4 CFU/mL. Both media of evaluated cultures resulted in vegetative cell count values without function of the incubation time. At time 0 the count was 2.30 and 1.69 log CFU/mL in BAT medium and orange juice, respectively. After 12h of incubation the count obtained in BAT medium was 8 log CFU/mL and 6.279 log CFU/mL in orange juice. In the tests carried out for spore quantification, thermal shock (80 °C/10min) was used, after only 9 hours of incubation it was possible to obtain a cell count, 4.47 log CFU/mL, these results indicate the spore germination due to shock. This detailed mapping assists in the search for alternatives for the monitoring and rapid control of *A. acidoterrestris* in industrial processing, since this species is being used as indicator of quality of orange juice.

Keywords: *Alicyclobacillus acidoterrestris*; orange juice; sporulation

1 INTRODUCTION

Brazil is responsible for more than half of the orange juice produced in the world, approximately 53%, and holds 76% of participation in the orange juice trade, according to a survey of the last five harvests carried out by the United States Department of Agriculture (USDA). Highlighting the concentrated and frozen orange juice - frozen concentrated orange juice (FCOJ). In 2017 alone, around 421 tonnes were exported (CITRUSBR, 2018).

Despite the low pH found in concentrated orange juices (3.5 - 4.0) and the high concentration of soluble solids (65 ºBrix) some microorganisms are able to remain in these conditions. Among them, there is the acid-resistant bacteria, *Alicyclobacillus acidoterrestris*, aerobic, Gram-positive, non-pathogenic microorganism and spore-forming (HIPPECHEN et al., 1981; GOTO et al., 2003).

The acid-thermophilic behavior of the spores of *A. acidoterrestris* allows survival in lethal stages of processing, which leads to the deterioration of citrus juices, characterized by the presence of undesirable sensory characteristics, such as unpleasant odor and taste. The development of these characteristics is attributed to the formation of the compounds 2,4-dibromophenol and 2-methoxyphenol (guaiacol) (OLIVEIRA and ABREU FILHO, 2012).

Guaiacol compound gives the juice an astringent flavor, which can be sensorially detected at a level of 2 µg/L (ORR et al., 2000). To date, there is no detailed knowledge about

the formation of guaiacol, although it is conventionally accepted that the compound can be produced by vanillic acid, an oxidized form of vanillin, by microorganisms such as: Bacillus megaterium, *Bacillus subtilis*, *Streptomyces setonii* and strains of *Streptomyces* (CRAWFORD and OLSON, 1978; ÁLVAREZ-RODRÍQUEZ et al., 2003). Its production is believed to occur by ferulic acid by *Paecilomyces variotii*, *Rhodotorula rubra* and *Sporotrichum thermophile* (RAHOUTI et al., 1989; HUANG et al., 1993; TOPAKAS et al., 2003). As for *Alicyclobacillus*, it is believed that its production by the microorganism occurs through the decarboxylation of vanillic acid. Guaiacol is produced through the non-oxidative decarboxylation of vanillic acid, the product of the oxidation of vanillin, a derivative of ferulic acid (CHANG and KANG, 2004).

For the concentrated orange juice industry, the absence of *A. acidoterrestris* is a quality requirement, so the industry investigates its presence in the final product through the quantification of spores. Meantime, until now, little is known about the sporulation process of this bacterium, how long it begins to form spores and what conditions affect the bacterial kinetics for sporulation, as well as when the guaiacol is produced and changes the sensory characteristics of the product.

Detection methods with quickness and lower cost favor the industry and the monitoring of the quality of the export juice, therefore studies that seek to map the behavior of this bacterium may contribute in the search for alternatives for the control of *A. acidoterrestris* in industrial processing.

Thus, this work aimed to evaluate the growth, enumeration, and sporulation of *Alicyclobacillus acidoterrestris* in the initial growth times, in a specific BAT medium and in reconstituted orange juice.

2 MATERIAL AND METHODS

2.1 ALICYCLOBACILLUS ACIDOTERRESTRIS STRAIN

Alicyclobacillus acidoterrestris used in our tests was acquired from the German Cultures Collection of Microorganisms and Cell Cultures (DSMZ - Deutsche Sammlung Von Mikroorganismen und Zellkulturen), which is deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI), located at the Pluridisciplinary Center for Chemical, Biological and Agricultural Research - CPQBA / UNICAMP.

A. acidoterrestris DSMZ 3922T - (CBMAI: 0244T) - Origin: Soil.

2.2 CULTURE MEDIUMS

Culture medium used in the tests was BAT (*Bacillus acidoterrestris*) (DEINHARD et al., 1987). The preparation of 1 liter of BAT medium: yeast extract (2 g), glucose (5 g), $(NH_4)2SO_4$ (0,2 g), $MgSO_4.7H_2O$ (0,5 g) were used , $CaCl_2.2H_2O$ (0,25 g), KH_2PO4 (3 g), Trace B (1 mL), which is composed of a solution of salts in trace elements (g / L): $CaCl_2.2H_2O$ (0,66 g); $ZnSO_4$.$7H_2O$ (0,18 g); $CuSO_4.5H_2O$ (0,16 g); $MnSO_4.4H_2O$ (0,02 g); $CoCl_2.6H_2O$ (0,18 g); H_3BO_3 (0.10 g); $Na_2MoO_4.2H_2O$ (0.30 g) (FARRAND et al., 1983).

Concentrated orange juice, used as a means of cultivation, was provided by an industry in the northwest region of the state of Paraná, free from contamination by *Alicyclobacillus*. The juice was reconstituted in sterile water to 11 °Brix.

2.3 PREPARATION OF THE VEGETATIVE CELL *A. ACIDOTERRESTRIS*

Alicyclobacillus acidoterrestris (CBMAI: 0244^T) kept in stock at a temperature of -20 ºC was activated in 3 mL of BAT broth and incubated at 45 ºC/24 hours. After incubation, it was transferred with the aid of a sowing loop to the plate with solidified BAT medium where cell viability was evaluated. This done, the plate with the inoculated microorganism was maintained, and the renovation was carried out periodically to maintain its viability. To start the experiment, 3 to 5 colonies were transferred from the plate with the solidified BAT medium to a tube containing 3 mL of BAT broth which was incubated at 45 °C/24 hours, then the bacterial suspension in solution was prepared. 0.85% saline to obtain a turbidity equivalent to the 0.5 McFarland scale standard which contains approximately $1.5x10^8$ CFU/mL. Then, a 1:10 dilution was performed in order to obtain a bacterial concentration of 10^7 CFU/mL, and when added to the tube containing the culture medium or orange juice, the final concentration was approximately 10^4 CFU/mL.

2.4 GROWTH CURVE FOR SPORULATION DETERMINATION AND ENUMERATION

For the realization of the growth curve, tubes containing 1000 µL of culture medium (BAT) and orange juice reconstituted in water were prepared. Then, 50 µL of vegetative cells of *A. acidoterrestris* prepared in 0.85% saline adjusted to the concentration of 10^8 UFC/mL and incubated at 45 ºC were added. The sporulation evaluation was performed at times 0, 3, 6, 9 and 12 hours, where after this incubation period, plating was performed in serial dilution in solid BAT medium, before and after thermal shock, 80 ºC for 10 minutes in water bath, in

order to eliminate the vegetative cells contained, followed by incubation for 24 hours and counting.

2.5 SPORE STAINING (WIRTZ-CONKLIN)

The spore staining methodology proposed by Wirtz-Conklin was used to show *A. acidoterrestris* spores during bacterial growth.

2.6 QUALITATIVE DETERMINATION OF GUAIACOL

In order to verify the qualitative formation of guaiacol, added media were prepared with the dye chromoazurol S. in the concentrations of 10 and 100 mg/L of BAT culture medium, values established according to the methodology used by Chang (2013). Tubes containing 3 mL of BAT medium were supplemented with 0.3 and 3 mg of the dye and added with bacterial suspensions of *Alicyclobacillus acidoterrestris* previously incubated at 45 °C for 24 hours and diluted to a concentration of 10^4 CFU/mL and then incubated at temperature and time determined by Chang (2013).

2.7 DETERMINATION OF THE INFLUENCE OF SUPPLEMENTATION OF THE CULTURE MEDIUM WITH VANILLIC ACID

In order to verify the effect of vanillic acid on the growth of the microorganism, the culture medium (BAT) was enriched with vanillic acid in concentrations of 10 and 100 mg/L, values established according to the methodology used by Chang (2013). Tubes containing 3 mL of BAT medium were supplemented with 0.3 mg and 3 mg of vanillic acid and added with bacterial suspensions of *Alicyclobacillus acidoterrestris* previously incubated at 45 °C for 24 hours and diluted to a concentration of 10^4 CFU/mL and then incubated in the temperature and time determined by Chang (2013).

3 RESULTS AND DISCUSSION

Through the quantification tests of planktonic cells and spores of *A. acidoterrestris* carried out with BAT culture medium and with the reconstituted orange juice, different counts were obtained in relation to the incubation time. These results are expressed in Table 1 and plotted in Figure 1.

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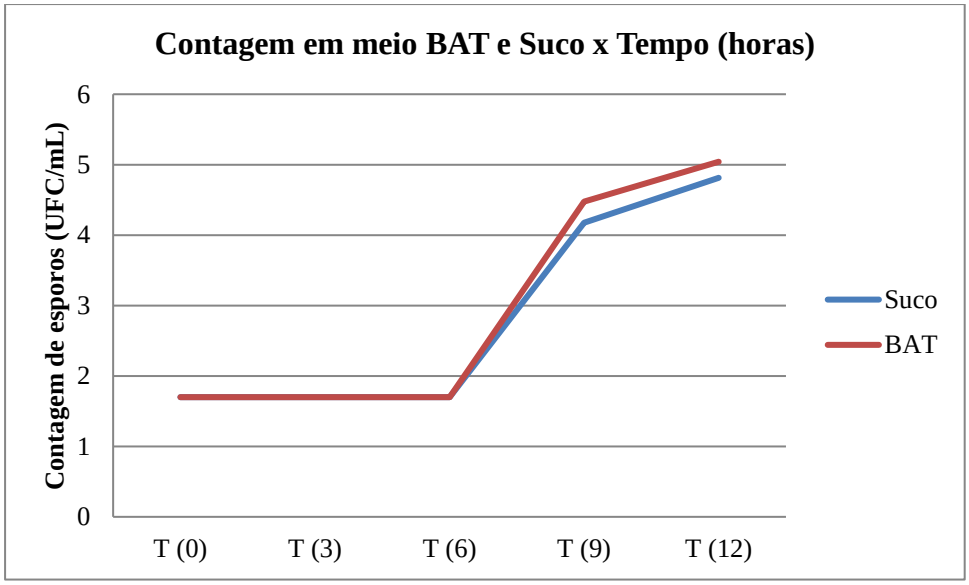
Table 1 – Counts of planktonic cells and spores at different incubation times at 45 ºC in BAT culture medium and in reconstituted orange juice and in reconstituted orange juice

Time (hours)	BAT		Orange Juice	
	Planktonic Cells (Log_{10} CFU/mL)	Spores (Log_{10} CFU/mL)	Planktonic Cells (Log_{10} CFU/mL)	Spores (Log_{10} CFU/mL)
T (0)	2,301	<1,699	1,699	<1,699
T (3)	3,176	<1,699	2,544	<1,699
T (6)	4,217	<1,699	4,000	<1,699
T (9)	6,602	4,477	6,217	4,176
T (12)	8,000	5,041	6,279	4,813

* Method detection limit = 1.699 Log_{10} CFU/mL.

Figure 1 - Spore count obtained through tests with BAT culture medium and orange juice reconstituted at 45 ºC as a function of incubation time (0, 3, 6, 9 and 12 hours)



In the present study, both the cultivated media evaluated, reconstituted orange juice and BAT medium, resulted in increased planktonic cell count values as a function of the incubation time in the tests performed without thermal shock (80 ºC/10 minutes, according to Terano 2005, with modifications). In the tests performed for the quantification of spores, where thermal shock was used, it was found that only after 9 hours of incubation it was possible to obtain cell count, showing the need for this period of spore germination and adaptation of the cell to the culture medium.

The spore formation occurs through a complex network of protein-DNA interaction in the stationary phase, in response to the adversities found in the environment. The most relevant factors are the scarcity of nutrients and population growth, factors that help to understand the reason that led to obtaining quantification of spores only after 9 hours of incubation (DRIKS, 1999; MCKENNEY ET AL., 2013; MILLER & BASSLER, 2001).

Initially in spore formation, genome duplication occurs, followed by cell division to form an asymmetric septum, in which the smallest compartment will result in the mature spore, while the largest (mother cell) will be responsible for nutrition. In the next stage, the pre-spore engulfs the mother cell and forms layers that will hold the mature spore. In the final stage, cell lysis occurs that will result in spore release (PIGGOT & COOTE, 1976).

The spores formed are capable of germinating, continuing the vegetative cycle. Spore germination and subsequent growth or inactivation, in general, can be induced by external factors, such as high hydrostatic pressure (VERCAMMEN et al., 2012; SOKOŁOWSKA et al., 2015; PORĘBSKA et al., 2015) or dioxide of supercritical carbon (BAE et al., 2009; PORĘBSKA et al., 2016). Other studies indicate that germination can also be induced by several nutrients, called germinants, such as: amino acids, purine nucleosides, sugars (LOVDAL et al., 2012), L-alanine (PAREDES-SABJA et al., 2011; KUWANA et al., 2013; CRUZ-MORA et al., 2015), ions and a mixture of asparagine, glucose, fructose and potassium ions (AGFK) (GOSH et al., 2012; STEWART et al., 2012). The germination process takes place in two stages, initially occurs with the release of dipicolinic acid (DPA) and partial rehydration of the nucleus, followed by hydrolysis of the cortex, rehydration of the nucleus and subsequent growth (PANDEY et al., 2013; SETLOW, 2003; YI & SETLOW, 2010).

Regarding the tests carried out to determine guaiacol through the reaction with the dye Cromoazurol S., it was not possible to observe a change in the coloring of the BAT culture medium when adding the dye, and it is not possible to identify a visual change resulting from the reaction of the dye with the guaiacol produced by the microorganism, as shown in Figure 2.

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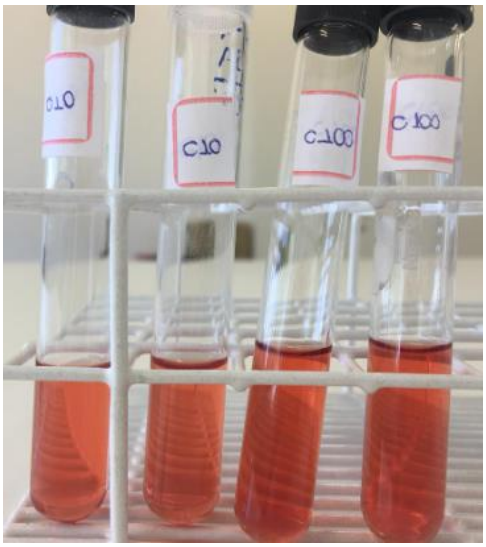
Figure 2 - BAT medium with addition of dye Chromoazurol S. in different concentrations



*C10 corresponds to the concentration of 10 mg/L of the dye Cromoazurol S. and C100 corresponds to 100 mg/

Chang et al. (2013) were able to identify the production of guaiacol by adding the dye Cromoazurol S. in the SK2 culture medium, however, the objective of the present study would be to evaluate the action of this dye in BAT medium, since it is a widely used medium. used and recommended by different regulatory bodies for the growth of Alicyclobacillus, due to its different composition, containing the presence of traces of different minerals. However, there was no success in adding the dye, as there was no visual change in color even using a guaiacol-producing reference strain.

In relation to the test performed to verify the influence of the supplementation of the BAT medium with vanillic acid on the growth of the microorganism, it was possible to observe a higher count of planktonic cells in relation to the non-supplemented medium. The results obtained are shown in Table 2.

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Table 2 - Count ± SD of planktonic cells obtained by incubating *A. acidoterrestris* for 24 hours at 45 ºC with BAT medium and BAT medium enriched with vanillic acid.

	BAT	**A10**	**A100**
Vanillic acid (mg/3 mL)	0,000	0,300	3,000
X ± σ (Log_{10} CFU/mL)	2,239 ± 0,088[a]	6,977 ± 0,281[b]	6,966 ± 0,017[b]

Different lower case letters between columns represent a significant difference according to the Tukey test ($p < 0.05$). *A10 corresponds to the concentration of 10 mg/L of vanillic acid and A100 corresponds to the concentration of 100 mg/L.

In the obtained results it is possible to observe that the supplementation in the concentration 10 mg / L presented count significantly higher than the non-supplemented medium. On the other hand, at a concentration of 100 mg/L the same was not observed. A result consistent with that found by Chang et al. (2013) when they evaluated the growth of *A. acidoterrestris* strains forming guaiacol in SK medium supplemented with vanillic acid in the same concentrations. These results may indicate that supplementation does not allow better growth performance of *A. acidoterrestris* in a directly proportional manner, thus demonstrating the need for further tests using higher concentrations of vanillic acid to prove the hypothesis raised.

4 CONCLUSION

In the present study, both the cultivation media evaluated, reconstituted orange juice and BAT medium, resulted in increasing planktonic cell count values as a function of the incubation time in the tests performed without thermal shock (80 ºC/10 minutes). In the tests performed for the quantification of spores, where thermal shock was used, only after 9 hours of incubation it was possible to obtain cell count, these results indicate the time necessary for the spore germination occurred due to the shock. In the tests carried out with the supplementation of the BAT medium with vanillic acid it was possible to observe that the supplementation in the concentration 10 mg/L presented a count significantly higher than the non-supplemented medium. However, at a concentration of 100 mg / L, the same was not observed. In the tests performed for the qualitative determination of guaiacol, it was not possible to observe a change in the color of the BAT medium with the addition of the dye

Cromoazurol S. even after incubation with *A. acidoterrestris*, reference strain capable of forming guaiacol.

REFERENCES

BAE, Y. Y.; LEE, H. J.; KIM, S. A.; RHEE, M. S. Inactivation of *Alicyclobacillus acidoterrestris* spores in apple juice by supercritical carbon dioxide. **International Journal of Food Microbiology**, v. 136(1), p. 95–100, 2009.

CHANG, S. S.; PARK, S. H.; KANG, D. H. Development of novel agar media for isolating guaiacol producing *Alicyclobacillus* spp. **International Journal of Food Microbiology**, v. 164, n. 1, p. 1-6, 2013.

CRUZ-MORA, J.; PÉREZ-VALDESPINO, A.; GUPTA, S.; WITHANGE, N.; KUWANA, R.; TAKAMATSU, H.; CHRISTIE, G.; SETLOW, P. The GerW protein is not involved in the germination of spores of *Bacillus* species. **PLoS One**, v. 10(3), 2015.

DEINHARD, G.; BLANZ, P.; PORALLA, K.; ALTAN, E. *Bacillus acidoterrestris* sp. nov., a new thermotolerant acidophile isolated from different soils. **Systematic and Applied Microbiology**, v. 10, n. 1, p. 47-53, 1987.

DRIKS, A. The *Bacillus subtilis* spore coat. **Microbiol Mol. Biol. Rev.,** v. 63, p. 1–20, 1999.

FARRAND, S. G.; LINTON, J. D.; STEPHRNSON, R. J.; MACCARTHY, W. V. The use of response surface analysis to study growth of *Bacillus acidocaldarius* throughout the growth range of temperature and pH. **Microbiology**, v. 135, n. 4, p.135-272, 1983.

GHOSH S.; SCOTLAND, M.; SETLOW, P. Levels of germination proteins in dormant and superdormant spores of *Bacillus subtilis*. **Journal of Bacteriology**, v. 194(9), p. 2221–2227, 2012.

KUWANA, R.; TAKAMATSU, H. The GerW protein is essential for L-alanine-stimulated germination of *Bacillus subtilis* spores. **Journal of Biochemistry**, *v.* 154(5), p. 409–417, 2013.

LOVDAL, I. S.; FROM, C.; MADSLIEN, E. H.; ROMUNDSET, K. C.; KLUFTERUD, E.; ROSNES, J. T.; GRANUM, P. E. Role of the gerA operon in L-alanine germination of *Bacillus lichenoformis* spores. **BMC Microbiology**, v. 12, p. 34, 2012.

MCKENNEY P. T.; DRIKS A.; EICHENBERGER P. The *Bacillus subtilis* endospore: assembly and functions of the multilayered coat. **Nat. Rev. Microbiol**., v. 11, p. 33-44, 2013.

MILLER, M. B.; BASSLER, B. L. Quorum sensing in bacteria. **Annual Rev. Microbiol**., v. 55, p. 165-199, 2001.

PANDEY, R.; TER BEEK, A.; VISCHER, N. O. E.; SMELT, J. P. P. M.; BRUL, S.; MANDERS, E. M. M. Live cell imaging of germination and outgrowth of individual *Bacillus subtilis* spores; the effect of heat stress quantitatively analyzed with spore tracker. **PLoS One**, v. 8(3), 2013.

PAREDES-SABJA, D.; SETLOW, P.; SARKER, M. R. Germination of spores of *Bacillus* and *Clostridiales* species: mechanisms and proteins involved. **Trends in Microbiology**, *v.* 19, p. 85–94, 2011.

PIGGOT, P. J.; COOTE, J. G. Genetic aspects of bacterial endospore formation. **Bacteriol. Rev.,** v. 40, p. 908–962, 1976.

PORĘBSKA, I.; RUTKOWSKA, M.; SOKOŁOWSKA, B. Decrease in optical density as a results of germination of *Alicyclobacillus acidoterrestris* spores under high hydrostatic pressure. **High Pressure Research**, v. 35, n. 1, p. 89-97, 2015.

PORĘBSKA, I.; SOKOŁOWSKA, B.; WOŹNIAK, Ł. Dipicolinic acid release and the germination of *Alicyclobacillus acidoterrestris* spores under nutrient germinants. **Polish Journal of Microbiology**, v. 1, p. 57–64, 2016.

SETLOW, P. Spore germination. **Current Opinion in Microbiology**, v. 6(6), p. 550–556, 2003.

SOKOŁOWSKA, B.; SKĄPSKA, S.; FONBERG-BROCZEK, M.; NIEZGODA, J.; PORĘBSKA, I.; DEKOWSKA, A.; RZOSKA, S. J. Germination and inactivation of *Alicyclobacillus acidoterrestris* spores induced by moderate hydrostatic pressure. **Polish Journal of Microbiology**, v. 64, p. 351-359, 2015.

STEWART, K. A.; YI, X.; GHOSH, S.; SETLOW, P. Germination protein levels and rates of germination of spores of *Bacillus subtilis* with overexpressed or deleted genes encoding germination proteins. **Journal of Bacteriology**, v.194, p. 3156–3164, 2012.

TERANO, H.; TAKAHASHI, K.; SAKAKIBARA, Y. Characterization of spore germination of a thermoacidophilic spore-forming bacterium, *Alicyclobacillus acidoterrestris*. **Bioscience, Biotechnology and Biochemistry,** v. 69, p. 1217–1220, 2005.

VERCAMMEN A.; VIVIJS, B.; LURGUIN, I.; MICHIELS, C. Germination and inactivation of *Bacillus* coagulans and *Alicyclobacillus acidoterrestris* spores by high hydrostatic pressure treatment in buffer and tomato sauce. **International Journal of Food Microbiology**, v. 152(3), p. 162–167, 2012.

YI, X.; SETLOW, P. Studies of the commitment step in the germination of spores of *Bacillus* species. **Journal of Bacteriology,** v. 192(13), p. 3424–3433, 2010.