ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
Food Science and Technology

DOI: https://doi.org/10.1590/fst.12120

Probiotic characterization of a commercial starter culture used in the fermentation of sausages

Jéssica Ferreira MAFRA[1], Alexsandra Iarlen Cabral CRUZ[1], Tiago Sampaio de SANTANA[1], Mariza Alves FERREIRA[1], Floricea Magalhães ARAÚJO[2], Norma Suely EVANGELISTA-BARRETO[1]*

Abstract

Probiotic starter culture does not only improve the safety and shelf-life of products but also extends health benefits to the consumer. This study investigated the probiotic potential of a commercial starter culture used in the fermentation of meat sausages. The starter culture tested, composed of *Lactobacillus sakei*, *Staphylococcus xylosus,* and *Staphylococcus carnosus,* was evaluated for resistance to antimicrobials, low pH values and bile salts; production of gas and capsules; acidification capacity; and growth after exposure to different pH values, temperatures, and curing salts. The antagonistic capacity was also assessed against *Escherichia coli* ATCC25922, *Salmonella* Enteritidis ATCC13076, *Vibrio parahaemolyticus*, *Staphylococcus aureus* ATCC43300, *Enterococcus faecalis* ATCC29212, and *Listeria monocytogenes* CERELA. The starter culture was susceptible to all tested antimicrobials and strongly inhibited pathogenic strains, with inhibition halos diameters > 30 mm. The culture was resistant to all concentrations of bile salts tested, did not produce gas or capsules, and could grow within a temperature range of 15 °C to 35 °C in saline medium containing healing salts (nitrite/nitrate). Although, the inability of the culture to withstand low pH, indicating intolerance to stomach acidity, limits its use as a live probiotic, beneficial health effects may be derived from the inactivated culture.

Keywords: antagonism; bile salts; antimicrobial susceptibility.

Practical Application: Probiotic starter cultures contribute to the production of functional fermented foods.

1 Introduction

Today, consumers are increasingly looking for foods that, in addition to their basic nutritional function, can provide health benefits such as reducing the risk of chronic and degenerative diseases (Behera & Panda, 2020). The high demand for healthier foods has encouraged researchers and the meat processing industry to develop products with new characteristics to attract consumers, for example, with the use of probiotic cultures in fermented products such as sausages (Slima et al., 2018).

According to a recent concept, probiotics can be defined as viable or inactivated microbial cells (vegetative or spore; intact or broken) healthy for the host (Zendeboodi et al., 2020). Probiotics produce a wide range of bioactive compounds, such as bacteriocins, enzymes, amino acids, peptides, short-chain fatty acids, vitamins, antioxidants, anti-inflammatory agents, immunomodulators, and exopolysaccharides (Chugh & Kamal-Eldin, 2020). Collectively, these metabolites act on the human body by strengthening the immune system, increasing nutrient absorption, decreasing blood cholesterol levels, blood pressure and heart rate, and improving digestion, food allergies, brain function, and inflammation (Guimarães et al., 2020; Roobab et al., 2020). Driven by health benefits, it is estimated that by 2023, the global probiotic market will earn about US $ 69.3 billion, with the food sector responsible for generating greater economic value (Barros et al., 2020). Currently, the most consumed probiotic products are yogurts and fermented milk (Behera & Panda, 2020). Although dairy products are the main vehicle for probiotics, studies have also demonstrated the feasibility of applying probiotic cultures in the preparation of fermented meat products, such as salami, representing an alternative for consumers intolerant to lactose and milk protein (Pavli et al., 2020).

In the preparation of sausages, initial cultures are generally added which are defined as microbial preparations, with a large number of cells from at least one species of microorganism, added to a raw material to produce a fermented food. These preparations accelerate and direct the fermentation process, improving product safety and extending shelf life by controlling pathogens and other microorganisms through competition, and producing new sensory properties (Farnworth & Champagne, 2016). In addition to these food benefits, starter cultures can confer health benefits to the consumer. However, to fully exploit these potential health benefits, the probiotic characteristics of starter cultures must be fully known.

Considering the potential benefits of adding probiotic starter cultures to food products, this study aimed to verify the probiotic potential of a commercial starter culture commonly used in the production of fermented sausages.

Received 15 Apr., 2020
Accepted 20 July, 2020
[1]*Centro de Ciências Agrárias, Ambientais e Biológicas – CCAAB, Núcleo de Estudos em Pesca e Aquicultura – NEPA, Universidade Federal do Recôncavo da Bahia – UFRB, Cruz das Almas, BA, Brasil*
[2]*Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia – UFBA, Salvador, BA, Brasil*
*Corresponding author: nsevangelista@yahoo.com.br

2 Materials and methods

2.1 Starter culture and growth conditions

The lyophilized starter culture, composed of *Staphylococcus xylosus, S. carnosus,* and *Lactobacillus sakei,* was acquired from Açogueiro Online, located in Jundiaí - São Paulo, and their probiotic characteristics were evaluated. The starter culture was activated in MRS broth (Man, Rogosa, and Sharpe) and inoculated in MRS agar and Baird-Parker agar for optimum growth of the three species. Confirmation of the growth of each species was carried out through morphological characterization and biochemical tests of fermentation of arabinose, sucrose, maltose, and xylose. The starter culture was activated in MRS broth at 37 °C for 24 hours, centrifuged (10,000 ×*g* for 10 min at 4 °C), washed twice in peptone water (0.1%), and stored in a medium supplemented with 20% glycerol at -20 °C (Kongkiattikajorn, 2015).

2.2 Probiotic characterization of the starter culture

Antimicrobial resistance

The resistance of the starter culture to the antimicrobials ampicillin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), nitrofurantoin (300 µg), tetracycline (30 µg), and vancomycin (30 µg) was determined by the disk-diffusion method, according to the recommendations of the Brazilian Committee on Antimicrobial Susceptibility Testing (2017). The disks were added to the surface of the MRS agar and the plates incubated at 37 °C for 24 hours in anaerobiosis. The results were expressed as sensitive (S), intermediate (I) and resistant (R) according to the standards recommended by Acar & Goldstein (1991).

Antagonistic activity

The starter culture was activated in MRS broth and incubated at 37 °C for 24 hours. Aliquots of 5 µL were inoculated onto plates containing MRS agar and incubated for 24 hours at 37 °C. A Brain Heart Infusion (BHI) agar overlay, containing indicator cultures (*Escherichia coli* ATCC25922, *Salmonella* Enteritidis ATCC13076, *Vibrio parahaemolyticus* (isolated from oysters), *Staphylococcus aureus* ATCC43300, *Enterococcus faecalis* ATCC29212, and *Listeria monocytogenes* CERELA), was added to the surface of the plates and incubated again for 24 hours at 37 °C. Antimicrobial activity was observed by the formation of an inhibition zone against indicator cultures (Vieira et al., 2020).

Determination of resistance to low pH values and bile salts

The resistance of the starter culture to low pH values and bile salts was investigated according to the methodology described by Muñoz-Quezada et al. (2013), with the following adaptations. 900 µL of MRS broth buffered to different pH values (2, 2.5, 3 and 7) and containing varying concentrations of bile salts (0%, 0.3%, 0.5%, and 0.7%) were inoculated with 100 µL of the standardized starter culture at 10^8 CFU mL^{-1} (colony forming units per mL). Subsequently, 100 µL of each treatment was diluted in peptone water (0.1%) at times 0, 0.5, 1, 1.5, and 2 hours. 10 µL of each dilution was plated onto MRS agar. Colony counting was performed after 48 hours of anaerobic incubation at 37 °C.

Gas production, acidification capacity and capsule production

Gas production and acidification capacity were determined according to Laslo et al. (2019). Capsule production was evaluated according to Hitchener et al. (1982), through negative staining by the Gins method.

Growth at different pH values and temperatures

The growth capacity of the starter culture was checked at pH adjusted to 3, 4, 5, and 6 with 5 M hydrochloric acid (HCl), and at temperatures of 4 °C, 15 °C, 25 °C, 35 °C, and 45 °C (Laslo et al., 2019).

Sensitivity to different concentrations of sodium chloride, and to the healing salts nitrite and sodium nitrate

For the resistance tests, sodium chloride was added to the MRS agar at concentrations of 1.5%, 2.5% and 3.0%. Tests for resistance to nitrite and sodium nitrate were performed using the same procedure with concentrations of 100, 120, 150 and 100, 200, 300 ppm, respectively (Bis-Souza et al., 2020).

Statistical analysis

Significant differences were assessed through analysis of variance (ANOVA) and Tukey's test using the RStudio program (RStudio Team, 2015), with a significance threshold of $p < 0.05$. Graphic analysis was performed with the aid of the Orange version 3.26.0 program (Orange, 2020).

3 Results and discussion

3.1 Probiotic characterization of the starter culture

Susceptibility to antimicrobials

The starter culture was susceptible to all tested antimicrobials, consistent with the findings of Müller et al. (2016), in which all *S. carnosus* isolates from starter cultures were susceptible to several antimicrobials, including ampicillin, ciprofloxacin, gentamicin, imipenem, tetracycline, and vancomycin, demonstrating that *S. carnosus* strains are widely susceptible to antimicrobials. Resistance to clinically relevant antimicrobials (Rychen et al., 2018) by *Lactobacillus* spp. involved in the fermentation of sausages were reported by Fraqueza (2015) and Rozman et al. (2020). Although the resistance of starter cultures to antimicrobials does not pose a direct risk to consumers because they are not pathogenic, susceptibility to antimicrobials is a desirable trait in probiotic cultures, as it ensures that the organisms do not contribute to the transmission of resistance genes to pathogens or commensal bacteria in the intestines (Zarzecka et al., 2020).

Antagonistic activity

The starter culture inhibited all pathogens tested, presenting inhibition zones with diameters greater than 30 mm. Greater

zones of inhibition were observed against Gram-positive bacteria *L. monocytogenes* and *S. aureus* (Table 1). Lactic acid bacteria isolated from sausages by Laslo et al. (2019) also showed an inhibitory effect against *S. aureus* (16.63 mm) and *E. coli* (14.47 mm), although with significantly smaller zones of inhibition than that found in this work.

The antagonistic activity of viable bacteria can occur through various mechanisms, such as competition for nutrients and adhesion sites, and the production of acidic compounds (lactic, acetic, and propionic acid), carbon dioxide, diacetyl, hydrogen peroxide and bacteriocins (Costa et al., 2018). However, inactivated bacteria can release bacterial components with antagonistic properties against pathogens, such as lipoteichoic acids, peptidoglycans, or exopolysaccharides (Sarkar & Mandal, 2016; Castro-Bravo et al., 2018). The inhibition of pathogens by the starter culture is important in the production of sausage-type sausages as it guarantees the microbiological safety of the product, in addition to its fundamental characteristic of modulating the intestinal microbiota, conferring benefits to the health of the host (Oliveira et al., 2018).

Resistance to low pH values

The effects of different pH values (2, 2.5, 3 and 7) on the growth of the starter culture are shown in Figure 1. The starter culture was not able to survive at pH 2. At pH 2.5, the culture could not survive above 30 minutes (count at last time-point (t = 0): 5.73 log CFU mL^{-1}), and at pH 3 it resisted up to 1 hour of incubation (count at last time-point (t = 0.5): 3.84 log CFU mL^{-1}).

Table 1. Average diameter of the inhibition halos (in mm) created by the starter culture when challenged with common food pathogens.

Pathogens	Inhibition zones (mm)
Staphylococcus aureus	36.02
Escherichia coli	31.18
Enterococcus faecalis	30.75
Salmonella Enteritidis	30.25
Listeria monocytogenes	37.33

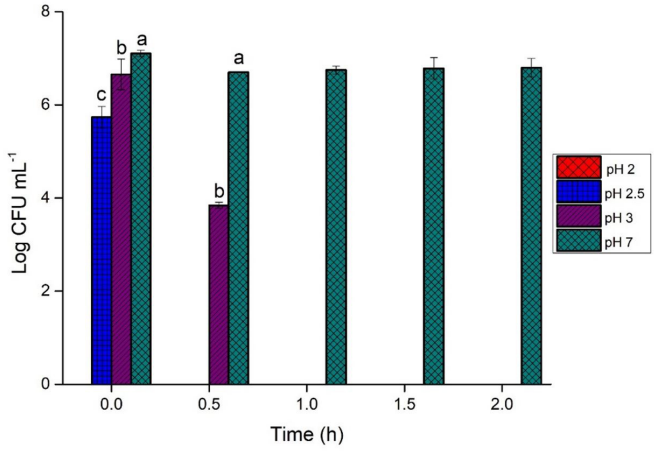

Figure 1. Growth of commercial starter culture at low pH values for 2 hours. Means followed by the same letters indicate no significant difference ($p < 0.05$), as analyzed using Tukey's test.

At pH 7, the viability of the culture was not affected, even after 2 hours (count at last time-point (t = 2): 7.10 log CFU mL^{-1}). Therefore, the starter culture showed intolerance to acidic pH, which represents stomach conditions, limiting its use as a live probiotic culture (Kandylis et al., 2016). In fact, strains of the genus Lactobacillus often have high sensitivity to acidic conditions, impairing their survival in adverse environments such as the stomach and fermented foods (Soares et al., 2019).

The observed bacterial suppression can be explained by the strong oxidizing action of the acid against biomolecules such as fatty acids, proteins, cholesterol, and DNA (Almada et al., 2016). Attractive options to reduce the deleterious effects of extreme gastric acid conditions (pH 1.5-3.5) and improve the performance and functionality of cultures may include protecting microorganisms through encapsulation or strategies based on adaptation mechanisms, where cells are previously exposed to low pH for a short period to induce tolerance and avoid acid stress (Chen et al., 2017; Kavitake et al., 2018; Zhao et al., 2020).

Studies have shown that most of the health benefits of probiotics can be produced by both viable and inactivated cells. There is evidence that preparations containing dead cells can exert relevant biological responses such as restoring intestinal homeostasis, inhibiting pathogens, and improving anxiety and stress (Nishida et al., 2017b; Vandenplas et al., 2017; Aguilar-Toalá et al., 2018; Piqué et al., 2019). In addition, probiotics retain their immunomodulatory activity even after the loss of cell viability (Rossoni et al., 2020), presenting a better immunological effect than live probiotics (Barros et al., 2020; Shripada et al., 2020). The modulation of the host's immune response seems to be associated with the structural components of dead cells, mainly the constituents of the cell wall (Rossoni et al., 2020).

Research also showed that one of the methods of inactivating probiotic microorganisms is a change in pH to induce cell membrane damage, chemical changes in fundamental components (ATP and DNA), and enzyme inactivation (Almada et al., 2016). In addition, studies pointed to the possibility of using food as a vehicle for delivering inactivated probiotics (Sawada et al., 2016, 2019; Sugawara et al., 2016; Nishida et al., 2017a). Thus, it is possible that the inactivation of the starter culture due to stomach acidity does not completely eliminate the beneficial health effects of probiotics.

Resistance to bile salts concentrations

At time 0, there was no significant difference ($p > 0.05$) between the effects of treatments containing 0.3% bile salts (7.17 log CFU mL^{-1}) and the control treatment (0% bile salts) (7.41 log CFU mL^{-1}). However, significant differences ($p < 0.05$) did emerge from the treatments of 0.5% (6.99 log CFU mL^{-1}) and 0.7% (7.00 log CFU mL^{-1}) bile salts, compared to control, demonstrating loss of viability of culture at these concentrations (Table 2). After 30 minutes, none of the bile salt treatments differed significantly from the control ($p > 0.05$), even after 2 hours of incubation (Table 2). This was corroborated by Han et al. (2017), who also found that *Lactobacillus* isolates from sausages showed high tolerance to bile salts. Bile salts

are important in the defense mechanism of the intestine, with normal physiological concentrations ranging from 0.3 to 0.5% (Muñoz-Quezada et al., 2013).

In the present study, the starter culture showed strong resistance to bile salts in concentrations of up to 0.7%. Bacteria can use several defense mechanisms against bile, including special transport mechanisms, the production of exopolysaccharides, or the synthesis of various types of surface proteins and fatty acids. In addition, several bacterial genera have the ability to enzymatically hydrolyze bile salts (Horáčková et al., 2018).

Tolerance to biliary stress is crucial for the survival of probiotics in the gastrointestinal tract. Evidence indicated that viable probiotic cultures are able to alter the synthesis of bile acids causing cholesterol reduction, which is beneficial in the treatment of hypercholesterolemia and hypertension (Sivamaruthi et al., 2020).

Technological characteristics

A probiotic culture must be able to survive food production conditions. In the case of fermented sausages, such as salami, the cultures must be able to acidify the medium, in addition to surviving varied temperatures and the presence of curing salts (Cruxen et al., 2019).

The results of the evaluation of the technological characteristics of the starter culture are shown in Table 3. The starter culture was able to tolerate temperatures of 15 °C, 25 °C and 35 °C, pH 5 and 6, different concentrations of sodium chloride (1.5%, 2.5% and 3%), nitrite (100, 120, and 150 ppm) and nitrate (100, 200, and 300 ppm), conditions commonly used in the production of sausages.

The ability of microorganisms to grow under adverse conditions has been shown to be dependent on species and lineage. The addition of 150 ppm of nitrite or sodium nitrate and the temperature of 15 °C proved to be a limiting factor to the growth of a strain of *Lactobacillus casei* (Bis-Souza et al., 2020), while *Staphylococcus* spp. generally survive NaCl concentrations of up to 15%, 150 ppm sodium nitrate, and temperatures between 15 and 40 °C (Cruxen et al., 2019). In addition, the starter culture reduced the pH of the medium from pH 7 to pH 4.88, after 48 hours of incubation. Rapid acidification is an important characteristic of cultures used in the manufacture of salami, as the drop in the pH of the meat gives stability to the product (Kunrath et al., 2017).

The starter culture did not produce gas (Table 3). The lack of gas production in sausage cultures is particularly important, as gas is associated with the formation of cavities within the product (Laslo et al., 2019). In addition, the starter culture did not produce a capsule (Table 3). Capsule production may be desirable for some fermented foods. However, during the processing of sausages, encapsulated microorganisms can adhere to the equipment and become a source of contamination for other products (Hitchener et al., 1982).

One of the great challenges of the food industry is to maintain the viability of starter cultures during the production process of fresh sausages and the storage period, an essential characteristic to preserve the food since these sausages do not undergo heat treatment. Mafra et al. (2019) demonstrated that a studied starter culture resisted the production conditions of a salami that used tilapia meat as a raw material, promoting an efficient reduction in pH (6.8-5.95). The acidic environment during the sausage fermentation process constitutes an obstacle to the survival of decay-causing and pathogenic microorganisms, providing microbiological stability, reducing water retention and, consequently, firm texture and feasibility to the product (Savoldi et al., 2019).

Table 2. Growth of commercial starter culture (log CFU mL^{-1}) in different concentrations of bile salts for 2 hours, with growth tested every 30 minutes. Means followed by the same letters indicate no significant difference ($p < 0.05$), as analyzed using Tukey's test.

Time (h)	Concentration of bile salts			
	0%	0.3%	0.5%	0.7%
0	7.41a	7.17ab	6.99b	7.00b
0.5	6.79	6.85	6.68	6.80
1	6.74	6.84	6.93	6.93
1.5	7.03	7.08	6.81	6.91
2	7.03	6.81	6.87	6.77

Means followed by equal letters on the same line do not show significant difference ($p > 0.05$) by the Tukey Test.

Table 3. Technological characteristics of the commercial starter culture.

Character	Starter culture
CO_2 production	-
Capsule production	-
Final pH (48 hours)	4.88
Multiplication a:	
4 °C	-
15 °C	++
25 °C	+++
37 °C	+++
45 °C	-
Multiplication in:	
pH 3.0	-
pH 4.0	-
pH 5.0	+++
pH 6.0	+++
Multiplication in:	
1.5%, 2.5%, 3.0% NaCl	+++
100, 120, 150 ppm $NaNO_2$	+++
100, 200, 300 ppm $NaNO_3$	+++

+++ strongly positive; ++ positive; - negative.

4 Conclusion

The commercial starter culture investigated in this study presents technological characteristics expected for application in sausage maturation processes. Although the culture did not present the characteristics for use as live probiotics due to its sensitivity to stomach acidity, it may still be considered a potential probiotic culture, since bacterial viability is not essential in the human health benefits afforded by probiotics. Future studies should focus on the use of inactive functional cells in food as

an important alternative for cases in which probiotics cannot survive processing, extensive shelf-life, or passage through the gastrointestinal tract. In addition, future research should determine the level of protection that adaptation mechanisms and encapsulation of probiotics can provide, allowing the use of starter cultures sensitive to acidic pH in the development of new products with functional properties.

Acknowledgements

The authors are grateful for financial support in part from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001 and Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB).

References

Acar, J. F., & Goldstein, F. W. (1991). Disk susceptibility test. In V. Lorian (Ed.), *Antibiotics in laboratory medicine* (chap. 2, pp. 17-52). New York: Williams & Wilkins.

Aguilar-Toalá, J. E., Garcia-Varela, R., Garcia, H. S., González, M.-H. V., Córdova, A. F., Vallejo-Córdoba, B., & Hernández-Mendoza, A. (2018). Postbiotics: an evolving term within the functional foods field. *Trends in Food Science & Technology*, 75, 105-114. http://dx.doi.org/10.1016/j.tifs.2018.03.009.

Almada, C. N., Almada, C. N., Martinez, R. C., & Sant'Ana, A. S. (2016). Paraprobiotics: evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. *Trends in Food Science & Technology*, 58, 96-114. http://dx.doi.org/10.1016/j.tifs.2016.09.011.

Barros, C. P., Guimarães, J. T. A., Esmerino, E., Duarte, M. C. K., Silva, M. C., Silva, R., Ferreira, B. M., Sant'ana, A. S., Freitas, M. Q., & Cruz, A. G. (2020). Paraprobiotics and postbiotics: concepts and potential applications in dairy products. *Current Opinion in Food Science*, 32, 1-8. http://dx.doi.org/10.1016/j.cofs.2019.12.003.

Behera, S. S., & Panda, S. K. (2020). Ethnic and industrial probiotic foods and beverages: efficacy and acceptance. *Current Opinion in Food Science*, 32, 29-36. http://dx.doi.org/10.1016/j.cofs.2020.01.006.

Bis-Souza, C. V., Penna, A. L. B., & Barretto, A. C. S. (2020). Applicability of potentially probiotic *Lactobacillus casei* in low-fat Italian type salami with added fructooligosaccharides: in vitro screening and technological evaluation. *Meat Science*, 168, 108186. http://dx.doi.org/10.1016/j.meatsci.2020.108186. PMid:32428692.

Brazilian Committee on Antimicrobial Susceptibility Testing – BrCAST. (2017). *Método de disco-difusão para teste de sensibilidade aos antimicrobianos. Versão 6.0*. Retrieved from http://brcast.org.br/download/documentos/Manual-Disco-Difusao-BrCAST-21092018.pdf

Castro-Bravo, N., Wells, J. M., Margolles, A., & Ruas-Madiedo, P. (2018). Interactions of Surface Exopolysaccharides from *Bifidobacterium* and *Lactobacillus* within the intestinal environment. *Frontiers in Microbiology*, 9, 2426. http://dx.doi.org/10.3389/fmicb.2018.02426. PMid:30364185.

Chen, M.-J., Tang, H.-Y., & Chiang, M.-L. (2017). Effects of heat, cold, acid and bile salt adaptations on the stress tolerance and protein expression of kefir-isolated probiotic *Lactobacillus kefiranofaciens* M1. *Food Microbiology*, 66, 20-27. http://dx.doi.org/10.1016/j.fm.2017.03.020. PMid:28576369.

Chugh, B., & Kamal-Eldin, A. (2020). Bioactive compounds produced by probiotics in food products. *Current Opinion in Food Science*, 32, 76-82. http://dx.doi.org/10.1016/j.cofs.2020.02.003.

Costa, W. K. A., Souza, G. T., Brandão, L. R., Lima, R. C., Garcia, E. F., Lima, M. S., Souza, E. L., Saarela, M., & Magnani, M. (2018). Exploiting antagonistic activity of fruit-derived *Lactobacillus* to control pathogenic bacteria in fresh cheese and chicken meat. *Food Research International*, 108, 172-182. http://dx.doi.org/10.1016/j.foodres.2018.03.045. PMid:29735046.

Cruxen, C. E. S., Funck, G. D., Haubert, L., Dannenberg, G. S., Marques, J. L., Chaves, F. C., Silva, W. P., & Fiorentini, Â. M. (2019). Selection of native bacterial starter culture in the production of fermented meat sausages: application potential, safety aspects, and emerging technologies. *Food Research International*, 122, 371-382. http://dx.doi.org/10.1016/j.foodres.2019.04.018. PMid:31229090.

Farnworth, E. R., & Champagne, C. P. (2016). Production of probiotic cultures and their incorporation into foods. In R. R. Watson & V. R. Preedy (Eds.), *Bioactives foods in promoting health* (chap. 1, pp. 303-318). Cambridge: Academic Press. http://dx.doi.org/10.1016/B978-0-12-802189-7.00020-4

Fraqueza, M. J. (2015). Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. *International Journal of Food Microbiology*, 212, 76-88. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.04.035. PMid:26002560.

Guimarães, J. T., Balthazar, C. F., Silva, R., Rocha, R. S., Graça, J. S. A., Esmerino, E., Silva, M. C., Sant'ana, A. S., Duarte, M. C. K. H., Freitas, M. Q., & Cruz, A. G. (2020). Impact of probiotics and prebiotics on food texture. *Current Opinion in Food Science*, 33, 38-44. http://dx.doi.org/10.1016/j.cofs.2019.12.002.

Han, Q., Kong, B., Chen, Q., Sun, F., & Zhang, H. (2017). *In vitro* comparison of probiotic properties of lactic acid bacteria isolated from Harbin dry sausages and selected probiotics. *Journal of Functional Foods*, 32(1), 391-400. http://dx.doi.org/10.1016/j.jff.2017.03.020.

Hitchener, B. J., Egan, A. F., & Rogers, P. J. (1982). Characteristics of lactic acid bacteria isolated from vacuum-packaged beef. *The Journal of Applied Bacteriology*, 52(1), 31-37. http://dx.doi.org/10.1111/j.1365-2672.1982.tb04369.x. PMid:7068524.

Horáčková, Š., Plocková, M., & Demnerová, K. (2018). Importance of microbial defence systems to bile salts and mechanisms of serum cholesterol reduction. *Biotechnology Advances*, 36(3), 682-690. http://dx.doi.org/10.1016/j.biotechadv.2017.12.005. PMid:29248683.

Kandylis, P., Pissaridi, K., Bekatorou, A., Kanellaki, M., & Koutinas, A. A. (2016). Dairy and non-dairy probiotic beverages. *Current Opinion in Food Science*, 7(2), 58-63. http://dx.doi.org/10.1016/j.cofs.2015.11.012.

Kavitake, D., Kandasamy, S., Devi, P. B., & Shetty, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods: a review. *Food Bioscience*, 21, 34-44. http://dx.doi.org/10.1016/j.fbio.2017.11.003.

Kongkiattikajorn, J. (2015). Potential of starter culture to reduce biogenic amines accumulation in *som-fug*, a thai traditional fermented fish sausage. *Journal of Ethnic Foods*, 2(4), 186-194. http://dx.doi.org/10.1016/j.jef.2015.11.005.

Kunrath, C. A., Savoldi, D. C., Mileski, J. P. F., Novello, C. R., Alfaro, A. T., Marchi, J. F., & Tonial, I. B. (2017). Application and evaluation of propolis, the natural antioxidant in italian-type salami. *Brazilian Journal of Food Technology*, 20(1), 1-10. http://dx.doi.org/10.1590/1981-6723.3516.

Laslo, É., György, É., & Czikó, A. (2019). Meat starter cultures: isolation and characterization of lactic acid bacteria from traditional sausages. *Acta Universitatis Sapientiae. Alimentaria*, 12(1), 54-69. http://dx.doi.org/10.2478/ausal-2019-0004.

Mafra, J. F., Santana, T. S., Cruz, A. I. C., Ferreira, M. A., & Evangelista-Barreto, N. S. (2019). Effect of the use of red propolis in salami of fish in the growth of fermenting lactic bacteria. *Higiene Alimentar*, 33, 2486-2490.

Müller, A., Reichhardt, R., Fogarassy, G., Danz, R. B., Gibis, M., Weiss, J., Schmidt, H., & Weiss, A. (2016). Safety assessment of selected *Staphylococcus carnosus* strains with regard to their application as meat starter culture. *Food Control*, 66, 93-99. http://dx.doi.org/10.1016/j.foodcont.2016.01.042.

Muñoz-Quezada, S., Chenoll, E., Vieites, J. M., Genovés, S., Maldonado, J., Bermúdez-Brito, M., Llorente, G. C., Matencio, E., Bernal, M. J., Romero, F., Suarez, U., Ramon, D., & Gil, A. (2013). Isolation, identification and characterization of three novel probiotic strains (*Lactobacillus paracasei* CNCM I-4034, *Bifidobacterium breve* CNCM I-4035 and *Lactobacillus rhamnosus* CNCM I-4036) from the faeces of exclusively breast-fed infants. *British Journal of Nutrition*, 109(2, Suppl. 2), 51-S62. http://dx.doi.org/10.1017/S0007114512005211. PMid:23360881.

Nishida, K., Sawada, D., Kawai, T., Kuwano, Y., Fujiwara, S., & Rokutan, K. (2017b). Para-psychobiotic Lactobacillus gasseri CP2305 ameliorates stress-related symptoms and sleep quality. *Journal of Applied Microbiology*, 123(6), 1561-1570. http://dx.doi.org/10.1111/jam.13594. PMid:28948675.

Nishida, K., Sawada, D., Kuwano, Y., Tanaka, H., Sugawara, T., Aoki, Y., Fujiwara, S., & Rokutan, K. (2017a). Daily administration of paraprobiotic *Lactobacillus gasseri* CP2305 ameliorates chronic stress associated symptoms in Japanese medical students. *Journal of Functional Foods*, 36, 112-121. http://dx.doi.org/10.1016/j.jff.2017.06.031.

Oliveira, M., Ferreira, V., Magalhães, R., & Teixeira, P. (2018). Biocontrol strategies for Mediterranean-style fermented sausages. *Food Research International*, 103, 438-449. http://dx.doi.org/10.1016/j.foodres.2017.10.048. PMid:29389634.

Orange. (2020). Retrieved from https://orange.biolab.si/download/#windows

Pavli, F. G., Argyri, A. A., Chorianopoulos, N. G., Nychas, G. J. E., & Tassou, C. C. (2020). Evaluation of *Lactobacillus plantarum* L125 strain with probiotic potential on physicochemical, microbiological and sensorial characteristics of dry-fermented sausages. *Lebensmittel-Wissenschaft + Technologie*, 118, 211-221. http://dx.doi.org/10.1016/j.lwt.2019.108810.

Piqué, N., Berlanga, M., & Miñana-Galbis, D. (2019). Health benefits of heat-killed (tyndallized) probiotics: an overview. *International Journal of Molecular Sciences*, 20(10), 2534. http://dx.doi.org/10.3390/ijms20102534. PMid:31126033.

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., & Aadil, R. M. (2020). Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science*, 32, 17-28. http://dx.doi.org/10.1016/j.cofs.2020.01.003.

Rossoni, R. D., Ribeiro, F. C., Barros, P. P., Mylonakis, E., & Junqueira, J. C. (2020). A prerequisite for health: probiotics. In M. E. Kambouris & A. Velegraki (Eds.), *Microbiomics: dimensions, applications, and translational implications of human and environmental microbiome research* (pp. 225-244). London: Academic Press. http://dx.doi.org/10.1016/B978-0-12-816664-2.00011-6.

Rozman, V., Mohar Lorbeg, P., Accetto, T., & Bogovič Matijašić, B. (2020). Characterization of antimicrobial resistance in lactobacilli and bifidobacteria used as probiotics or starter cultures based on integration of phenotypic and in silico data. *International Journal of Food Microbiology*, 314, 108388. http://dx.doi.org/10.1016/j.ijfoodmicro.2019.108388. PMid:31707173.

RStudio Team. (2015). *RStudio: integrated development environment for R*. Retrieved from http://www.rstudio.com

Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., Villa, R. E., Wallace, R. J., Wester, P., Glandorf, B., Herman, L., Kärenlampi, S., Aguilera, J., Anguita, M., Brozzi, R., & Galobart, J. (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, 16(3), 5206. http://dx.doi.org/10.2903/j.efsa.2018.5206. PMid:32625840.

Sarkar, A., & Mandal, S. (2016). Bifidobacteria: insight into clinical outcomes and mechanisms of its probiotic action. *Microbiological Research*, 192, 159-171. http://dx.doi.org/10.1016/j.micres.2016.07.001. PMid:27664734.

Savoldi, D. C., Kunrath, C. A., Oliveira, D. F., Novello, C. R., Coelho, A. R., Marchi, J. F., & Tonial, I. B. (2019). Características físicas e sensoriais de Salame Tipo Italiano com adição de própolis. *Revista de Ciências Agroveterinárias*, 18(2), 212-221. http://dx.doi.org/10.5965/223811711812019212.

Sawada, D., Kuwano, Y., Tanaka, H., Hara, S., Uchiyama, Y., Sugawara, T., Fujiwara, S., Rokutan, K., & Nishida, K. (2019). Daily intake of *Lactobacillus gasseri* CP2305 relieves fatigue and stress-related symptoms in male university Ekiden runners: a double-blind, randomized, and placebo-controlled clinical trial. *Journal of Functional Foods*, 57, 465-476. http://dx.doi.org/10.1016/j.jff.2019.04.022.

Sawada, D., Sugawara, T., Ishida, Y., Aihara, K., Aoki, Y., Takehara, I., Takano, K., & Fujiwara, S. (2016). Effect of continuous ingestion of a beverage prepared with *Lactobacillus gasseri* CP2305 inactivated by heat treatment on the regulation of intestinal function. *Food Research International*, 79, 33-39. http://dx.doi.org/10.1016/j.foodres.2015.11.032.

Shripada, R., Gayatri, A.-J., & Sanjay, P. (2020). Paraprobiotics. In J. Faintuch & S. Faintuch (Eds.), *Precision medicine for investigators, practitioners and providers* (pp. 39-49). London: Academic Press. http://dx.doi.org/10.1016/B978-0-12-819178-1.00005-8.

Sivamaruthi, B. S., Fern, L. A., Ismail, D. S. N. R. P. H., & Chaiyasut, C. (2020). The influence of probiotics on bile acids in diseases and aging. *Biomedicine and Pharmacotherapy*, 128, 110310. http://dx.doi.org/10.1016/j.biopha.2020.110310. PMid:32504921.

Slima, S. B., Ktari, N., Triki, M., Trabelsi, I., Abdeslam, A., Moussa, H., Makni, I., Herrero, A. M., Jiménez-Colmenero, F., & Ruiz-Capillas, C. (2018). Effects of probiotic strains, *Lactobacillus plantarum* TN8 and *Pediococcus acidilactici*, on microbiological and physico-chemical characteristics of beef sausages. *Lebensmittel-Wissenschaft + Technologie*, 92, 195-203. http://dx.doi.org/10.1016/j.lwt.2018.02.038.

Soares, M. B., Martinez, R. C., Pereira, E. P., Balthazar, C. F., Cruz, A. G., Ranadheera, C. S., & Sant'ana, A. S. (2019). The resistance of *Bacillus, Bifidobacterium*, and *Lactobacillus* strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Research International*, 125, 108542. http://dx.doi.org/10.1016/j.foodres.2019.108542. PMid:31554104.

Sugawara, T., Sawada, D., Ishida, Y., Aihara, K., Aoki, Y., Takehara, I., Takano, K., & Fujiwara, S. (2016). Regulatory effect of paraprobiotic *Lactobacillus gasseri* CP2305 on gut environment and function. *Microbial Ecology in Health and Disease*, 27(1), 30259. PMid:26979643.

Vandenplas, Y., Bacarea, A., Marusteri, M., Bacarea, V., Constantin, M., & Manolache, M. (2017). Efficacy and safety of APT198K for the treatment of infantile colic: a pilot study. *Journal of Comparative Effectiveness Research*, 6(2), 137-144. http://dx.doi.org/10.2217/cer-2016-0059. PMid:28114795.

Vieira, K. C. O., Ferreira, C. S., Bueno, E. B. T., Moraes, Y. A., Toledo, A. C. C. G., Nakagaki, W. R., Pereira, V. C., & Winkelstroter, L.

K. (2020). Development and viability of probiotic orange juice supplemented by *Pediococcus acidilactici* CE51. *Lebensmittel-Wissenschaft + Technologie*, 130, 109637. http://dx.doi.org/10.1016/j.lwt.2020.109637.

Zarzecka, U., Zadernowska, A., & Chajęcka-Wierzchowska, W. (2020). Starter cultures as a reservoir of antibiotic resistant microorganisms. *Lebensmittel-Wissenschaft + Technologie*, 127, 109424. http://dx.doi.org/10.1016/j.lwt.2020.109424.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, 32, 103-123. http://dx.doi.org/10.1016/j.cofs.2020.03.009.

Zhao, M., Huang, X., Zhang, H., Zhang, Y., Gänzle, M., Yang, N., Nishinari, K., & Fang, Y. (2020). Probiotic encapsulation in water-in-water emulsion via heteroprotein complex coacervation of type: a gelatin/sodium caseinate. *Food Hydrocolloids*, 105, 105790. http://dx.doi.org/10.1016/j.foodhyd.2020.105790.