FRANCIELLY SOARES OLIVEIRA

GENOMIC CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF *Pediococcus pentosaceus* ST65ACC

Thesis submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Luís Augusto Nero

Co-adviser: Antônio Fernandes de Carvalho

VIÇOSA - MINAS GERAIS
2023

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
O48g
2023

Oliveira, Francielly Soares, 1991-
Genomic characterization and antimicrobial activity of *Pediococcus pentosaceus* ST65ACC / Francielly Soares Oliveira. – Viçosa, MG, 2023.
1 tese eletrônica (113 f.): il. (algumas color.).

Texto em inglês.
Orientador: Luis Augusto Nero.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Tecnologia de Alimentos, 2023.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2023.720
Modo de acesso: World Wide Web.

1. *Pediococcus*. 2. Preservação biológica. 3. Bacteriocinas. 4. Pediocina. 5. Genômica. I. Nero, Luis Augusto, 1975-. II. Universidade Federal de Viçosa. Departamento de Tecnologia de Alimentos. Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. III. Título.

CDD 22. ed. 579.35

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

FRANCIELLY SOARES OLIVEIRA

GENOMIC CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF *Pediococcus pentosaceus* ST65ACC

Thesis submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

APPROVED: October 06, 2023.

Assent:

Documento assinado digitalmente
gov.br
FRANCIELLY SOARES OLIVEIRA
Data: 17/11/2023 10:36:06-0300
Verifique em https://validar.iti.gov.br

Francielly Soares Oliveira
Author

Documento assinado digitalmente
gov.br
LUIS AUGUSTO NERO
Data: 20/11/2023 10:46:11-0300
Verifique em https://validar.iti.gov.br

Luís Augusto Nero
Adviser

ACKNOWLEDGEMENTS

I thank God for his guidance and strength, especially in the most difficult times.

To my parents, João and Santa, and to my brothers, Fernanda, Paula, and Tiago for all the support and encouragement... I love you so much.

To Professor Nero, for his guidance, for all the knowledge shared, for his understanding and patience, all my thanks.

To all my friends and colleagues at InsPOA and Viçosa, thank you so much for making these years easier. In particular, I thank Rafa for her friendship and fundamental support in carrying out this work, in planning, carrying out the analyzes, and support in writing and revising the articles.

To Professor Ricardo and Professor Antônio for all the support, thank you!

To all DVT students and technicians who contributed to the realization of this work, especially Morine, Dagô, and Seu Luis.

To the Federal Agency for Support and Evaluation of Graduate Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasília, DF, Brazil–FinanceCode 001), forgranting the scholarship. Also, my acknowledgment to the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasília, DF, Brazil) and the Minas Gerais State Research Support Foundation (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG, Belo Horizonte, MG, Brazil), for supporting the research.

Finally, to the Federal University of Viçosa, especially the Department of Food Technology, for the opportunity to complete the Graduate training.

ABSTRACT

OLIVEIRA, Francielly Soares, D.Sc., Universidade Federal de Viçosa, October, 2023. **Genomic characterization and antimicrobial activity of *Pediococcus pentosaceus* ST65ACC**. Adviser: Luís Augusto Nero. Co-adviser: Antônio Fernandes de Carvalho.

The bacteriocinogenic strain *Pediococcus pentosaceus* ST65ACC was previously isolated from Brazilian artisan cheese (BAC) and showed strong bactericidal activity against *Listeria monocytogenes*. This strain also showed beneficial properties, such as the ability to survive gastrointestinal conditions and to aggregate with *L. monocytogenes*, indicating it as a potential probiotic candidate. The present study aimed to genomically characterize the *P. pentosaceus* ST65ACC strain through whole-genome sequencing (WGS), analyzing its technological and beneficial properties, bacteriocin clusters, and safety profiles, and also evaluating its antimicrobial activity against *L. monocytogenes* Scott A in different culture media. The genome of *P. pentosaceus* ST65ACC comprises 1,933,194 bp witha GC content of 37.00 %, and contains1,950 protein coding sequences (CDSs) with 62 RNA genes (6 rRNA, 55 tRNA, 1 tmRNA). Genomic analysis revealed the presence of operons that encode the bacteriocins pediocin PA-1/AcH and penocin-A, and also identified the presence of genes related to beneficial properties, such as stress adaptation genes and adhesion genes. Regarding the strain safety profiles, genes encoding biogenic amines and virulence genes were not detected. Genes related to antibiotic resistance were identified in the genome, but not in prophage regions, and no plasmids were detected, thus presenting a low risk of transferring to other bacteria. The results obtained fromthe interaction of *P. pentosaceus* ST65ACC and *L. monocytogenes* Scott A in MRS broth, BHI broth, milk, and meat broth for 96 h showed that the antimicrobial activity of the strain was more effective in MRS broth. *L. monocytogenes* was inhibited toun detectable levels by the strain in MRS broth during the 96 h of analysis, with high levels of bacteriocin production (3,200 – 12,800 AU/ml) being identified. However, lower inhibitory activities were recorded in BHI, milk, and meat broth, with low or no production of bacteriocins at the times analyzed. Thus, verifying that the composition of the media can interfere with the production/activity of bacteriocins and, consequently, with the antagonistic activity of

P. pentosaceus ST65ACC on *L. monocytogenes* Scott A. The results obtained through genomic characterization confirmed the beneficial potential and safety of *P. pentosaceus* ST65ACC, indicating that this strain can be considered suitable for application in food biopreservation, as well as a promising probiotic candidate.

Keywords: *Pediococcus*. Biopreservation. Bacteriocins. Pediocin. Genomic analysis.

RESUMO

OLIVEIRA, Francielly Soares, D.Sc., Universidade Federal de Viçosa, outubro de 2023. **Caracterização genômica e atividade antimicrobiana de *Pediococcus pentosaceus* ST65ACC**. Orientador: Luís Augusto Nero. Coorientador: Antônio Fernandes de Carvalho.

A cepa bacteriocinogênica *Pediococcus pentosaceus* ST65ACC foi previamente isolada de queijo artesanal brasileiro, e apresentou forte atividade bactericida contra *Listeria monocytogenes*. Esta cepa também apresentou propriedades benéficas, como capacidade de sobreviver a condições gastrointestinais e de se agregar com *L. monocytogenes*, indicando-a como potencial candidata probiótica. O presente estudo teve como objetivo caracterizar genomicamente a cepa *P. pentosaceus* ST65ACC através do sequenciamento total do genoma (WGS), analisando suas propriedades tecnológicas e benéficas, agrupamentos de bacteriocinas e perfis de segurança, e avaliar a atividade antimicrobiana da cepa sobre *L. monocytogenes* Scott A em diferentes meios de cultura. O genoma de *P. pentosaceus* ST65ACC apresentou 1.933.194 pb e um conteúdo de GC de 37,00 %, e tem 1.950 sequências codificadoras de proteínas (CDSs) com 62 genes de RNA (6 rRNA, 55 tRNA, 1 tmRNA). A análise genômica revelou a presença de operonsque codificam as bacteriocinas pediocina PA-1/AcH e penocina-A, e também identificou a presença de genes relacionados a propriedades benéficas, como genes de adaptação ao estresse e genes de adesão. Em relação aos perfis de segurança da cepa, não foram detectados genes codificadores de aminas biogênicas e genes de virulência. Genes relacionados à resistência a antibióticos foram identificados no genoma, mas não em regiões de profagos e nenhum plasmídeo foi detectado, apresentando baixo risco de serem transferidos para outras bactérias. Os resultados obtidos com a interação de *P. pentosaceus* ST65ACC e *L. monocytogenes* Scott A em caldo MRS, caldo BHI, leite e caldo de carne durante 96 h mostraram que a atividade antimicrobiana da cepa foi mais eficaz em caldo MRS. A população de *L. monocytogenes* foi inibida a níveis não detectáveis pela cepa em caldo MRS durante as 96 h de análise, sendo identificados altos níveis de produção de bacteriocinas (3.200 – 12.800 UA/ml). No entanto, menores atividades inibitórias foram registradas em BHI, leite e caldo de carne, com baixa ou nenhuma produção de bacteriocinas

nos tempos analisados. Dessa forma, o estudo revelou que a composição dos meios pode interferir na produção/atividade das bacteriocinas e, consequentemente, na atividade antagonista de *P. pentosaceus* ST65ACC sobre *L. monocytogenes* Scott A. Os resultados obtidos através da caracterização genômica confirmaram o potencial benéfico e segurança de *P. pentosaceus* ST65ACC, indicando que essa cepa pode ser considerada adequada para aplicação na biopreservação de alimentos, bem como um candidato probiótico promissor.

Palavras-chave: *Pediococcus*. Biopreservação. Bacteriocinas. Pediocina. Análise genômica.

SUMMARY

GENERAL INTRODUCTION

Lactic acid bacteria (LAB) constitute a group of microorganisms widely distributed in nature. They are found in dairy products, meat products, vegetables, in the gastrointestinal and urogenital tract of humans and animals, as well as soil and water (Liu et al., 2014). BAL have the ability to produce lactic acid as the main end product of carbohydrate metabolism and are generally described as Gram-positive, facultative anaerobic or microaerophilic, catalase negative, non-spore-forming, and cocci- or rod-shaped. They have a long history of use in the food industry, being fundamental for fermentation processes, as they confer beneficial characteristics (nutritional, sensory, and technological) to fermented foods and also play an important role in protecting these products (Said et al., 2019). The production of organic acids by LAB and the consequent reduction in pH constitute the main biopreservation mechanisms of fermented foods, in addition to other metabolites, such as hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins (Porto et al., 2017; Bintsis, 2018).

Several studies have been carried out over the years with the aimed at identifying and characterizing new LAB strains with antimicrobial activity for food bioprotection, focusing on the spectrum of action and characterization of the antimicrobial peptides produced (Cavicchioli et al., 2017; Said et al., 2018; Castilho et al., 2019). Bacteriocins can be produced by a wide variety of bacteria and are of particular interest to the industry due to their performance in the control and inhibition of pathogens and spoilage microorganisms, and may have important activities and properties for use in food, as well as in the veterinary and pharmaceutical segments. Thus, several bacteriocins have been identified, sequenced, and detailed in several databases, such as BACTIBASE and BAGEL4 (Hammami et al., 2007; van Heel et

al., 2018).

The functionality of a microorganism is considered important in selecting a culture for fermentation or use as a bioprotective culture. However, the main regulatory concern is its safety (Pariza et al., 2015; Coton et al., 2018). Many BAL species are considered safe (Generally Regarded as Safe, GRAS status) by the Food and Drug Administration (FDA), and have also received QPS (Qualified Assumption of Security) status in the European Union for food applications (Bourdichon et al., 2012; EFSA, 2018). However, despite this status, an efficient evaluation of the safety criteria of promising strains for application in food is necessary. Some LAB may have the capacity to produce toxic compounds, such as biogenic amines (BA), and strains of *Lactococcus*, *Enterococcus*, *Pediococcus*, and lactobacilli have already been associated with high concentrations of these compounds in fermented foods (Özogul & Hamed, 2018; Barbieri et al., 2019; Tsanasidou et al., 2021). Furthermore, the transfer of antimicrobial resistance (AMR) genes among LAB strains and pathogenic microorganisms has already been described in several studies (Nunziata et al., 2022). Thus, the European Food Safety Authority (EFSA) recommends analyzing the WGS data of potential probiotic candidates, in addition to their phenotypic resistance profiles to verify the presence of AMR genes, ensuring that there are low risks of spreading mobile genetic elements associated with antimicrobial resistance (Coton et al., 2018; Nunziata et al., 2022).

Next-generation sequencing has facilitated the identification and proper characterization of promising probiotic strains, providing in just one assay to obtain data that are explored by genomic analyzes (Morovic et al., 2016; 2017). Through genomic analysis, it is possible to detect genetic elements associated with the production of desirable compounds, such as bacteriocin or undesirable ones, such as

genes related to AMR and virulence (Wu et al., 2017). The identification of possible genetic determinants of AMR, genes encoding virulence factors, or enzymes responsible for the production of biogenic amines can qualify and disqualify the application of strains in food production (Morovic et al., 2017; Markusková et al., 2018).

Pediococcus spp., belonging to the LAB genus, are of great importance to the food industry due to their important role in the developing desirable sensory characteristics, controlling the fermentation process, and acting as food biopreservatives (Porto et al., 2017). In previous studies, the strain *P. pentosaceus* ST65ACC was isolated from artisanal raw milk cheese (Cavicchioli et al., 2017) and characterized as bacteriocinogenic with strong anti-listerial activity (Cavicchioli et al., 2017; 2019), showing beneficial properties that characterize it as a potential probiotic candidate (Cavicchioli et al., 2019). Furthermore, this strain showed very low frequencies of virulence markers and did not show BA production. However, the strain showed some characteristics of antibiotic resistance (Cavicchioli et al., 2019).

Therefore, this work aimed to genomically characterize *P. pentosaceus* ST65ACC, regarding its beneficial and safety characteristics for its application in the food industry, and to evaluate its antimicrobial activity and production of bacteriocins in different culture media.

References

Barbieri F, Montanari C, Gardini F, Tabanelli G (2019) Biogenic amine production by lactic acid bacteria: a review. Foods 8(1):17. https://doi.org/10.3390/foods8010017

Bintsis T (2018) Lactic acid bacteria: their applications in foods. J Bacteriol Mycol Open Access 6(2):89–94. https://doi.org/10.15406/jbmoa.2018.06.00182

Bourdichon F, Berger B, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, Harnett J, Huys G, Laulund S, Ouwehand A, Powell IB, Prajapati JB, Seto Y,Ter Schure E, Van Boven A, Vankerckhoven V, Zgoda A, Bech Hansen E (2012) A safety assessment of microbial food cultures with history of use in fermented dairy products. Bull Int Dairy Fed 455:2–12.

Castilho NPA, Nero LA, Todorov SD (2019) Molecular screening of beneficial and safety determinants from bacteriocinogenic lactic acid bacteria isolated from Brazilian artisanal calabresa. Lett Appl Microbiol 69(3):204-211. https://doi.org/10.1111/lam.13194

Cavicchioli VQ, Camargo AC, Todorov SD, Nero LA (2017) Novel bacteriocinogenic *Enterococcus hirae* and *Pediococcus pentosaceus* strains with antilisterial activity isolated from Brazilian artisanal cheese. J Dairy Sci 100:2526–2535. https://doi.org/10.3168/jds.2016-12049

Cavicchioli VQ, Camargo AC, Todorov SD, Nero LA (2019) Potential control of *Listeria monocytogenes* by bacteriocinogenic *Enterococcus hirae* ST57ACC and *Pediococcus pentosaceus* ST65ACC strains isolated from artisanal cheese. Probiotics Antimicrob Proteins 11:696–704.https://doi.org/10.1007/s12602-018-9449-0

Coton M,Lebreton M, Leyva Salas M, Garnier L, Navarri M,Pawtowski A, Le Blay G, Valence F, Coton E, Mounier J(2018) Biogenic amine and antibiotic resistance profiles determined for lactic acid bacteria and a *Propionibacterium* prior to use as antifungal bioprotective cultures. International Dairy Journal 85:21–26. https://doi.org/10.1016/j.idairyj.2018.05.001

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, 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 J 16(3):e05206. https://doi.org/10.2903/j.efsa.2018.5206

Hammami R, Zouhir A, Ben Hamida J, Fliss I (2007) BACTIBASE: a new web-accessible database for bacteriocin characterization. BMC Microbiol 7:89. https://doi.org/10.1186/1471-2180-7-89

Liu W, Pang H, Zhang H, Cai Y (2014) Biodiversity of lactic acid bacteria. In: Zhang H, Cai Y (eds) Lactic Acid Bacteria. Springer, Dordrecht, pp103–203. https://doi.org/10.1007/978-94-017-8841-0_2

Markusková B, Lichvariková A, Szemes T, Korenová J, Kuchta T, Drahovská H (2018) Genome analysis of lactic acid bacterial strains selected as potential starters for traditional Slovakian bryndza cheese. FEMS Microbiol Lett 365(23). https://doi.org/10.1093/femsle/fny257

Morovic W, Hibberd AA, Zabel B, Barrangou R, Stahl B (2016) Genotyping by PCR and high-throughput sequencing of commercial probiotic products reveals composition biases. Front Microbiol 7:1747. https://doi.org/10.3389/fmicb.2016.01747

Morovic W, Roper JM, Smith AB, Mukerji P, Stahl B, Rae JC, Ouwehand AC (2017) Safety evaluation of HOWARU® Restore (*Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium animalis* subsp. *lactis* Bl-04 and *B. lactis* Bi-07) for antibiotic resistance, genomic risk factors, and acute toxicity. Food Chem Toxicol 110:316-324. https://doi.org/10.1016/j.fct.2017.10.037

Nunziata L, Brasca M, Morandi S, Silvetti T (2022) Antibiotic resistance in wild and commercial non-enterococcal Lactic Acid Bacteria and Bifidobacteria strains of dairy origin: An update. Food Microbiol 104:103999. https://doi.org/10.1016/j.fm.2022.103999

Pariza MW, Gillies KO, Kraak-Ripple SF, Leyer G, Smith AB (2015) Determining the safety of microbial cultures for consumption by humans and animals. Regul Toxicol Pharmacol 73(1):164-71. https://doi.org/10.1016/j.yrtph.2015.07.003

Porto MC, Kuniyoshi TM, Azevedo PO, Vitolo M, Oliveira RP (2017) *Pediococcus*spp.: An important genus of lactic acid bacteria and pediocin producers. Biotechnol Adv 35:361-374. https://doi.org/10.1016/j.biotechadv.2017.03.004

Özogul F, Hamed I (2018) The importance of lactic acid bacteria for the prevention of bacterial growth and their biogenic amines formation: A review. Crit Rev Food Sci Nutr 58:1660–1670. https://doi.org/10.1080/10408398.2016.1277972.

Said LB, Gaudreau H, Dallaire L, Tessier M, FlissI (2019) Bioprotective culture: A new generation of food additives for the preservation of food quality and safety. Industrial Biotechnology 15(3):138-147.https://doi.org/10.1089/ind.2019.29175.lbs

Tsanasidou C, Asimakoula S, Sameli N, Fanitsios C, Vandera E, Bosnea L, Koukkou A-I, Samelis J (2021) Safety evaluation, biogenic amine formation, and enzymatic activity profiles of autochthonous enterocin-producing greek cheese isolates of the *Enterococcus faecium / durans* group. Microorganisms 9(4):777. https://doi.org/10.3390/microorganisms9040777

van Heel AJ, de Jong A, Song C, Viel JH, Kok J, Kuipers OP (2018) BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. Nucleic Acids Res 46:W278–W281. https://doi.org/10.1093/nar/gky383

Wu C, Huang J, Zhou R (2017) Genomics of lactic acid bacteria: Current status and potential applications. Crit Rev Microbiol 43(4):393-404. https://doi.org/10.1080/1040841X.2016.1179623

OBJECTIVES

The aim of this study was to carry out a genomic characterization of the *P. pentosaceus* ST65ACC strain based on whole genome sequencing (WGS) and to evaluate its antimicrobial activity against *Listeria monocytogenes* in different culture media. Considering this main objective, the specific objectives were:

- ✓ Conduct a literature review on *Pediococcus* spp.;
- ✓ Perform whole-genome sequencing (WGS) of *P. pentosaceus* ST65ACC;
- ✓ Compare its genomic sequence with sequences from other strains;
- ✓ Identify biotechnological properties, bacteriocin gene clusters, and safety-related genes through genome exploration and comparative analyses;
- ✓ Evaluate the interaction between *P. pentosaceus* ST65ACC and *L. monocytogenes* Scott A in MRS broth, BHI, reconstituted skim milk, and meat broth;
- ✓ Assess the influence of culture media on the production of bacteriocins by the strain *P. pentosaceus* ST65ACC.

CHAPTER 1: *Pediococcus* spp.: bioprotective and probiotic potential

Francielly Soares Oliveira et al.

Manuscript prepared for submission to *Brazilian Journal of Microbiology*

Titlepage

Francielly Soares Oliveira[1,2], Rafaela da Silva Rodrigues[1,2], Valéria Quintana Cavicchioli[3], Antônio Fernandes de Carvalho[2], Luís Augusto Nero[1*]

Pediococcus spp.: bioprotective and probiotic potential

[1]InsPOA – Laboratório de Inspeção de Produtos de Origem Animal, Departamento de Veterinária, Universidade Federal de Viçosa, 36570 900, Viçosa MG, Brazil

[2]InovaLeite – Laboratório de Pesquisa em Leites e Derivados, Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, 36570 900, Viçosa MG, Brazil

[3]Centro de Pesquisa em Alimentos, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, 74690 900, Goiânia, GO, Brazil

*Corresponding author: Luís Augusto Nero (nero@ufv.br)

Abstract

Pediococcus spp. are widely found in nature, being of great importance given their increasing use in the food industry and health. These bacteria not only contribute to the development of technological, organoleptic and nutritional characteristics of foods, but can also play an important role in their biopreservation through the production of antimicrobial metabolites, such as bacteriocins. Pediocins, small unmodified peptides, are the main bacteriocins produced by *Pediococcus* and have the capacity to inhibit different pathogenic microorganisms, mainly *Listeria monocytogenes*. Several studies on pediococci have been developed on the production of bacteriocins, their spectrum of action, and their applications in food and silage. In addition to these important roles in biopreservation processes, some *Pediococcus* species are beneficial to human and animal health, due to their potential to reduce enteric diseases, modulation of the intestinal microbiota, anti-inflammatory capacity, among other benefits. In this review we present the main characteristics of *Pediococcus* spp., their bioprotective and probiotic properties, produced bacteriocins and current examples of the commercial use of these microorganisms.

Keywords: *Pediococcus*, biopreservation, pediocin, probiotics

Introduction

Pediococcus, a genus of lactic acid bacteria (LAB), are Gram-positive bacteria, shaped like cocci in pairs or tetrads, and occur naturally in a variety of foods, such as dairy products, fermented vegetables, and meat products (1, 2). In addition to contributing to the texture, aroma and flavor of foods, they can play an important role in their preservation by producing different antimicrobial metabolites, including organic acids, hydrogen peroxide, diacetyl, and bacteriocins (3). Bacteriocins are antimicrobial peptides synthesized ribosomally by different bacteria and are capable of inhibiting closely related microorganisms or foodborne pathogens (4).

Bacteriocins produced by *Pediococcus* have been widely studied and applied in food production and have shown potential as biopreservatives and as antimicrobial agents in human and veterinary medicine (5, 6). *Pediococcus* bacteriocinogenic strains normally produce pediocin PA-1/AcH, a low molecular weight class IIa bacteriocin with a consensus motif of YGNG(V/L), and show bactericidal activity against several pathogenic bacteria, most notably *Listeria monocytogenes* (7, 8). These bacteriocins are characterized as excellent biopreservatives because they are generally recognized as safe (GRAS), active at low concentrations and in a wide pH range, and because they have high heat stability (9).

Many *Pediococcus* strains are also strong candidates for probiotics, as they are resistant to acidity and survive passage through the gastrointestinal tract, thus providing benefits to the health of the host (10, 11). Beneficial results from the use of *Pediococcus* spp. both in animal and human practices have been highlighted, such as intestinal colonization capacity (11, 12), infection control (13), intestinal microbiota modulation (14, 15, 16), anticancer activity (17, 18), improvement in the productive performance and growth of animals (19, 20), among other properties.

Thus, in this review we aimed to present the main characteristics of *Pediococcus* spp., their bacteriocins produced and use as a biopreservative, and their potential as a probiotic candidate.

Taxonomy and characteristics of *Pediococcus*

Pediococcus spp. belong to the family *Lactobacillaceae* in the order *Lactobacillales*, characterized as Gram-positive homofermentative and facultative aerobic bacteria, they present cocci morphology in pairs or tetrads, with diameter varying between 0.5 and 1.0 µm (1, 21, 22). The arrangement of cells in tetrads is the result of alternating division in two perpendicular directions, just like the genus *Tetragenococcus* spp. (1, 21). They are immobile, catalase and oxidase negative, and do not produce spores (2, 23). Although, some strains of *Pediococcus pentosaceus* have been reported as presenting pseudo-catalase activity when grown in media with low carbohydrate concentrations (24).

The genus *Pediococcus* currently includes the species *P. acidilactici*, *P. pentosaceus*, *P. damnosus*, *P. parvulus*, *P. inopinatus*, *P. claussenii*, *P. cellicola* and *P. stilesii* (1, 8). This classification underwent several changes over the years and due to advances in taxonomic identification techniques it was possible to reclassify some species. Collins et al. (25), through analysis of 16S rRNA gene sequences, demonstrated that *P. urinaeequi* (26) was a member of the genus *Aerococcus*. However, it was Felis et al. (27) who described the taxonomic status of *P. urinaeequi* and proposed its reclassification under the name of *Aerococcus urinaeequi*, based on comparative analysis of 16S rRNA gene sequence and DNA-DNA hybridization. Collins et al. (25) transferred *P. halophilus* (28) to a new genus and renamed it

Tetragenococcus halophilus, based on sequence analysis of the 16S rRNA gene. Strains formerly known as *P. cerevisiae* have also been reclassified and distributed among the species *P. damnosus*, *P. pentosaceus*, and *P. acidilactici* (28-29). Colins et al. (25) and Dobson et al. (30) suggested the reclassification of *P. dextrinicus* (31) to the genus *Lactobacillus*, since the strain is not closely related to the other *Pediococcus*, as demonstrated by the similarity of the 16S rDNA sequence. Consequently, Haakensen et al. (32) proposed a formal reclassification of *P. dextrinicus* to *Lactobacillus dextrinicus*, subsequently reclassified as *Lapidilactobacillus dextrinicus* (33).

Pediococcus spp. have been isolated from different sources such as plants, fruits, vegetables (34, 36), meat and meat products (37, 38), dairy products (39-42), beverages and fermented foods (43, 44), silage (45), animals, and humans (46, 47, 48). They are generally found in the same environments as other LABs such as lactobacilli, *Leuconostoc* and *Weissella* because they have similar growth requirements (23). *Pediococcus* strains are also frequently found in wines (2, 49), beers (30, 50, 51) and in the environment where these beverages are produced (52). *P. pentosaceus* and *P. acidilactici* are the species of this genus most frequently isolated and associated with food, being used in the production of pediocin, fermentation processes, and probiotic supplements for humans and animals (7, 8).

All *Pediococcus* species demonstrate optimal growth between 25-35 °C, pH range of 6.0-6.5, and tolerate concentrations of 4 to 5 % NaCl (1, 22). However, the optimal conditions depend on each species. All species grow at pH 4.5 (22), and most *Pediococcus* grow at pH 7.5, with the exception of *P. damnosus* (24), while *P. stilesii* can grow at pH 9.6 (35). Notably, *P. acidilactici* and *P. pentosaceus* are able to with stand concentrations of up to 10 % NaCl and can grow at pH 8.5.

Phenotypically, the strains of *P. acidilactici* and *P. pentosaceus* are difficult to separate, differing only in the inability of *P. acidilactici* to ferment maltose and show the ability to grow at temperatures of 50 °C, while *P. pentosaceus* tolerates temperatures of 39-45 °C. Classified as homofermentative bacteria, *Pediococcus* spp. can metabolize glucose via Embden-Meyerhof-Parnas (EMP) producing lactic acid as a final product, without the production of CO_2 (23). *P. claussenii* produce L(+)-lactate unlike other *Pediococcus* which produce DL-lactate from glucose (1). *P. inopinatus* and *P. parvulus* are incapable of using pentoses (24), which differentiates them from other species. Particularly demanding in terms of nutrients for cultivation, *Pediococcus* requires media rich in amino acids, minerals, and vitamins (2).

Genotypically, lactobacilli is the closest group to the genus *Pediococcus*, with 16S rRNA gene sequence similarities ranging from 85 to 94 %. The sequence similarity of the 16S rRNA gene within the genus *Pediococcus* shows values between 91.3-99.3 %, with *P. damnosus* and *P. inopinatus* sharing the greatest similarity (23, 53). The genetic material of this genus presents a guanine and cytosine (G+C) content between 35 to 44 % (1).

Pediococcus **biocontrol potential**

Pediococcus spp., as well as other LABs, can produce various antimicrobial compounds such as organic acids, hydrogen peroxide, antimicrobial proteins or bacteriocins. These compounds can contribute to the control of pathogens and spoilage bacteria, there by prolonging storage and increasing food safety (3, 54). The capacity of some strains to produce bacteriocins holds considerable importance, due to their potential application as bioprotective agents in both foods and clinical settings

(5, 55-57).

Bacteriocins are antimicrobial peptides synthesized ribosomally by various bacteria and released into the extracellular environment as a mechanism to combat other microorganisms in their natural environments (58-59). Bacteriocins produced by LAB can present antagonistic activity against a wide variety of bacteria, typically inhibiting genetically related bacteria (4, 60, 61). They are of particular interest to the food industry due to their heat stability and their classification as safe for application in food production (GRAS status) (62). These peptides can be divided into two classes according to their nature: Class I, comprising small peptides (≤ 5 kDa) post-translationally modified (antibiotics); Class II – peptides (≤ 10 kDa) that are not post-translationally modified, further divided into five subclasses, IIa (pediocin-like bacteriocins), IIb (two-component bacteriocins), IIc (cyclic bacteriocins), IId (linear peptides, not similar to pediocin), and IIe (bacteriocins that contain a carboxy-terminal region rich in serine) (5).

Bacteriocins produced by *Pediococcus* spp. are called pediocins or pediocin-like, and are often encoded by plasmids, but genes encoding bacteriocins can be located on chromosomes (63-65). Pediocins are small unmodified peptides, belonging to the class IIa of bacteriocins, and are also known as heat-stable peptides with strong antilisterial activity (7-8).Pediocin-like bacteriocins (PLBs) contain 36 to 48 amino acid residues and exhibit 40-60 % amino acid sequence similarity (58). These peptides share a conserved N-terminal “pediocin box” motif YGNG(V/L), usually in a larger sequence stabilized by a disulfide bond, YGNG(V/L)X1CX2(K/N)X3X4C, and a C-terminus region amphiphilic or hydrophobic less conserved (7, 65, 66). The conserved N-terminal region forms a structure similar to a three-stranded antiparallel β sheet, stabilized by a disulfide and several

hydrogen bonds. One side of this structure harbors hydrophobic residues, including two cysteines, while the other side is composed of hydrophilic residues. The C-terminal region consists of anα-helix followed by an extended tail that folds back into the central α-helix, forming a hairpin-like structure responsible for cell recognition (67).

The biosynthesis of class II bacteriocins involves a gene cluster composed of four main genes, a structural peptide precursor of the bacteriocin, an immunity protein, an ABC transporter (ATP-binding cassette), which exports the bacteriocin out of the cell, and an accessory protein, responsible for the regulation of expression (8, 64, 66). Pediocin is synthesized by an inactive prebacteriocin (*pedA*) of 66 amino acid residues, with an N-terminal length of 18 amino acids, which is further processed and cleaved after a double residue of glycine by proteolytic activity during export by the ABC transporter (*pedC*) and the accessory protein (*pedD*), producing an active peptide of 44 amino acids (7, 64, 68). The accessory protein also ensures the native conformation of the peptide, including the correct arrangement of disulfide bridges (68, 69). The producing strain protects itself by expressing a cognate immunity protein (*pedB*), which guarantees protection from the action of its own bacteriocins (70).

Pediocin PA-1/AcH was initially characterized from *P. acidilactici* PAC 1.0 strains (71, 72). Since then, other strains and species of *Pediococcus*, mainly *P. pentosaceus*, *P. acidilactici*, *P. damnosus*, have been described as producing pediocin and its variants, such as pediocin PA-1 (41, 73-75), pediocin SA-1 (76), pediocin ST18 (77), and pediocin PD-1 (63). Some studies have shown that strains of *Pediococcus* spp. may also harbor genes related to the production of other bacteriocins, such as penocin A (75, 78, 79), plantaricin (80), coagulin (11, 81), and

enterolysin A (79-80, 82). In a study carried out by Rodrigues Blanco et al. (80), when evaluating the genome of 175 *P. pentosaceus* strains, 13 bacteriocins were identified. In addition to the PLBs penocin A, coagulin A, pediocin PA-1, and plantaricin 423, which corresponded to approximately 54 % of the bacteriocins detected, were identified the bacteriocins enterolysin A, bovicin, carnocin, and pentocin.

The inhibitory action of class IIa bacteriocins is based on the specific interaction with proteins incorporated into the membrane of the mannose phosphotransferase system (Man-PTS), which is involved in the transport and metabolism of carbohydrates in bacteria (70, 83). In a recent study, Zhu et al. (67) demonstrated that the man-PTS system is responsible for the sensitivity of *L. monocytogenes* to pediocin PA-1 using cryogenic electron microscopy. The N-terminal region of the β sheet of pediocin binds to the target cell membrane and the C-terminal domain permeabilizes the membrane and interacts with the hydrophobic nucleus, causing the formation of pores in the membrane, dissipation of the transmembrane potential, exhaustion of reserves of ATP, losses of amino acids, ions and other compounds (65, 67). The producer strain protects itself by the interaction of the C-terminal portion of the immunity protein with the C-terminal domain of the bacteriocin, which may act by interfering with the formation of pores in the membrane or by blocking the pore itself, preventing membrane leakage induced by the bacteriocin (67, 84, 85).

Several *Pediococcus* spp. strains have been shown to restrict the growth of *Listeria* spp., such as strains of *P. acidilactici* (76, 86-88), *P. pentosaceus* (10, 45, 89), *P. parvulus* (90). In addition to its high anti-listerial activity, some *Pediococcus* spp. strains have been shown to be effective against other pathogens such as

Clostridium perfringens (87, 91), *Clostridium botulinium* (92), and *Staphylococcus aureus* (86, 93, 94). The bacteriocinogenic strain *P. pentosaceus* GS4 has shown inhibitory activity against different pathogens, including *S. aureus*, *L. monocytogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* (93). Ladha and Jeevaratnam (95) reported that the bacteriocin produced by *P. pentosaceus* LJR1 was capable to inhibit the pathogens *Salmonella* Typhi MTCC134 and *L. monocytogenes* MTCC 1143. Furthermore, its addition to contaminated shrimp resulted in a reduction of approximately 1 log unit of *L. monocytogenes* on day 1, which was maintained for 7 days of storage. *P. pentosaceus* T1 effectively inhibited the growth of *L. monocytogenes* in salmon fillet and improved kimchi quality by suppressing LAB such as *Leuconostoc mesenteroides* and *Lactobacillus sakei* (96). *P. pentosaceus* TC48 was used for fermentation of triticale silage, resulting in not only improved quality silage but also demonstrated efficient antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *Enterococcus faecalis* (97). In work carried out by Fugaban et al. (88), *P. acidilactici* ST3522BG and *P. pentosaceus* ST3633BG strains showed strong bacteriocinogenic activity against *L. monocytogenes* and vancomycin-resistant *Enterococcus* spp. Furthermore, both strains were able to inhibit mycotoxin-producing fungi, including *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium expansum*.

In summary, the bacteriocins produced by *Pediococcus* spp. perform well as antimicrobial agents, showing the ability to inhibit a wide variety of pathogens.

Probiotic properties and health benefits of *Pediococcus*

Certain microorganisms, after ingestion, exhibit various beneficial effects on

the host health, commonly referred to as “probiotics”. This term is defined by Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (98). This definition was revised and further supported by the International Scientific Association for Probiotics and Prebiotics (ISAPP), resulting in a minor grammatical correction, and now probiotics are defined to “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (99). The most commonly reported probiotics include various species of lactobacilli, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Propionibacterium*, *Enterococcus*, as well as certain yeast species such as *Candida pintolopesii* and *Saccharomyces boulardii*, and molds such as *Aspergillus niger* and *Aspergillus oryzae* (100). *Pediococcus* spp. has been extensively studied for both human and animal probiotic applications (13, 17-20,101, 102)

However, for a microorganism to achieve probiotic status, it must meet specific criteria assessed through various methods that typically involve assessing their stress tolerance ability (related to host-associated stress resistance), adhesion ability (for persistence in the intestine), antimicrobial activity, and safety assessment (103). Several *Pediococcus* strains have been investigated based on these criteria, isolated from various sources including dry-cured meat (104), fruit processing residues (105), human sources such as milk (106) and ileal epithelium (107), fermented food like cheese (42, 108), salted and fermented sea-food (109), fermented cucumber (110), meat product (11), and fermented cereal-dairy product (12), and animal sources such as gastrointestinal tract of broilers (111) and cow manure (112). Among these strains, *P. pentosaceus* and *P. acidilactici* are particularly notable due to the presence of

multiple strains identified with probiotic potential.

Studies have confirmed the probiotic properties of *Pediococcus* spp. with regards to their ability to overcome biological barriers to establish colonization in the intestine. Strains have demonstrated resistance to digestive enzymes, such as lysozyme, pepsin, and pancreatin, as well as tolerance to bile salts and low pH (12, 42, 106, 110). Subsequently, a potentially probiotic strain must have a good adhesion capacity for successful intestinal colonization and be active against pathogenic bacteria. *Pediococcus* strains have phenotypically demonstrated hydrophobic surface, auto-aggregation, coaggregation with pathogens, antibacterial activity, *in vitro* cell adhesion, as well as inhibition of pathogen adhesion (11, 12, 110).

Approaches investigating functional properties related to health-promoting effects can also be included in the selection of probiotic microorganisms (103). Well-defined *Pediococcus* strains may have the potential to benefit human and animal health. *In vitro* tests have shown anticancer activity of *Pediococcus* by inducing apoptosis in human cancer cell line (MCF-7) (18). In addition, exopolysaccharides produced by *Pediococcus* have anticancer activity on the human colon cancer cell line (HCT116), with strong cytotoxic and antiproliferative effects (17). Another potential application as a probiotic has been demonstrated in the control of enterotoxigenic *Escherichia coli* (ETEC) infection in porcine intestinal epithelial cells, where it reduced *E. coli* adhesion to intestinal epithelial cells and down-regulated the expression of proinflammatory genes (13).

In vivo assays involving oral administration of *Pediococcus* spp. in mice or rats have showed diverse health-promoting potentials, like protective effect against hyperlipidemia by modulating the intestinal microbiota and regulating gene expression profiles in the liver (15), ameliorate intestinal inflammation by modulated

the gut microbial composition and function, immunological profiles, and gut barrier function (14), and blood cholesterol reduction (113). This type of research has also shown that *Pediococcus* can be used as a therapeutic intervention to mitigate the adverse effects of toxicity compounds such as cadmium (114). Another alleviation of toxic effect was observed for mycotoxin ochratoxin A, thus showing the potential of *Pediococcus* as a beneficial feed additive for animals exposed to this risk (112).

However, in order to ensure the appropriate selection of probiotics for human use, clinical trials play a crucial role. Rigorous clinical trials designs carried out with double-blind, randomized, and placebo-controlled methods have found results indicating that *Pediococcus* increases the secretory of protective immunoglobulin IgA, which acts in host defense against pathogens (102). When combined with *Bifidobacterium longum*, a clinical trial showed that they reduce crying times of colicky infants and improve fecal consistency (101). Additionally, in a clinical trial combining *Pediococcus* with *Lactiplantibacillus plantarum*, it has been shown to decrease abdominal pain associated with irritable bowel syndrome (115). Similarly, studies conducted on farmed animals have also investigated the application of probiotics in veterinary medicine. *Pediococcus* has shown potential as an antibiotic replacement to improve growth performance in pigs (20). Furthermore, a positive effect on the productive performance of laying hens was observed when *Pediococcus* was administered as a dietary supplement (19). A diet supplemented with *P. pentosaceus* SL001 significantly promoted the growth of grass carp and affected the structure of the intestinal microbiota, resulting in a reduction of pathogens and an increase in potential probiotics (81).

Industrial and commercial use of *Pediococcus*

LABs have a long history of safe use in food and most groups and genera, such as lactobacilli, *Lactococcus*, *Leoconostoc* and *Pediococcus* have been granted GRAS status by the Food and Drug Administration (FDA) and have Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA) (5). Studies focused on the genus *Pediococcus* have received great attention, especially the species *P. pentosaceus* and *P. acidilactici,* through the characterization of their genetic, molecular and physiological aspects. These species can be applied in the production of fermented foods, such as vegetables, dairy products, and meat products, and are capable of promoting their biopreservation through the production of organic acids, bacteriocins and other metabolites (8).

Bacteriocins can be applied to foods basically in three ways: purified or partially purified, in the form of a crude fermented product containing bacteriocins, and by the addition of bacteriocin-producing protective cultures (116). Until now, only nisin and pediocin PA-1 have been marketed as food additives (3, 117). Nisin, produced by strains of *Lactococcus lactis* is marketed in several countries under the name Nisaplin™ (Danisco, E234), in the manufacture of dairy and meat products (3). Despite several studies on the application of bacteriocins in food, the use of purified bacteriocins in the industry remains constrained. This limitation is frequently attributed to the ineffectiveness of isolated bacteriocin to eliminate pathogens in the food matrix and also due to the high cost of isolation and purification. Consequently, the use of the bacteriocin-producing strain may be more effective when applied to food (117).

Commercially available products containing bacteriocins from *Pediococcus* are

widely used in the food industry. Examples include ALTA 2431™ (Quest International, Sarasota, FL, USA), which contains pediocin PA-1 produced by *P. acidilactici* for use in meat products to control *L. monocytogenes*, and Danisco's FDA-approved MicroGARD TM (DuPont-Danisco, Thomson, IL, USA) range, which also contain pediocin PA-1 from *P. acidilactici* (3, 5, 73).

Protective cultures of *Pediococcus* are marketed for application in food and feed as initial or adjunct cultures, with the aim of inhibiting spoilageand pathogenic bacteria, resulting in improved quality and sensory attributes (8). Examples include the commercial product Bactoferm™ F-LC (Chr. Hansen Holding A/S, Hoersholm, Denmark), which is composed of *Lactobacillus curvatus* producing sakacin A, *P. acidilactics* producing pediocin PA-1/AcH, and *Staphylococcus xylosus*, intended for controlling *L. monocytogenes* in meat products (116). The commercial product Bactocell® (Lallemand Animal Nutrition, Rexdale, Ontario), derived from the strain *P. acidilactici* CNCM I-4622, has been considered safe and effective by the European Food Safety Authority (EFSA). It is currently authorized in the European Union for use in silage and as a zootechnical additive in animal feed and water for all fattening and breeding pig species except sows, all avian species, all fish, shrimp, and crustaceans (118-120). Additionally, there is a commercially available preparation containing the strain *P. pentosaceus* DSM 16.244, which is authorized by the Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) for use as a safe feed additive in feed for all animal species (121).

Conclusions and Perspectives

In recent years, *Pediococcus* spp. have received great attention, playing a

very important role in the food industry, assisting in fermentation processes and improving the quality and safety of products, as well as improving human and animal health and nutrition. Several *Pediococcus* strains, mainly *P. pentosaceus* and *P. acidilactici*, have demonstrated considerable potential as biopreservatives and potential probiotic candidates.

One of the latest trends is the direct incorporation of bacteriocin in its pure or semi-purified form in bioactive films and coatings directly on food surfaces and packaging, or even the inclusion of bacteriocin-producing LABs. However, the application of bacteriocins as food additives may be limited for several reasons, such as their effectiveness in inhibiting pathogenic bacteria in complex matrices or the high price of purification. Thus, a very important step in identifying the beneficial properties of *Pediococcus* is the comprehensive evaluation of its characteristics and disadvantages. Further studies should be conducted with the aim of effectively applying these bacteria and/or their bacteriocins in sectors such as the food industry, agriculture, livestock, and clinical environments.

References

1. Holzapfel WH, Franz CMAP, Ludwig W, Dicks LMT (2015) *Pediococcus*. In: Whitman WB (ed) Bergey's manual of systematic of archaea and bacteria. John Wiley & Sons. Hoboken, NJ (2014), pp 359-376. https://doi.org/10.1002/9781118960608.gbm00606

2. Wade ME, Strickland MT, Osborne JP, Edwards CG (2019) Role of *Pediococcus* in winemaking. Aust. J. Grape Wine Res 25:7–24. https://doi.org/10.1111/ajgw.12366

3. Alvarez-Sieiro P, Montalbán-López M, Mu D, Kuipers OP (2016) Bacteriocins of lactic acid bacteria: extending the family. Appl Microbiol Biotechnol 100:2939–2951. https://doi.org/10.1007/s00253-016-7343-9

4. Cotter PD, Ross RP, Hill C (2013) Bacteriocins - a viable alternative to

antibiotics?. Nat Rev Microbiol 11(2):95-105. https://doi.org/10.1038/nrmicro2937

5. O'Connor PM, Kuniyoshi TM, Oliveira RP, Hill C, Ross RP, Cotter PD (2020) Antimicrobials for food and feed; a bacteriocin perspective. Curr Opin Biotechnol 61:160-167. https://doi.org/10.1016/j.copbio.2019.12.023

6. Fernandes A, Jobby R (2022) Bacteriocins from lactic acid bacteria and their potential clinical applications. Appl Biochem Biotechnol 194:4377–4399. https://doi.org/10.1007/s12010-022-03870-3

7. Papagianni M, Anastasiadou S (2009) Pediocins: The bacteriocins of Pediococci. Sources, production, properties and applications. Microb Cell Fact 8:3. https://doi.org/10.1186/1475-2859-8-3

8. Porto MC, Kuniyoshi TM, Azevedo PO, Vitolo M, Oliveira RP (2017) *Pediococcus*spp.: An important genus of lactic acid bacteria and pediocin producers. Biotechnol Adv 35:361-374. https://doi.org/10.1016/j.biotechadv.2017.03.004

9. Devi SM, Halami PM (2011) Detection and characterization of Pediocin PA-1/AcH like bacteriocin producing lactic acid bacteria. Curr Microbiol 63:181–185. https://doi.org/10.1007/s00284-011-9963-8

10. Cavicchioli VQ, Camargo AC, Todorov SD, Nero LA (2019) Potential control of *Listeria monocytogenes* by bacteriocinogenic *Enterococcus hirae* ST57ACC and *Pediococcus pentosaceus* ST65ACC strains isolated from artisanal cheese. Probiotics Antimicrob Proteins 11:696–704. https://doi.org/10.1007/s12602-018-9449-0

11. Zommiti M, Bouffartigues E, Maillot O, Barreau M, Szunerits S, Sebei K, Feuilloley M, Connil N, Ferchichi M (2018) *In vitro* assessment of the probiotic properties and bacteriocinogenic potential of *Pediococcus pentosaceus* MZF16 isolated from artisanal tunisian meat "Dried Ossban". Front Microbiol 9:2607. https://doi.org/10.3389/fmicb.2018.02607

12. Vasiee A, Falah F, Behbahani BA, Tabatabaee-Yazdi F (2020) Probiotic characterization of *Pediococcus* strains isolated from Iranian cereal-dairy fermented product: Interaction with pathogenic bacteria and the enteric cell line Caco-2. J Biosci Bioeng 130(5):471-479. https://doi.org/10.1016/j.jbiosc.2020.07.002

13. Yin H, Ye P, Lei Q, Cheng Y, Yu H, Du, J, Pan H, Cao Z (2020) *In vitro* probiotic properties of *Pediococcus pentosaceus* L1 and its effects on enterotoxigenic *Escherichia coli*-induced inflammatory responses in porcine intestinal epithelial cells. Microb Pathog 144:104163. https://doi.org/10.1016/j.micpath.2020.104163

14. Dong F, Xiao F, Li X, Li Y, Wang X, Yu G, Zhang T, Wang Y (2022) *Pediococcus pentosaceus* CECT 8330 protects DSS-induced colitis and regulates the intestinal microbiota and immune responses in mice. J Transl Med 20(1):33. https://doi.org/10.1186/s12967-022-03235-8

15. Zhang Q, Guo WL, Chen GM, Qian M, Han JZ, Lv XC, Chen LJ, Rao PF, Ai LZ, Ni L (2022) *Pediococcus acidilactici* FZU106 alleviates high-fat diet-induced lipid

metabolism disorder in association with the modulation of intestinal microbiota in hyperlipidemic rats. Curr Res Food Sci 5:775-788. https://doi.org/10.1016/j.crfs.2022.04.009

16. Zhang D, Zhang J, Kalimuthu S, Liu J, Song ZM, He BB, Cai P, Zhong Z, Feng C, Neelakantan P, Li YX (2023) A systematically biosynthetic investigation of lactic acid bacteria reveals diverse antagonistic bacteriocins that potentially shape the human microbiome. Microbiome 11(1):91. https://doi.org/10.1186/s40168-023-01540-y

17. Kumar R, Bansal P, Singh J, Dhanda S (2020) Purification, partial structural characterization and health benefits of exopolysaccharides from potential probiotic *Pediococcus acidilactici* NCDC 252. 99:79-86. Process Biochem 99:79-86. https://doi.org/10.1016/j.procbio.2020.08.028

18. Jafari-Nasab T, Khaleghi M, Farsinejad A, Khorrami S (2021) Probiotic potential and anticancer properties of *Pediococcus* sp. isolated from traditional dairy products. Biotechnol Rep 29:e00593. https://doi.org/10.1016/j.btre.2021.e00593

19. Mikulski D, Jankowski J, Mikulska M, Demey V (2020) Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on productive performance, egg quality, and body composition in laying hens fed diets varying in energy density. Poult Sci 99:2275-2285. https://doi.org/10.1016/j.psj.2019.11.046

20. Pupa P, Apiwatsiri P, Sirichokchatchawan W, Pirarat N, Maison T, Koontanatechanon A, Prapasarakul N (2021) Use of *Lactobacillus plantarum* (strains 22F and 25F) and *Pediococcus acidilactici* (strain 72N) as replacements for antibiotic-growth promotants in pigs. Scient Rep 11:12028. https://doi.org/10.1038/s41598-021-91427-5

21. Axelsson L (2004) Lactic acid bacteria: classification and physiology. In: Salminen, S., Wright, A.V. and Ouwehand, A., Eds., Lactic acid bacteria: microbiological and functional aspects, 3rd Edition, Marcel Dekker, New York, pp 1-67. https://doi.org/10.1201/9780824752033.

22. Dicks L, Endo A (2014) The family *Lactobacillaceae*: Genera other than *Lactobacillus*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The Prokaryotes. Springer, Berlin, Heidelberg pp 203-212. https://doi.org/10.1007/978-3-642-30120-9_207

23. Franz CMAP, Endo A, Abriouel H, Reenen CAV, Gálvez A, Dicks LMT (2014) The genus *Pediococcus*. Ch. 21. Holzapfel WH, Wood BJB (Eds). NJ, USA: Wiley Online Library, pp 359–376. https://doi.org/10.1002/9781118655252.ch21

24. Simpson WJ, Taguchi H (1995) The genus *Pediococcus*, with notes on the genera *Tetratogenococcus* and *Aerococcus*. In: Wood, B.J.B., Holzapfel, W.H. (eds) The genera of lactic acid bacteria. The lactic acid bacteria, vol 2. Springer, Boston, MA, pp 125-172. https://doi.org/10.1007/978-1-4615-5817-0_5

25. Collins MD, Williams AM, Wallbanks S (1990) The phylogeny of *Aerococcus* and *Pediococcus* as determined by 16S rRNA sequence analysis: description of *Tetragenococcus* gen. nov. FEMS microbial lett 58(3):255–262. https://doi.org/10.1016/s0378-1097(05)80004-7

26. Garvie EI (1988) *Pediococcus urinaeequi* nom. rev. In validation of the publication of new names and combinations previously effectively published outside the IJSB List no 25 Int J Syst Bacteriol 38:220–222. https://doi.org/10.1099/00207713-38-2-220

27. Felis GE, Torriani S, Dellaglio F (2005) Reclassification of *Pediococcus urinaeequi* (*ex* Mees 1934) Garvie 1988 as *Aerococcus urinaeequi* comb. Nov. Int J Syst Evol Microbiol 55(3):1325-1327. https://doi.org/10.1099/ijs.0.63324-0

28. Garvie EI. *Pediococcus urinaeequi* nom. rev. In validation of the publication of new names and combinations previously effectively published outside the IJSB List no 25. Int J Syst Bacteriol. 1988;38:220–222. https://doi.org/10.1099/00207713-38-2-220

29. Raccach M (1987) Pediococci and biotechnology. https://doi.org/10.3109/10408418709104442

30. Dobson CM, Deneer H, Lee S, Hemmingsen S, Glaze S, Ziola B (2002) Phylogenetic analysis of the genus *Pediococcus*, including *Pediococcus claussenii* sp. nov., a novel lactic acid bacterium isolated from beer. Int J Syst Evol Microbiol 52(Pt 6):2003-2010. https://doi.org/10.1099/00207713-52-6-2003

31. Coster E, White HR (1964) Further studies of the genus *Pediococcus*. J. Gen. Microbiol. 26: 185– 97

32. Haakensen M, Dobson CM, Hill JE, Ziola B (2009) Reclassification of *Pediococcus dextrinicus* (Coster and White 1964) Back 1978 (Approved list 1980) as *Lactobacillus dextrinicus* comb. nov., and emended description of the genus *Lactobacillus*. Int J Syst Evol Microbiol 59: 615–21. https://doi.org/10.1099/ijs.0.65779-0

33. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S (2020) A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol 70(4):2782-2858. https://doi.org/10.1099/ijsem.0.004107

34. Todorov SD, Dicks LMT (2009) Bacteriocin production by *Pediococcus pentosaceus* isolated from *marula* (*Scerocarya birrea*). Int J Food Microbiol, 132(2-3):117-126. https://doi.org/10.1016/j.ijfoodmicro.2009.04.010

35. Franz CMAP, Vancanneyt M, Vandemeulebroecke K, De Wachter M, Cleenwerck I, Hoste B, Schillinger U, Holzapfel WH, Swings J (2006) *Pediococcus stilesii* sp. nov., isolated from maize grains Int J Syst Evol Microbiol 56(Pt 2):329–333. https://doi.org/10.1099/ijs.0.63944-0

36. Halami PM, Badarinath V, Manjulata Devi S, Vijayendra SVN (2011) Partial characterization of heat-stable, antilisterial and cell lytic bacteriocin of *Pediococcus pentosaceus* CFR SIII isolated from a vegetable source. Ann Microbiol, 61:323-330. https://doi.org/10.1007/s13213-010-0145-x

37. Albano H, Todorov SD, van Reenen CA, Hogg T, Dicks LMT, Teixeira P (2007)

Characterization of a bacteriocin produced by *Pediococcus acidilactici* isolated from “Alheira”, a fermented sausage traditionally produced in Portugal. Int J Food Microbiol 116:239-247. https://doi.org/10.1016/j.ijfoodmicro.2007.01.011

38. Anastasiadou S, Papagianni M, Filiousis G, Ambrosiadis I, Koidis P (2008) Growth and metabolism of a meat isolated strain of *Pediococcus pentosaceus* in submerged fermentation: Purification, characterization and properties of the produced pediocin SM-1. Enzyme Microb Technol 43(6):448-454. https://doi.org/10.1016/j.enzmictec.2008.05.007

39. Gurira OZ, Buys EM (2005) Characterization and antimicrobial activity of *Pediococcus* species isolated from South African farm-style cheese. Food Microbiol 22(2-3):159-168. https://doi.org/10.1016/j.fm.2004.08.001

40. Cavicchioli VQ, Camargo AC, Todorov SD, Nero LA (2017) Novel bacteriocinogenic *Enterococcus hirae* and *Pediococcus pentosaceus*strains with antilisterial activity isolated from Brazilian artisanal cheese. J Dairy Sci 100:2526–2535. https://doi.org/10.3168/jds.2016-12049

41. Gutierrez-Cortes C, Suarez H, Butirago G, Nero LA, Todorov SD (2018) Characterization of bacteriocins produced by strains of *Pediococcus pentosaceus* isolated from Minas cheese. Ann Microbiol 68(6):383–398. https://doi.org/10.1007/s13213-018-1345-z

42. Bhagat D, Raina N, Kumar A, Katoch M, Khajuria Y, Slathia PS, Sharma P (2020) Probiotic properties of a phytase producing *Pediococcus acidilactici* strain SMVDUDB2 isolated from traditional fermented cheese product, Kalarei. Sci Rep, 10(1):1926. https://doi.org/10.1038/s41598-020-58676-2

43. Reda FM, Refaie AZ (2019) Purification and characterization of pedioxanthin (carotenoid pigment) produced by *Pediococcus pentosaceus* N33 strain isolated from pickles. Food Biotechnol 33:217-236. https://doi.org/10.1080/08905436.2019.1617166

44. Otunba AA, Osuntoki AA, Okunowo W, Olukoya DK, Babalola BA (2022) Characterization of novel bacteriocin PB2 and comprehensive detection of the pediocin gene ped-A1 from *Pediococcus pentosaceus* PB2 strain isolated from a sorghum-based fermented beverage in Nigeria. Biotechnol rep 36:e00772. https://doi.org/10.1016/j.btre.2022.e00772

45. Fugaban JII, Vazquez Bucheli JE, Kim B, Holzapfel WH, Todorov SD (2021) Safety and beneficial properties of bacteriocinogenic *Pediococcus acidilactici* and *Pediococcus pentosaceus* isolated from silage. Lett Appl Microbiol 73:6, 725–734. https://doi.org/10.1111/lam.13562

46. Hudson JA, Cai Y, Corner RJ, Morvan B, Joblin KN (2000) Identification and enumeration of oleic acid and linoleic acid hydrating bacteria in the rumen of sheep and cows. J Appl Microbiol 88(2):286-292. https://doi.org/10.1046/j.1365-2672.2000.00968.x

47. Mathys S, von Ah U, Lacroix C, Staub E, Mini R, Cereghetti T, Meile L (2007) Detection of the pediocin gene pedA in strains from human faeces by real-time PCR

and characterization of *Pediococcus acidilactici* UVA1. BMC Biotechnol 7:55. https://doi.org/10.1186/1472-6750-7-55

48. Millette M, Dupont C, Archambault D, Lacroix M (2007) Partial characterization of bacteriocins produced by human *Lactococcus lactis* and *Pediococccus acidilactici* isolates. J Appl Microbiol 102(1):274-282. 10.1111/j.1365-2672.2006.03044.x

49. Beneduce L, Spano G, Vernile A, Tarantino D, Massa S (2004) Molecular characterization of lactic acid populations associated with wine spoilage. J basic microbiol 44(1):10–16. https://doi.org/10.1002/jobm.200310281

50. Iijima K, Suzuki K, Asano S, Kuriyama H, Kitagawa Y (2007) Isolation and identification of potential beer-spoilage *Pediococcus inopinatus* and beer-spoilage *Lactobacillus backi*strains carrying the horA and horC gene clusters. J Inst Brew 113(1):96-101. https://doi.org/10.1002/j.2050-0416.2007.tb00262.x

51. Snauwaert I, Stragier P, De Vuyst L, Vandamme P (2015) Comparative genome analysis of *Pediococcus damnosus* LMG 28219, a strain well-adapted to the beer environment. BMC Genomics 16(1):267. https://doi.org/10.1186/s12864-015-1438-z

52. Zhang B, Tong H, Dong X (2005) *Pediococcus cellicola* sp. nov., a novel lactic acid coccus isolated from a distilled-spirit-fermenting cellar. Int J Syst Evol Microbiol 55: 2167–2170. https://doi.org/10.1099/ijs.0.63778-0

53. Collins MD, Rodrigues U, Ash C, Aguirre M, Farrow JAE, Martinez-Murcia A, Phillips BA, Williams AM,Wallbanks S (1991) Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. FEMS Microbiol Lett 77:5-12. https://doi.org/10.1111/j.1574-6968.1991.tb04313.x

54. Eijsink VGH, Axelsson L, Diep DB, Havarstein LS, Holo H, Nes IF (2002) Production of class II bacteriocins by lactic acid bacteria; an example of biological warfare and communication. Antonie Van Leeuwenhoek 81 (1-4)639-654. https://doi.org/10.1023/A:1020582211262

55. Asami K, Kondo A, Suda Y, Shimoyamada M, Kanauchi M (2017) Neutralization of Lipopolysaccharide by Heat Shock Protein in *Pediococcus pentosaceus* AK-23. J Food Sci 82(7):1657–1663. https://doi.org/10.1111/1750-3841.13679

56. Hao L, Cheng Y, Su W, Wang C, Lu Z, Jin M, Wang F, Wang Y (2021) *Pediococcus pentosaceus* ZJUAF-4 relieves oxidative stress and restores the gut microbiota in diquat-induced intestinal injury. Appl Microbiol Biotechnol 105(4):1657–1668. https://doi.org/10.1007/s00253-021-11111-6

57. Jiang S, Cai L, Lv L, Li L (2021) *Pediococcus pentosaceus*, a future additive or probiotic candidate. Microb Cell Fact 20(1):45. https://doi.org/10.1186/s12934-021-01537-y

58. Papagianni M (2003) Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function, and applications. Biotechnol Adv 21:465-499. https://doi.org/10.1016/S0734-9750(03)00077-6

59. van Heel AJ, de Jong A, Song C, Viel JH, Kok J, Kuipers OP (2018) BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. Nucleic Acids Res 46:W278–W281. https://doi.org/10.1093/nar/gky383

60. Mills S, Stanton C, Fitzgerald GF, Ross RP (2011) Enhancing the stress responses of probiotics for a lifestyle from gut to product and back again. Microb Cell Fact 10 Suppl 1(Suppl 1):S19. https://doi.org/10.1186/1475-2859-10-S1-S19

61. Field D, Ross RP, Hill C (2018) Developing bacteriocins of lactic acid bacteria into next generation biopreservatives. CurrOpin Food Sci 20:1-6. https://doi.org/10.1016/j.cofs.2018.02.004

62. Bourdichon F, Berger B, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, Harnett J, Huys G, Laulund S et al. (2012) Safety demonstration of microbial food cultures (MFC) in fermented food products. Bull Int Dairy Fed 455:62.

63. Bauer R, Chikindas ML, Dicks LM (2005) Purification, partial amino acid sequence and mode of action of pediocin PD-1, a bacteriocin produced by *Pediococcus damnosus* NCFB 1832. Int J Food Microbiol 101(1):17–27. https://doi.org/10.1016/j.ijfoodmicro.2004.10.040

64. Drider D, Fimland G, Hechard Y, Mc Mullen LM, Prévost H (2006) The continuing story of class IIa bacteriocins. Microbiol Mol Biol Rev 70(2):564-582. https://doi.org/10.1128/MMBR.00016-05

65. Balandin SV, Sheremeteva EV, Ovchinnikova TV (2019) Pediocin-like antimicrobial peptides of bacteria. Biochem (Mosc) 84:464-478. https://doi.org/10.1134/S000629791905002X

66. Zhang T, Zhang Y, Li L, Jiang X, Chen Z, Zhao F, Yi Y (2022) Biosynthesis and production of class II bacteriocins of food-associated lactic acid bacteria. Fermentation 8:217. https://doi.org/10.3390/fermentation8050217

67. Zhu, L., Zeng, J., Wang, C., & Wang, J. (2022). Structural basis of pore formation in the mannose phosphotransferase system by pediocin PA-1. Appl Environ Microbiol 88(3), e0199221. https://doi.org/10.1128/AEM.01992-21

68. Antoshina DV, Balandin SV, Ovchinnikova TV (2022) Structural features, mechanisms of action, and prospects for practical application of class II bacteriocins. Biochem (Mosc) 87:1387–1403. https://doi.org/10.1134/S0006297922110165

69. Oppegård C, Fimland G, Anonsen JH, Nissen-Meyer J (2015) The pediocin PA-1 accessory protein ensures correct disulfide bond formation in the antimicrobial peptide pediocin PA-1. Biochem 54 (19):2967-2974. https://doi.org/10.1021/acs.biochem.5b00164

70. Ríos Colombo NS, Chalón MC, Navarro SA, Bellomio A (2018) Pediocin-like bacteriocins: new perspectives on mechanism of action and immunity. Curr genet 64(2):345–351. https://doi.org/10.1007/s00294-017-0757-9

71. Henderson JT, Chopco AL, van Wassenaar PD (1991) Purification and primary

structure of pediocin PA-1 produced by *Pediococcus acidilactici* PAC1 0. Arch Biochem Biophys 295: 5-12. https://doi.org/10.1016/0003-9861(92)90480-K

72. Marugg JD, Gonzalez CF, Kunka BS, Ledeboer AM, Pucci MJ, Toonen MY, Walker SA, Zoetmulder LC, Vandenbergh PA (1992). Cloning, expression, and nucleotide sequence of genes involved in production of pediocin PA-1, and bacteriocin from *Pediococcus acidilactici* PAC1.0. Appl Environ Microbiol 58(8):2360–2367. https://doi.org/10.1128/aem.58.8.2360-2367.1992

73. Simha BV, Sood SK, Kumariya R, Garsa AK (2012) Simple and rapid purification of pediocin PA-1 from *Pediococcus pentosaceus* NCDC 273 suitable for industrial application. Microbiol Res 167(12):544–549. https://doi.org/10.1016/j.micres.2012.01.001

74. Kuniyoshi TM, Mendonça CMN, Vieira VB, Robl D, Franco BDGM, Todorov SD, Tomé E, O'Connor PM, Converti A, Araujo WL, Vasconcellos LPSP, Varani AM, Cotter PD, Rabelo SC, Oliveira RPS (2021) Pediocin PA-1 production by *Pediococcus pentosaceus* ET34 using non-detoxified hemicellulose hydrolysate obtained from hydrothermal pretreatment tof sugarcane bagasse. Bioresource Technol 338:125565. https://doi.org/10.1016/j.biortech.2021.125565

75. Oliveira, FS, Rodrigues FS, Carvalho AF, Nero LA (2023) Genomic analyses of *Pediococcus pentosaceus* ST65ACC, a bacteriocinogenic strain isolated from artisanal raw-milk cheese. Probiotics Antimicrob 15(3):630–645. https://doi.org/10.1007/s12602-021-09894-1

76. Anastasiadou S, Papagianni M, Filiousis G, Ambrosiadis I, Koidis P (2008) Pediocin SA-1, an antimicrobial peptide from *Pediococcus acidilactici* NRRL B5627: production conditions, purification and characterization. Bioresour technol 99(13):5384–5390. https://doi.org/10.1016/j.biortech.2007.11.015

77. Todorov S, Dicks LMT (2005) Pediocin ST18, an anti-listerial bacteriocin produced by *Pediococcus pentosaceus* ST18 isolated from boza, a traditional cereal beverage from Bulgaria. Process Biochem 40(1):365–370. https://doi.org/10.1016/j.procbio.2004.01.011

78. Martino ME, Maifreni M, Marino M, Bartolomeoli I, Carraro L, Fasolato L, Cardazzo B (2013) Genotypic and phenotypic diversity of *Pediococcus pentosaceus* strains isolated from food matrices and characterisation of the penocin operon. Antonie Van Leeuwenhoek 103:1149–1163. https://doi.org/10.1007/s10482-013-9897-1

79. Jiang J, Yang B, Ross RP, Stanton C, Zhao J, Zhang H, Chen W (2020) Comparative genomics of *Pediococcus pentosaceus* isolated from different niches reveals genetic diversity in carbohydrate metabolism and immune system. Front in microbiol 11:253. https://doi.org/10.3389/fmicb.2020.00253

80. Rodrigues Blanco I, José Luduverio Pizauro L, Victor Dos Anjos Almeida J, Miguel Nóbrega Mendonça C, de Mello Varani A, Pinheiro de Souza Oliveira R (2022) Pan-genomic and comparative analysis of *Pediococcus pentosaceus* focused on the *in silico* assessment of pediocin-like bacteriocins. Comput Struct Biotechnol J

20:5595-5606. https://doi.org/10.1016/j.csbj.2022.09.041

81. Gong L, He H, Li D, Cao L, Khan TA, Li Y, Pan L, Yan L, Ding X, Sun Y, Zhang Y, Yi G, Hu S, Xia L (2019) A new isolate of *Pediococcus pentosaceus* (SL001) with antibacterial activity against fish pathogens and potency in facilitating the immunity and growth performance of grass carps. Front Microbiol 10:1384. https://doi.org/10.3389/fmicb.2019.01384

82. Surachat K, Kantachote D, Deachamag P, Wonglapsuwan M (2021) Genomic insight into *Pediococcus acidilactici* HN9, a potential probiotic strain isolated from the traditional thai-style fermented beef nhang. Microorganisms 9(1):50. https://doi.org/10.3390/microorganisms9010050

83. Jeckelmann JM, Erni B (2020) The mannose phosphotransferase system (Man-PTS) - Mannose transporter and receptor for bacteriocins and bacteriophages. Biochim Biophys Acta Biomembr 1862(11):183412. https://doi.org/10.1016/j.bbamem.2020.183412

84. Johnsen L, Fimland G, Nissen-Meyer J (2005) The C-terminal domain of pediocin-like antimicrobial peptides (class IIa bacteriocins) is involved in specific recognition of the C-terminal part of cognate immunity proteins and in determining the antimicrobial spectrum. J Biol Chem 280:9243–9250. https://doi.org/10.1074/jbc.M412712200

85. Zhu L, Zeng J, Wang J (2022) Structural basis of the immunity mechanisms of pediocin-like bacteriocins. Appl Environ Microbiol 88(13):e0048122. https://doi.org/10.1128/aem.00481-22

86. Mandal B, Chowdhury R, Jee CB (2014) Purification and characterization of pediocin produced by *Pediococcus acidilactici* NCIM 2292. Int J Pharm Pharmaceut Sci 6(6):357–361

87. Nieto-Lozano JC, Reguera-Useros JI, Pelaez-Martinez MC, Sacristan-Perez-Minayo G, Gutierrez-Fernandez AJ, Torre AH (2010) The effect of the pediocin PA-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. Food Control 21:679–685. https://doi.org/10.1016/j.foodcont.2009.10.007

88. Fugaban JII, Vazquez Bucheli JE, Park YJ, Suh DH, Jung ES, Franco BDGM, Ivanova IV, Holzapfel WH, Todorov SD (2022) Antimicrobial properties of *Pediococcus acidilactici* and *Pediococcus pentosaceus* isolated from silage. J Appl Microbiol 132(1):311–330. https://doi.org/10.1111/jam.15205

89. Todorov SD, Furtado DN, Saad SMI, Tomé E, Franco BDGM (2011) Potential beneficial properties of bacteriocin-producing lactic acid bacteria isolated from smoked salmon. J Appl Microbiol 110:971–986. https://doi.org/10.1111/j.1365-2672.2011.04950.x

90. Bennik M, Smid EJ, Gorris L (1997) Vegetable-associated *Pediococcus parvulus* produces pediocin PA-1. Appl environ microbial 63(5):2074–2076. https://doi.org/10.1128/aem.63.5.2074-2076.1997

91. Bédard F, Hammami R, Zirah S, Rebuffat S, Fliss I, Biron E (2018) Synthesis, antimicrobial activity and conformational analysis of the class IIa bacteriocin pediocin PA-1 and analogs thereof. Sci rep 8(1):9029. https://doi.org/10.1038/s41598-018-27225-3

92. Bagenda DK, Hayashi K, Yamazaki K, Kawai Y (2008) Characterization of an antibacterial substance produced by *Pediococcus pentosaceus* lz3. 13 isolated from Japanese fermented marine food. Fish Sci 74:439–448. https://doi.org/10.1111/j. 1444-2906.2008.01542.x

93. Ghosh B, Sukumar G, Ghosh AR (2019) Purification and characterization of pediocin from probiotic *Pediococcus pentosaceus* GS4, MTCC 12683. Folia Microbiol (Praha) 64:765–778. https://doi.org/10.1007/s12223-019-00689-0

94. Pato U, Yusmarini Y, Fitriani S, Tartila, Yeni R, Fadillah F, Husnanini L (2020) Enhancement of the growth and antimicrobial activity of *P. pentosaceus* Strain 2397 against *Staphylococcus aureus*. Biotechnology 20(1):8–14. https://doi.org/10.3923/biotech.2021.8.14

95. Ladha G, Jeevaratnam K (2020) Characterization of purified antimicrobial peptide produced by *Pediococcus pentosaceus* LJR1, and its application in preservation of white leg shrimp. World J Microbiol Biotechnol 36:72. https://doi.org/10.1007/s11274-020-02847-w

96. Jang S, Lee D, Jang IS, Choi HS, Suh HJ (2015) The culture of *Pediococcus pentosaceus* T1 inhibits *listeria* proliferation in salmon fillets and controls maturation of kimchi. Food Technol Biotechnol 53(1):29–37. https://doi.org/10.17113/ftb.53.01.15.3754

97. Soundharrajan I, Kim D, Kuppusamy P, Muthusamy K, Lee HJ, Choi KC (2019) Probiotic and triticale silage fermentation potential of *Pediococcus pentosaceus* and *Lactobacillus brevis* and their impacts on pathogenic bacteria. Microorganisms 7(9):318. https://doi.org/10.3390/microorganisms7090318

98. FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: report of a Joint FAO/WHO Expert Consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Amerian Córdoba Park Hotel, Córdoba, Argentina, 1-4, 2001.

99. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11(8):506-14. https://doi.org/10.1038/nrgastro.2014.66

100. Kaur S, Kaur R, Rani N, Sharma S, Joshi M (2021) Sources and selection criteria of probiotics. In: Goel, G., Kumar, A. (eds) Advances in probiotics for sustainable food and medicine. Microorganisms for Sustainability 21:27-43. https://doi.org/10.1007/978-981-15-6795-7_2

101. Chen K, Liu C, Li H, Lei Y, Zeng C, Xu S, Li J, Savino F (2021) Infantile Colic

Treated With *Bifidobacterium longum* CECT7894 and *Pediococcus pentosaceus* CECT8330: A Randomized, Double-Blind, Placebo-Controlled Trial. Front Pediatr 9:635176. https://doi.org/10.3389/fped.2021.635176

102. Kawashima T, Ikari N, Kouchi T, Kowatari Y, Kubota Y, Shimojo N, Tsuji NM (2018) The molecular mechanism for activating IgA production by *Pediococcus acidilactici* K15 and the clinical impact in a randomized trial. Sci Rep 8(1):5065. https://doi.org/10.1038/s41598-018-23404-4

103. Pereira GVM, Coelho BO, Júnior AIM, Thomaz-Soccol V, Soccol CR (2018) How to select a probiotic? A review and update of methods and criteria. Biotechnol Adv 36(8):2060-2076. https://doi.org/10.1016/j.biotechadv.2018.09.003

104. Topçu KC, Kaya M, Kaban G (2020) Probiotic properties of lactic acid bacteria strains isolated from pastırma. LWT 134:110216. https://doi.org/10.1016/j.lwt.2020.110216

105. Trindade DPA, Barbosa JP, Martins EMF, Tette PAS (2022) Isolation and identification of lactic acid bacteria in fruit processing residues from the Brazilian Cerrado and its probiotic potential. Food Biosci 48:101739. https://doi.org/10.1016/j.fbio.2022.101739

106. Osmanagaoglu O, Kiran F, Ataoglu H (2010) Evaluation of in vitro probiotic potential of *Pediococcus pentosaceus* OZF isolated from human breast milk. Probiotics Antimicrob Proteins 2(3):162-74. https://doi.org/10.1007/s12602-010-9050-7

107. Vasudevan L, V J, M S, Ts C (2021) Mucosa-adherent *Pediococcus pentosaceus* l44 isolated from healthy human and effect of oleic acid on its probiotic properties. Curr Res Microb Sci 2:100058. https://doi.org/10.1016/j.crmicr.2021.100058

108. Khaleghi M, Khorrami S, Jafari-Nasab T (2023) *Pediococcus acidilactici* isolated from traditional cheese as a potential probiotic with cytotoxic activity against doxorubicin-resistant MCF-7 cells. Biotech 13(6):170. https://doi.org/10.1007/s13205-023-03597-w

109. Lee KW, Park JY, Sa HD, Jeong JH, Jin DE, Heo HJ, Kim JH (2014) Probiotic properties of *Pediococcus* strains isolated from jeotgals, salted and fermented Korean sea-food. Anaerobe 28:199-206. https://doi.org/10.1016/j.anaerobe.2014.06.013

110. Shukla R, Goyal A (2014) Probiotic potential of *Pediococcus pentosaceus* CRAG3: a new isolate from fermented cucumber. Probiotics Antimicrob Proteins 6(1):11-21https://doi.org/10.1007/s12602-013-9149-8

111. Reuben RC, Roy PC, Sarkar SL, Alam RU, Jahid IK (2019) Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC Microbiol 19(1):253. https://doi.org/10.1186/s12866-019-1626-0

112. Tang J, Yin L, Zhao Z, Ge L, Hou L, Liu Y, Chen X, Huang K, Gan F (2023)

Isolation, identification and safety evaluation of OTA-detoxification strain *Pediococcus acidilactici* NJB421 and its effects on OTA-induced toxicity in mice. Food Chem Toxicol 172:113604. https://doi.org/10.1016/j.fct.2023.113604

113. Jang WJ, Kim CE, Jeon MH, Lee SJ, Lee JM, Lee EW, Hasan MT (2021) Characterization of *Pediococcus acidilactici* FS2 isolated from Korean traditional fermented seafood and its blood cholesterol reduction effect in mice. J Funct Foods 87:104847. https://doi.org/10.1016/j.jff.2021.104847

114. Dubey V, Mishra AK, Ghosh AR, Mandal BK (2019) Probiotic *Pediococcus pentosaceus* GS4 shields brush border membrane and alleviates liver toxicity imposed by chronic cadmium exposure in Swiss albino mice. J Appl Microbiol 126(4):1233-1244. https://doi.org/10.1111/jam.14195

115. Barraza-Ortiz DA, Pérez-López N, Medina-López VM, Minero-Alfaro JI, Zamarripa-Dorsey F, Fernández-Martínez NDC, Llorente-Ramón A, Ramos-Aguilar GA (2021) Combination of a probiotic and an antispasmodic increases quality of life and reduces symptoms in patients with irritable bowel syndrome: a pilot study. Dig Dis 39(3):294-300. https://doi.org/10.1159/000510950

116. Chikindas ML, Weeks R, Drider D, Chistyakov VA, Dicks LM (2018) Functions and emerging applications of bacteriocins. Curr Opin Biotechnol 49:23-28. https://doi.org/10.1016/j.copbio.2017.07.011

117. Silva CCG, Silva SPM, Ribeiro SC (2018). Application of bacteriocins and protective cultures in dairy food preservation. Front microbial 9:594. https://doi.org/10.3389/fmicb.2018.00594

118. Commission Implementing Regulation (EU) 2020/151 of 4 February 2020 concerning the authorisation of *Pediococcus acidilactici* CNCM I-4622 as a feed additive for all porcine species for fattening and for breeding other than sows, all avian species, all fish species and all crustaceans and repealing Regulations (EC) No 911/2009, (EU) No 1120/2010 and (EU) No 212/2011 and Implementing Regulations (EU) No 95/2013, (EU) No 413/2013 and (EU) 2017/2299 (holder of authorisationDanstar Ferment AG represented in the Union by Lallemand SAS). OJ L 33, 5.2.2020, p. 12. http://data.europa.eu/eli/reg_impl/2020/151/oj

119. Commission Implementing Regulation (EU) No 1119/2012 of 29 November 2012 concerning the authorisation of preparations of *Pediococcus acidilactici* CNCM MA 18/5 M DSM 11673, *Pediococcus pentosaceus* DSM 23376, NCIMB 12455 and NCIMB 30168, *Lactobacillus plantarum* DSM 3676 and DSM 3677 and *Lactobacillus buchneri* DSM 13573 as feed additives for all animal species. OJ L 330, 30.11.2012, p. 14. http://data.europa.eu/eli/reg_impl/2012/1119/oj

120. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Bampidis V, Azimonti G, Bastos MD, Christensen H, Dusemund B, Kouba M, Durjava MK, Lopez-Alonso M, Puente SL, Marcon F et al.6 Assessment of the application for renewal of authorisation of Bactocell (R) (*Pediococcus acidilactici* CNCM I-4622) as a feed additive for all fish and shrimps and its extension of use for all crustaceans. EFSA J. 2019;17(4):e05691. http://doi.org/10.2903/j.efsa.2019.5691

121. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, … Pettenati E. Assessment of the application for renewal of the authorisation of *Pediococcus pentosaceus* DSM 16244 as a feed additive for all animal species. EFSA J. 2020;18(6):e06166. https://doi.org/10.2903/j.efsa.2020.6166

CHAPTER 2: Genomic Analyses of *Pediococcus pentosaceus* ST65ACC, a Bacteriocinogenic Strain Isolated from Artisanal Raw-Milk Cheese

Francielly Soares Oliveira et al.

The content of this part has been published in:

Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-021-09894-1

Titlepage

Francielly Soares Oliveira[1,2]; Rafaela da Silva Rodrigues [1,2]; Antônio Fernandes de Carvalho[2]; Luís Augusto Nero[1*]

Genomic analyses of *Pediococcus pentosaceus* ST65ACC, a bacteriocinogenic strain isolated from artisanal raw-milk cheese

[1] InsPOA - Laboratório de Inspeção de Produtos de Origem Animal, Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, 36570 900, MG, Brazil

[2] Inovaleite - Laboratório de Pesquisa em Leite e Derivados, Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Viçosa, 36570 900, MG, Brazil

* Author for correspondence. Tel.: + 55 31 3899 1463, fax: + 55 31 3899 1457, Email: nero@ufv.br

Abstract

Pediococcus pentosaceus ST65ACC was obtained from a Brazilian artisanal cheese (BAC) and characterized as bacteriocinogenic. This strain presented beneficial properties in previous studies, indicating its potential as a probiotic candidate. In this study, we aimed to carry out a genetic characterization based on whole-genome sequencing (WGS), including taxonomy, biotechnological properties, bacteriocin clusters and safety related genes. WGS was performed using the Illumina MiSeq platform and the genome was annotated with the Prokaryotic Genome Annotation (Prokka). *P. pentosaceus* ST65ACC taxonomy was investigated and bacteriocin genes clusters were identification by BAGEL4, metabolic pathways were analyzing by Kyoto Encyclopedia of Genes and Genomes (KEGG) and safety related genes were checked. *P. pentosaceus* ST65ACC had a total draft genome size of 1,933,194 bp with a GC content of 37.00 %, and encoded 1,950 protein coding sequences (CDSs), 6 rRNA, 55 tRNA, 1 tmRNA and no plasmids were detected. The analysis revealed absence of a CRISPR/Cas system, bacteriocin gene clusters for pediocin PA-1/AcH and penocin-A were identified. Genes related to beneficial properties, such as stress adaptation genes and adhesion genes, were identified. Furthermore, genes related to biogenic amines and virulence related genes were not detected. Genes related to antibiotic resistance were identified, but not in prophage regions. Based on the obtained results, the beneficial potential of *P. pentosaceus* ST65ACC was confirmed, allowing its characterization as a potential probiotic candidate.

Keywords: *Pediococcus pentosaceus*, whole-genome sequencing, bacteriocin, comparative genomics

Introduction

Pediococcus pentosaceus ST65ACC was isolated from a Brazilian artisanal cheese (BAC) and characterized as bacteriocinogenic in a previous study carried out by Cavicchioli et al. [1]. Many strains of *P. pentosaceus* are described as producing pediocin PA-1/AcH [2-4] and may also harbor operons of other bacteriocins of the class IIa, such as peniocin-A and enterolysin A, class III [3, 5]. Class IIa bacteriocins are particularly attractive because they have an efficient bactericidal effect against *Listeria monocytogenes* [6-8] and can inhibit others foodborne pathogenic such as *Clostridium botulinum* [9], *Clostridium perfringens* [10], *Staphylococcus aureus* [11], making them potentially useful for use as bio-preservatives.

A previous study also demonstrated that the *P. pentosaceus* strain ST65ACC has desired beneficial properties, such as resistance to simulated gastrointestinal conditions, auto-aggregation capacity and aggregation with *L. monocytogenes* [12], which may facilitate the elimination of this pathogen and adhesion to intestinal mucosa. Also, some *P. pentosaceus* strains have been associated with anti-inflammatory capacity, antioxidant properties and lipid-lowering effect [13-16]. *P. pentosaceus* strains and their bacteriocins are being increasingly considered as supplements in the food industry and in the gut health [17].

Advances in genome sequencing technologies and the increasing availability of tools for sequence analysis have enabled the genomic characterization of microorganisms and has become mandatory for defining the exact taxonomy of new strains and for evaluating all technological/beneficial information and related to their safety [18, 19]. The study of genomic sequences allows careful safety assessment, such as determination of antimicrobial resistance profiles, assessment of the presence of virulence factors and production of biogenic amines, which are

necessary for the use of new strains in food [18, 20]. *P. pentosaceus* ST65ACC is a strain with excellent bacteriocinogenic capacity and high ability to with stand gastrointestinal conditions [1, 12] and, because of that, it needs to be better analyzed for the presence of characteristics and/or potential problems. In this context, the aim of this study was to perform the whole genome sequencing (WGS) of *P. pentosaceus* ST65ACC [1, 12], identify the taxonomy, analyze the biotechnological and beneficial properties, bacteriocin clusters and factors related to their safety through genetic exploration of the genome and comparative analysis.

Material and methods

Bacterial strain and culture conditions

P. pentosaceus ST65ACC was isolated from artisanal cheese, produced from raw cows' milk (Nova Venécia, ES, Brazil), characterized as a bacteriocinogenic strain and initially identified as *P. pentosaceus* by sequencing the 16S rRNA gene. The strain ST65ACC was stored at -80 °C in Man Rogosa Sharpe broth (MRS, Becton, Dickinson and Co., Franklin Lakes, NJ) supplemented with 20 % glycerol [1].

Genome sequencing, assembly and annotation

P. pentosaceus ST65ACC strain was sent for WGS by Illumina sequencing using the Neoprospecta Microbiome Technologies genomic services (Florianópolis, SC, Brazil). For genome assembly, the A5-miseq pipeline [21] was used, the assembly correction using the IDBA-UD program [22] in conjunction with the SPAdes program [23], and the GMCloser program [24] was used to close the gaps in the

scaffolds. After evaluations of assembly quality, the genome was annotated with the Prokaryotic Genome Annotation (Prokka) program [25].

The *P. pentosaceus* ST65ACC genome map was predicted using CGView (http://stothard.afns.ualberta.ca/cgview_server/) [26]. Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg) database was utilized for the annotation and classification of metabolic pathways [27], using KEEG Automatic Annotation Server (KAAS, https://www.genome.jp/kegg/kaas/) [28].Packages in R (Version 4.0.2) were used for data analysis and graph plotting included dplyr, plyr, ggplot2, and ggstance. Tools on the KEGG server were used to search for genes involved in the metabolic pathways of glycolysis, use of lactose, pyruvate and proteolytic system. In addition, prophages were searched with PHASTER webserver (www.phaster.ca) [29]; the presence of clustered palindromic interspaced palindromic repeats (CRISPR) regions and *cas* genes were searched with CRISPRCasFinder (https://crisprcas.i2bc.paris-saclay.fr/) [30]; insertion sequences (IS) were identified with ISfinder(www-is.biotoul.fr) [31] and plasmids were searched with PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) [32].

Phylogenetic Analysis

The taxonomy of the *P. pentosaceus* ST65ACC was investigated using the following methods: average nucleotide identity (ANI) based on the BLASTN algorithm (ANIb); ANI based on the MUMMER ultra-fast alignment tool (ANIm); tetranucleotide frequency correlation coefficients (TETRA); and digital DNA:DNA hybridization (DDH) using the genome-to-genome distance (GGDC) method, based on *P. pentosaceus* complete genomes published in the NCBI (Supplementary Table1). The ANIb, ANIm and TETRA frequencies were calculated using the JSpeciesWS software

(http://jspecies.ribohost.com/jspeciesws/) [33].The GGDC was calculated using a web service (http://ggdc.dsmz.de/) [34] with BLAST method, and the GGDC results were based on the recommended formula 2. In addition, a phylogenomic tree was built using the Type Strain Genome Server (TYGS; https://tygs.dsmz.de), for a taxonomic analysis based on the complete genome [35]: the ST65ACC genome was compared with deposited genomes of the same type lineage and a phylogenetic tree was visualized using iTol v.4.2.

Comparative Genomic Analysis

To infer orthologous gene groups from 15 strains of *P. pentosaceus* (Supplementary Table1), all genomes were annotated with the Prokka [25]. Posteriorly, OrthoFinder v.2.3.3 with the default settings [36] was used for determining groups of orthologous gene pairs (orthogroups) and an upset plot was created using the R software (version 4.0.2) with the UpsetR package [37]. Core orthogroups shared among *P. pentosaceus* strains were further annotated to Clusters Orthologous Groups (COG) categories [38] using the online server Batch CD-Search (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) [39].

Multiple Genome Alignments

To better analyze the evolutionary relationships between species, the genomes grouped in the same clade as the phylogenetic tree of the *P. pentosaceus* ST65ACC were selected, and the Mauve alignment tool [40] was used in order to perform alignments of the whole genome at the nucleotide level, to verify synteny between large blocks of genomic sequences [41]. Additionally, a comparative

analysis of the whole genome was performed using BLAST Ring Image Generator (BRIG) v.0.95 [42], in order to have a broad visualization of the identity of *P. pentosaceus* strains (Supplementary Table1), using the strain ATCC25745 as a reference genome.

Identification of carbohydrate active enzyme (CAZyme)

The genes associated with families of CAZymes were identified through searches in the Carbohydrate Active Enzyme Database (CAZy, http://www.cazy.org/) [43]. All of the annotated protein sequences from the genome annotation process were scanned using the dbCAN server (http://bcb.unl.edu/dbCAN2/index.php) [44] with HMMER v.3.3.2 [45] against the CAZy database. The E-value of $1e^{-15}$ and coverage of 0.35 were used as the cut-off limit to identify the CAZyme class.

Clusters of bacteriocin genes

Genome analysis for the presence of bacteriocin gene clusters was performed using the web tool BAGEL4 (http://bagel4.molgenrug.nl/index.php) [46]. Subsequently, the bacteriocin domains were manually confirmed using BLASTp against databases of non-redundant protein sequences (nr) (https://blast.ncbi.nlm.nih.gov/). The conservation of the bacteriocin structure was provided by Weblogo (https://weblogo.berkeley.edu/logo.cgi) [47].

Safety assessment and identification of antibiotic resistance genes

Antimicrobial resistance genes (AMR) were researched using the databases

Comprehensive Antibiotic Resistance Database (CARD) [48], ResFinder [49], ARG_ANNOT [50] and NCBI AMRFinder Plus [51].Virulence factor genes were detected using the database VFDB [52]. The screening using these databases was performed using ABRicate (https://github.com/tseemann/abricate) with default parameters. The probability of being a human pathogen was determined using the PathogenFinder (http://cge.cbs.dtu.dk/services/PathogenFinder/) [53]. In addition, the KEGG database (https://www.kegg.jp/) using KAAS search tool was inspected for virulence factors, biogenic amine (BA) and AMR genes [28]. The AMR genes were inspected in "Brite ko01504: Antimicrobial resistance genes".

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAJHSK000000000. The version described in this paper is version JAJHSK010000000.

Results

General characteristics of the *P. pentosaceus* ST65ACC genome

The draft genome sequence of *P. pentosaceus* ST65ACC comprises 1,933,194 bp, with a GC content of 37.00 %, as can be seen in Fig. 1. General information of *P. pentosaceus* ST65ACC genome and its genomic features, including prophages, CRISPR region, and AMR gene, are given in Table 1. A total of 2,012 genes were identified in the genome, including 1,950 protein coding sequences (CDS), 6 rRNA, 55 tRNA and 1 tmRNA, according to Prokka's prediction [25]. No plasmids were found in the *P. pentosaceus* ST65ACC genome.

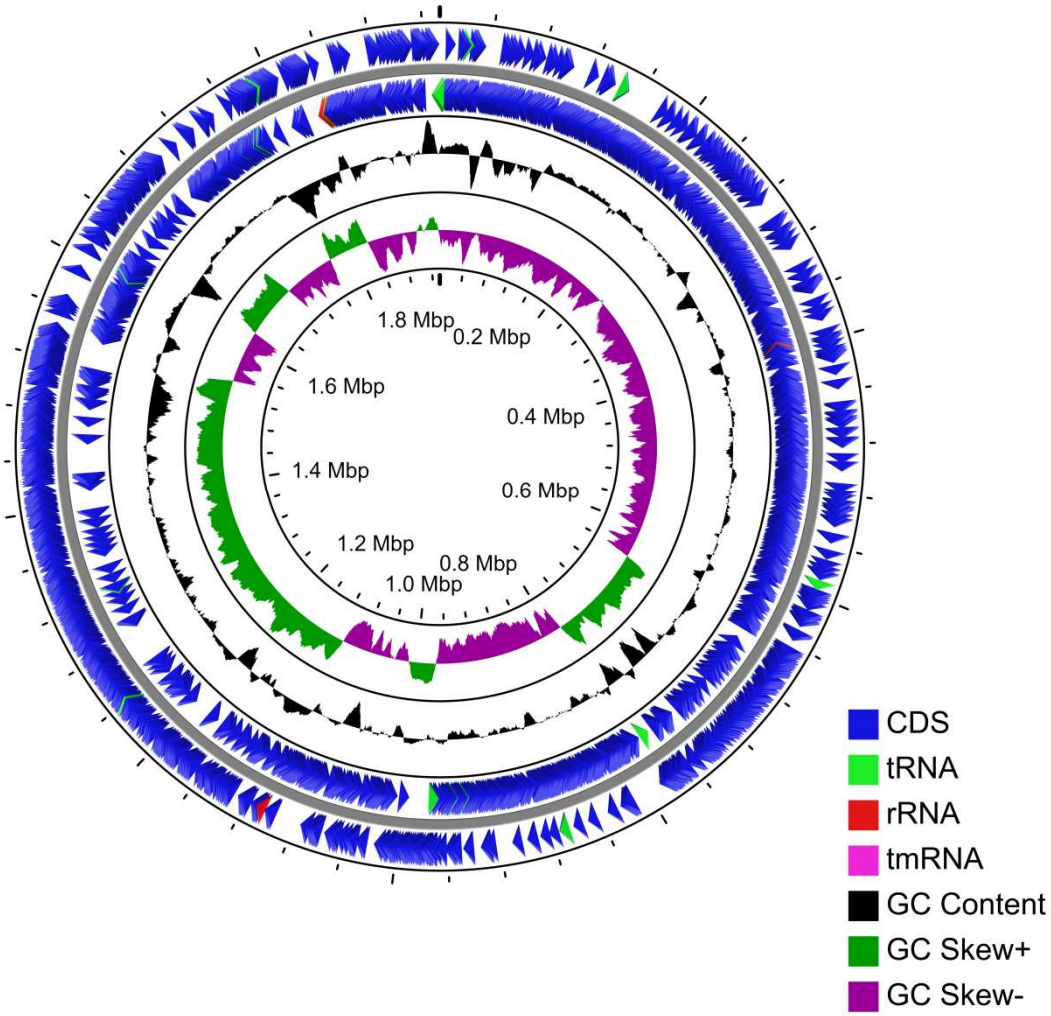

Fig. 1 Circular genome map of *P. pentosaceus* ST65ACC. The circular illustration was visualized using the CGView server and contains four rings. The outermost circle and the second circle show the positions of the CDSs in forward and reverse strand directions, respectively. The tRNA, tmRNA e rRNA genes are represented by green, red and pink arrows, respectively. The next rings show the following information: GC content, GC skew+, and GC skew-, respectively.

Table 1 Genomic features of *P. pentosaceus* ST65ACC

Features	Genome
Size (pb)	1,933,194
GC content (%)	37.00 %
Number of genes	2,012
Number of CDS	1,950
tRNA	55
rRNA	6
tmRNA	1
Plasmid	0
CRISPR	2 (questionable)
Cas proteins	2
Prophages	1 intact, 1 questionable
Antimicrobial resistance (AR)	7
Probability of being a human pathogen	0.171

Phylogenetic Analysis

The similarity of the *P. pentosaceus* strain ST65ACC compared to other genomes of *P. pentosaceus* deposited in the NCBI (Supplementary Table 1) were evaluated using the ANIb, ANIm, TETRA and GGDC algorithms. The relatedness values of the strain ST65ACC with all the evaluated *P. pentosaceus* strains are above the limit established for each algorithm (ANIb: 95 %, ANIm: 95 %, TETRA: 0.999 and GGDC: 70 %), as can be seen in Supplementary Table 2. *P. pentosaceus* SRCM102736 was identified as the closest neighbor of the strain, with a similarity of 99.31 % for ANIb, 99.71 % for ANIm, 0.99878 for TETRA and 97.20 % for GGDC. The high similarity values between the genomes of *P. pentosaceus* can also be observed in the phylogenomic tree inferred from GBDP distances in TYGS (Fig. 2).

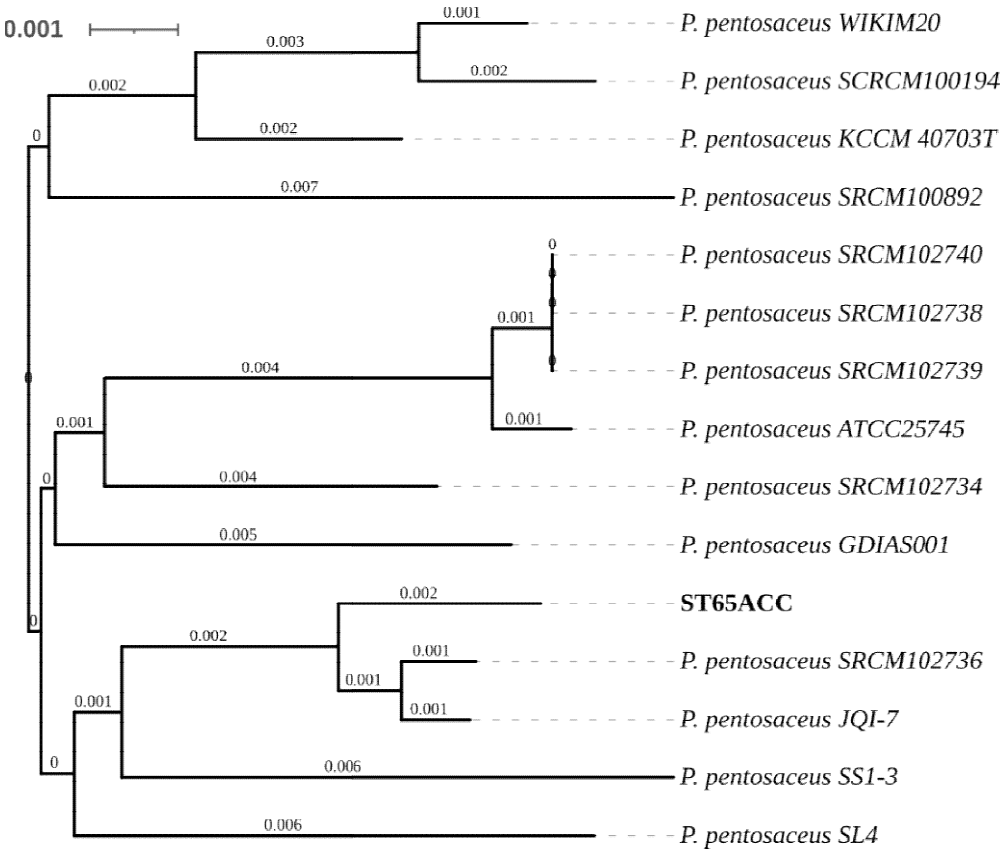

Fig. 2 Phylogenomic tree built using the TYGS (https://tygs.dsmz.de), for taxonomic analysis based on the complete genome and determination of closely related strains [35]. Tree inferred with FastME 2.1.6.1 [54] from Genome Blast Distance Phylogeny (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula *d5*. The numbers above branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 5.5 %. The tree was rooted at the midpoint [55].

Comparative Genomic Analysis

In total, 27,091 genes were present in the 15 genomes of the *P. pentosaceus* evaluated (Supplementary Table 1) and, of these genes, 26,326 (97.2 %) were grouped into 2,216 orthogroups by OrthoFinder [36], from which, 1,410 were identified as core orthogroups and 806 were identified as accessory orthogroups (Fig. 3). *P. pentosaceus* ST65ACC was present in 1,804 (81.4 %) orthogroups, and only one orthogroup was species-specific, containing 2 unique species-specific genes that encode a phage associated protein XkdX. For more information on the properties of

orthogroups shared across strains, the core orthogroups were later classified in functional COG categories through the online server Batch CD-Search [39]. Of the 1,410 core orthogroups, 997 (70.71 %) genes were classified into the COG families comprising 21 functional categories (Figure 4). The most of the orthogroups were related to “Translation, ribosomal structure and biogenesis” category (J; 16.76 %), followed by “Transcription” activities (K; 8.79 %), “General function prediction only” (R; 6.88 %), “Carbohydrate transport and metabolism” (G; 6.70 %), “Cell wall/membrane/envelope biogenesis” (M; 6.43 %), “Replication, recombination and repair” (L; 6.43 %) and “Nucleotide transport and metabolism” (E; 6.25 %).

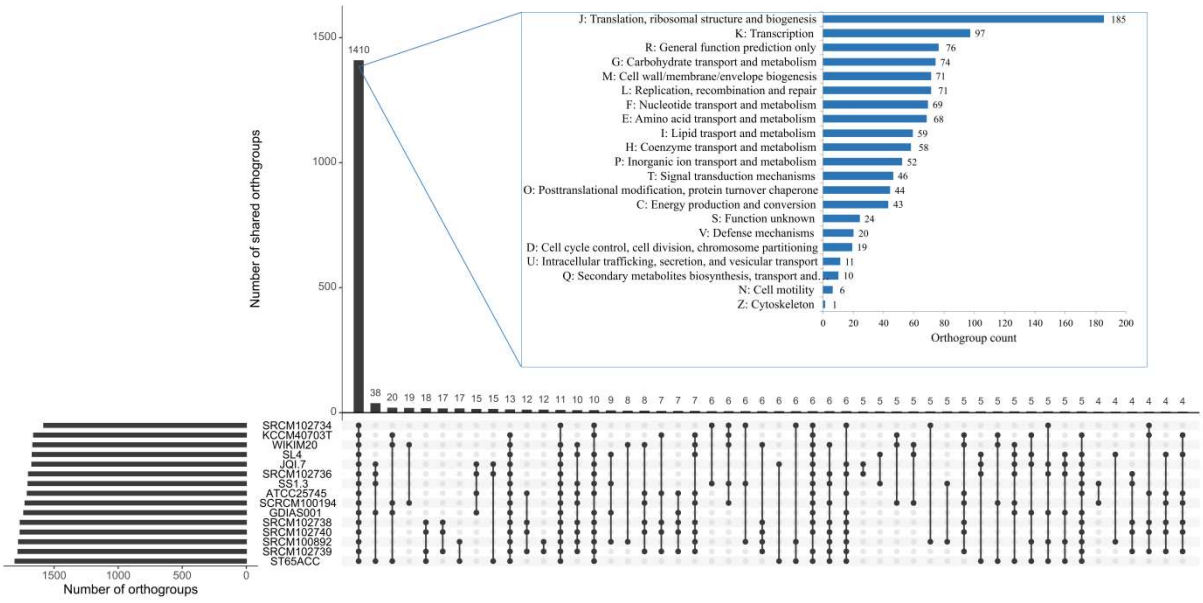

Fig. 3 Upset plot comparing shared orthogroups between the fifteen *P. pentosaceus* group species. Box plot shows the distributions of orthogroup protein length means. Bars represent a number of orthogroups showing specific conservation patterns with numbers also given above each bar. The chart in blue shows the functional category of core orthogroups based on COG classification using server Batch CD-search [39].

Multiple genome alignment

P. pentosaceus strains SRCM102736 and JQI-7 were selected for multiple

genome alignment, as they are closer to the strain ST65ACC based on their locations in the phylogenetic tree (Fig. 2), as shown in Fig. 4. The three strains show similarities, sharing thirteen regions, however, the genes are in different positions and in some cases with inversions within each chromosome. Furthermore, the *P. pentosaceus* strain ST65ACC does not have the dark blue color region present in the initial position of *P. pentosaceus* SRCM102736 and in the position 800,000 of *P. pentosaceus* JQI-7. This illustrates how one strain of the same species can diverge from the others and provides important evidence for the reconstruction of the strain ST65ACC.

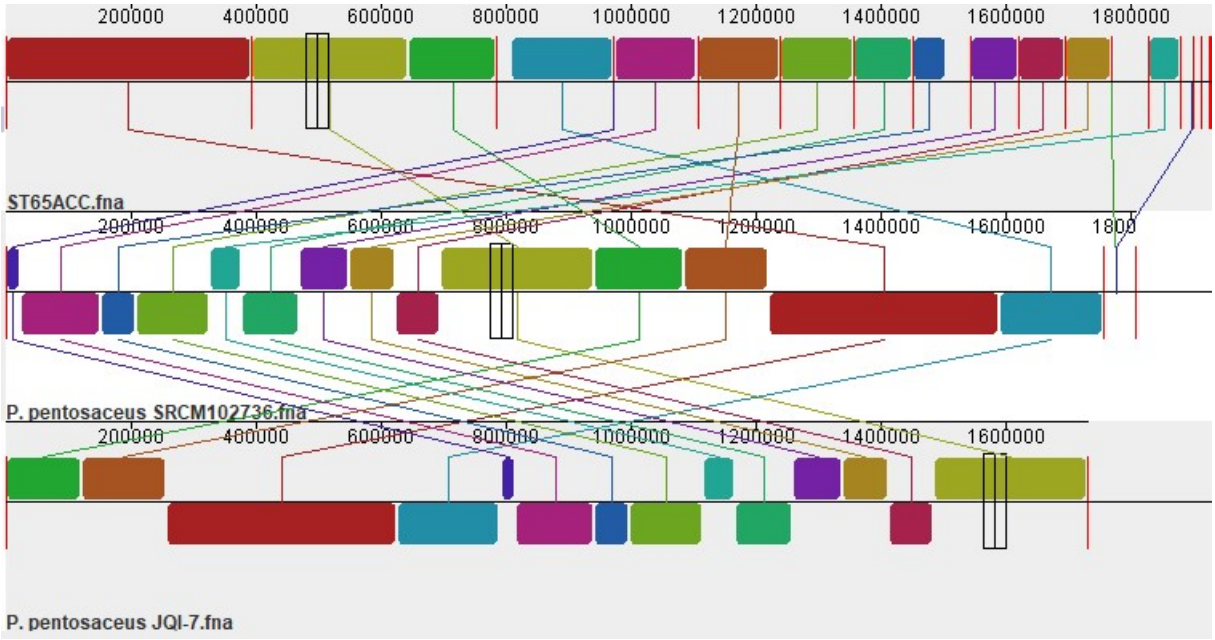

Fig. 4 Multiple genome alignment of *Pediococcus pentosaceus* strains, including ST65ACC, SRCM102736 and JQI-7. Visualization of alignment is arranged into one horizontal panel per genome sequence, with the label of the genome sequence name on the bottom-left of each panel. The homologous blocks are represented with the same color and are connected within the genome by lines.

In order to have a broad visualization of coding sequences between the strain ST65ACC and the other *P. pentosaceus* strains (Supplementary Table1), all

genomes were compared to the reference genome *P. pentosaceus* ATCC25745 using BRIG [42], can be viewed on the Fig. 5. The similarity between the genomes is represented by the solid part of the circle, while the variability is represented as a blank. This means open reading frames that are present in the reference genome but absent in the remaining genomes, which also correlate with differences in assembly quality [3].

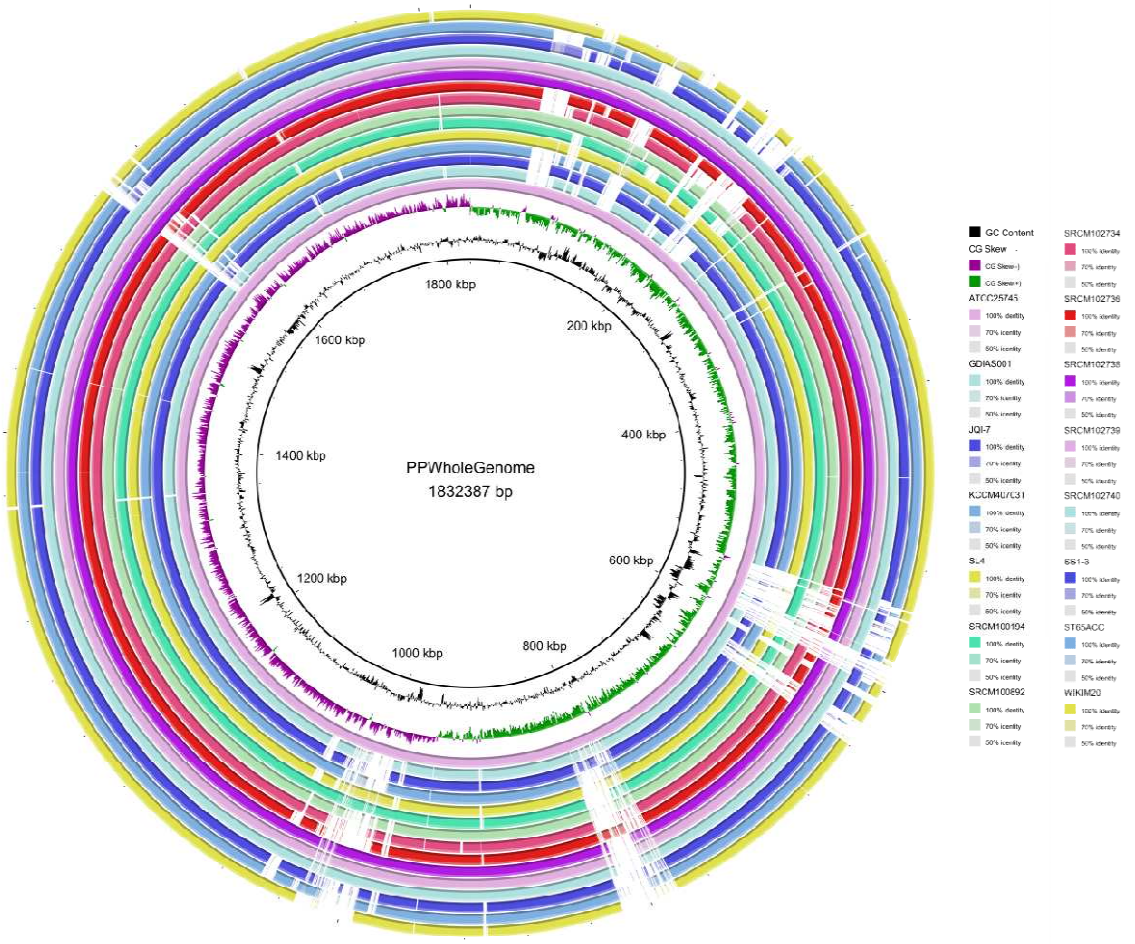

Fig. 5 The whole genome comparison. Nucleotide alignments of fifteen *P. pentosaceus* genomes generated with the BRIG.

Identification of active carbohydrate enzymes

CAZymes is a classification based on a sequence of enzymes that are

involved in the metabolism of complex carbohydrates. The enzymes were investigated by comparing predicted genes in the *P. pentosaceus* ST65ACC genome with the CAZY database. Thirty-five genes have been identified in three classes of CAZymes, including glycosyltransferase (GT), glycoside hydrolase (GH) and carbohydrate esterase (CE) (Fig. 6). The CAZymes analysis revealed the GT family as the main category, with 21 genes distributed in 6 GT families, followed by the GH family (11 genes) and CE (3 genes).

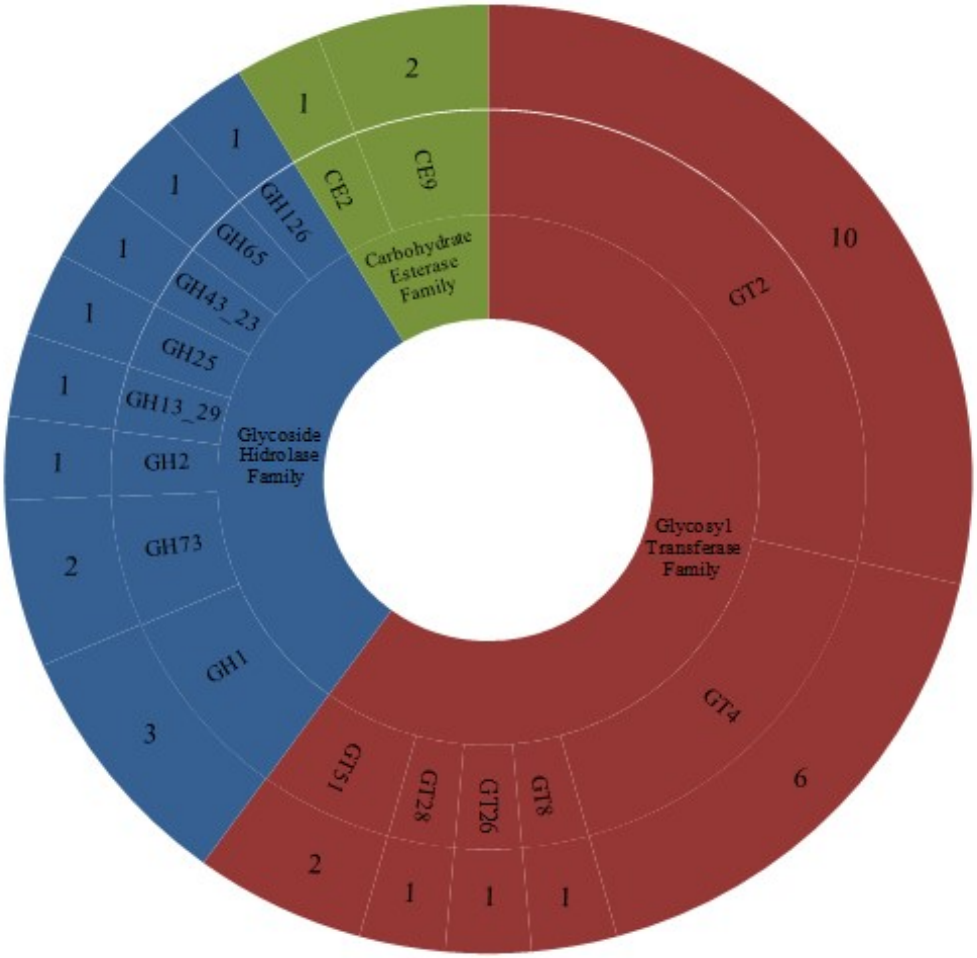

Fig. 6 CAZymes distribution in ST65ACC genome. CAZymes were assigned by searching against the CAZy database using the dbCAN webserver. Different colors represent different classes of CAZymes found in the genome. The representation from the inner to outer rings are, CAZyme classes, CAZyme families, and the number of genes identified in each family, respectively.

Bacteriocin genes clusters

Gene clusters of two pediocin-like bacteriocins were detected for the *P. pentosaceus* ST65ACC genome, pediocin PA-1/AcH and penocin A, can be seen in Fig. 7A. A highly conserved sequence of the precursor peptide of pediocin PA-1, the *pedA* gene was identified at 62 aa (Fig 7B), with the motif YGNGV consensus at its N-terminus, showing 100 % coverage and 100 % amino acid sequence identity with reference sequence P29430.2 [56]. The *pedB* gene is located downstream of *pedA*, and encodes the immunity protein (112 aa). The *pedC* gene encodes a bacteriocin maturation protein (82 aa) and the *pedD* gene (LanT) encoding an ABC transporter, transport protein and ATP-ligase processing (724 aa). Upstream of the *pedA* gene, the presence of other genes that may also be involved in the expression of pediocin PA-1 was detected.

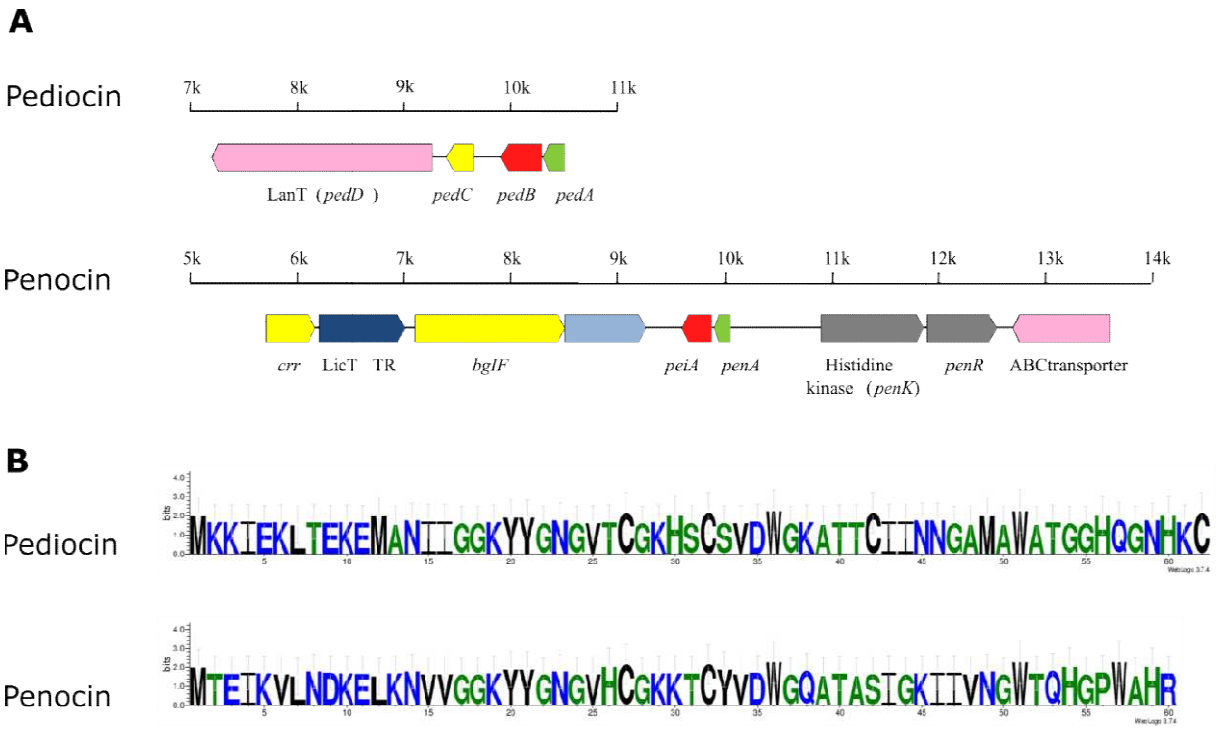

Fig. 7A) Putative operons for the predicted bacteriocin in *P. pentosaceus* ST65ACC. TR, transcriptional regulator. The numbers above the arrow indicate the genomic position of the group of genes. **B)** The Weblogo of pediocin and penocin consensus.

In the penocin-A operon, *penA*gene was annotated as leader protein (60 aa),

with a consensus of double-glycine (GG)-leader at the N-terminus (Fig.7B), with 100 % coverage and 98.33 % amino acid sequence identity with the reference sequence AGC70505.1 [57]. The *peiA* gene encodes an immunity protein with 92 aa and three other genes are found in a different amount of the *penA* gene, the gene encoding the protein histidine kinase (*penK*) and the response regulator *penR*, which are oriented in a different direction, and then the *penT* gene encoding the ABC transporter (304 aa), as well as identified in *P. pentosaceus*strain ATCC 25745 [5]. Transcriptional regulators of the *LicT* family (276 aa) and the glucose transporter system PTS (encoded by *bglF* and *crr*) were also identified (Fig. 7A).

Pathway analysis of *P. pentosaceus* ST65ACC

In total, 974 genes were mapped into 162 KEGG pathways, divided into 6 functional categories and subdivided into 38 subcategories (Fig. 8A). The most abundant functional categories were associated with carbohydrate metabolism (18.68 %), translation (8.11 %), amino acid metabolism (7.39 %), replication and repair (6.67 %), nucleotide metabolism (6.47 %) and membrane transport (6.47 %). For better visualization of the metabolic pathways of the ST65ACC genome, the subcategories of carbohydrate, amino acid and lipid metabolism were shown in Fig. 8B. These pathways are critical to niche adaptation and provide an overview of the strain's ability to metabolize different energy sources.

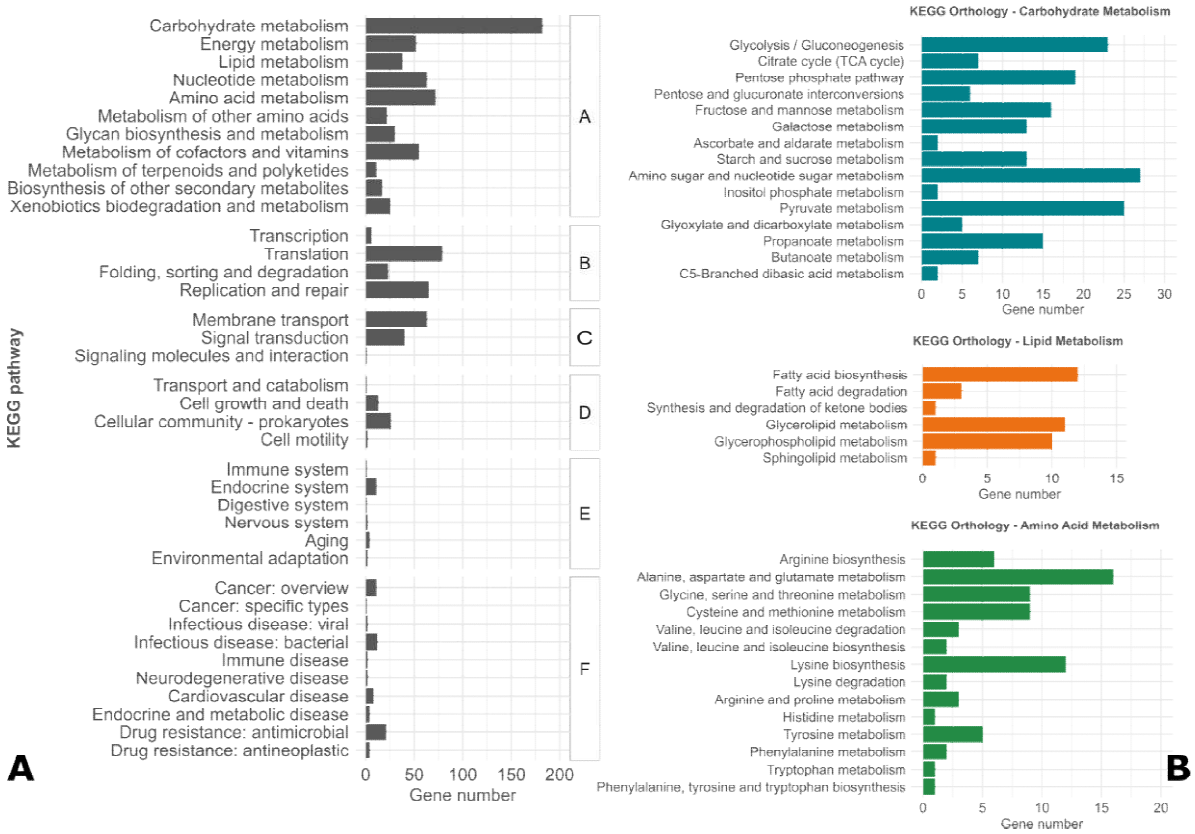

Fig. 8 Kyoto Encyclopedia of Genes and Genomes (KEGG) functional annotation. **a)** Detailed representation of the functional classes belonging to six main functional categories.**b)** Subcategories of carbohydrate, amino acid and lipid metabolism. Functional categories: A- Metabolism; B- Genetic Information Processing; C- Environmental Information Processing; D- Cellular Processes; E- Organismal Systems; F- Human Diseases.

The KEGG metabolic pathways showed that the *P. pentosaceus* ST65ACC genome exhibits homolactic fermentative capabilities, presenting the complete pathways for glucose, galactose and pentose phosphate metabolism. The genes and their positions in the genome are shown in Supplementary Table 3. An important feature of lactic acid bacteria (LAB) is their ability to metabolize lactose in milk, and the genome presented genes involved in the lactose utilization, such as the *lacZ* (beta-galactosidase), *lacY* (OHS family lactose permease) and *lacI* (LacI transcriptional regulator family) gene, shown in Supplementary Table 3. Genes that encode proteins of the Leloir metabolic pathway, responsible for the catabolism of D-

galactose in UDP-glucose, were also identified in the genome [58]: galactokinase (*galK*) genes, UDP-glucose--hexose-1-phosphate uridylyl-transferase (*galT*), UDP-glucose 4-epimerase (*galE*) genes and aldose 1-epimerase (*galM*) genes.

P. pentosaceus ST65ACC genome presents metabolic pathways for the conversion of pyruvate, through the regeneration of NAD, to the production of diacetyl and/or acetoin (Supplementary Table 3). The genes encoding α-acetolactate synthase (*als*), α-acetolactate decarboxylase (*alsD*), 2,3-butanediol dehydrogenase/meso-butanediol dehydrogenase (*budC*) and diacetyl reductase (*butA*) are present in the sequences. However, the strain did not present a complete pathway for using citrate. The strain also shows the pathways for conversion of pyruvate to acetyl-CoA by a pyruvate dehydrogenase complex (*pdhABCD*), and further conversion to acetate via the phosphate acetyltransferase-acetate kinase (*pta*, *ackA*). Furthermore, pyruvate can be converted to carbon dioxide and diacetyl phosphate by pyruvate oxidase (*poxL*) when oxygen is available and later converted to acetate by the enzyme acetate kinase (*ackA*) and/or acylphosphatase (*acyP*). Alternatively, under optimal conditions pyruvate can be converted to acetate by pyruvate dehydrogenase (*poxB*).

P. pentosaceus ST65ACC genome did not present complete pathways for amino acid synthesis, however, a variety of components of the proteolytic system were identified in the genome, genes encoding peptidases (*pepC*, *pepN*, *pepX*, *pepF*, *pepQ*, *pepS*, *pepO*, *pepDA, pepDB*, *pepT*), amino acid permeases (*glnH*, *glnP*, *metQIN*) and oligopeptide transport systems (*oppABCDF*) to the intracellular space. Cell-envelope proteinases (CEPs) were not found in the genome. The genes present and their positions in the genome are shown in the Supplementary Table 4.

Genes related to stress resistance, tolerance to acids and bile salts were also

present in the *P. pentosaceus* ST65ACC genome (Supplementary Table 5). ORFs encoding the F_0F_1-ATP synthase complex proteins (*atpF1ABGDE*, *atpF0AB*) and Na^+:H^+antiporter (*nhaC*, *nhaK*) were identified in the genome. As well as genes related to the arginine deiminase system (arginine deiminase (*arcA*), carbamate kinase (*arcC*), and arginine/ornithine (*arcD*) antiporters) and genes encoding heat stress proteins, including heat shock protein Hsp20 and molecular chaperones *hslO*, *groEL*, *grpE*, *groES*, *dnaK* and *dnaJ*were found in the genome. The ATP-dependent intracellular proteases *clpP*, *clpX*, *clpE*, *clpL* and *hslV* were also identified in the *P. pentosaceus* ST65ACC genome, which can act in the repair of macromolecules. Regarding adhesion and aggregation properties, the genome presented genes that encode lipoteicoic acid (*ltaS*), which helps of the bacterial surface the intestinal mucosa. The genes sortase A (*srtA*), surface-associated enolase (*eno*), *clp* protease and peptidyl-prolyl cis-trans isomerase (*ppiB*) and some clandestine proteins such as glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), transaldolase (*tal*), elongation factors (*tuf*, *tsf*, *fusA*) were detected in the *P. pentosaceus* ST65ACC genome.

Mobile genetic element

P. pentosaceus ST65ACC genome was investigated for the presence of mobile genetic elements such as prophages, insertion elements (IS) and CRISPR regions. Two prophage regions were found by the PHASTER server, with only intact and questionable prophages considered in this study (Table 2). Most of the components in every phage region are hypothetical proteins and phage-related proteins of the genera *Lactobacillus* and *Listeria*.

Table 2 Prophages predicted in *P. pentosaceus* ST65ACC using PHASTER

Region	Region length	Score	Completeness	Total CDS	Region position	GC (%)	Most common GC phago (%)
1	46.4Kb	150	intact	48	366-46811	36.37	PHAGE_Lactob_phigle_NC_004305 (15)
2	39.7Kb	90	questionable	61	50497-90271	35.73	PHAGE_Lister_B025_NC_009812 (11)

IS were identified in some contings of *P. pentosaceus* ST65ACC genome. Most they belonged to the species *P. pentosaceus* (ISPp1), *Leuconostoc mesenteroides* (IS1165) and *Lactobacillus plantarum* (ISLpl1). Also found IS from the species *Enterococcus hirae* (IS1310), *L. monocytogenes* (ISLmo3) and the genus *Bacillus*: *B. cereus* (ISBce15, ISBce18, IS240C), *B. thuringiensis* (ISBth6, IS240F), *B. weihenstephanensis* (ISBwe2, ISBwe3), and Gram-negative bacteria, *Acinetobacter bereziniae* (ISAbe16), *Escherichia coli* (IS1397), *Pseudomonas syringae* (ISPsy8) and *Chromohalobacter japonicas* (ISChja3). Thus, revealing the adaptation mechanism of the strain ST65ACC for survival via integration of these elements into its genome. The sorting of CRISPR sequences by the CRISPRcasFinder web server resulted in two CRISPR regions (2 questionable) in the genome of *P. pentosaceus* ST65ACC, not associated with a Cas system. CRISPR1 and CRISPR2 blocks contain a spacer and two short direct repeat sequences. Two Cas3 proteins were found in the ST65ACC genome not associated with CRISPR sequences.

Safety assessment and identification of antibiotic resistance genes

Pathogenic genes, AMR genes and virulence factors were investigated to assess the safety of using *P. pentosaceus* ST65ACC. No antibiotic resistance (AR) genes and virulence factors were identified using CARD, ARG-ANNOT, ResFinder, NCBI AMRFinderPlus and VFDB databases. Subsequently, further research was

carried out using the KEEG database and seven AR genes were identified (*lsa*, *penP*, *dltABCD*, *abcA* / *amrA*), which can be seen in Complementary Table 6. It was also identified in the *P. pentosaceus* ST65ACC genome the *hlyIII* gene, which encodes the hemolysin III toxin. Genes related to BA production were not identified. The risk score for *P. Pentosaceus* ST65ACC was 0.171, which characterizes the strain as a non-human pathogen by the Pathogen Finder.

Discussion

In this study, the WGS of *P. pentosaceus* ST65ACC, genomic characterization and comparative analyzes between related species were performed. The ANI, TETRA and GGDC analyzes clearly identified the phylogenetic proximity between the *P. pentosaceus* ST65ACC genome and the *P. pentosaceus* strains available at the NCBI. Here, a core orthogroup is defined as an orthogroup in which all analyzed genomes are present and all other orthogroups are defined as accessory orthogroups. Thus, the genomes of *P. pentosaceus* share 1,410 orthogroups, and most of the genes present in these orthogroups are genes involved in maintenance functions, fundamental to the growth and survival of the species.

CAZimas are directly involved in biosynthesis (glycosyltransferases, GTs), degradation (glycoside hydrolases (GHs), polysaccharide lyases (PLs), carbohydrate esterases (CEs) and enzymes for auxiliary activities, AAs) and recognition (carbohydrate binding module, CBM) of a variety of complex carbohydrates, playing a key role in sugar metabolism. There are different types of GTs involved in the biosynthesis of disaccharides, oligosaccharides and polysaccharides, which help in the formation of glycosidic bonds [43]. Of the six GT families identified in the *P.*

pentosaceus ST65ACC genome, enzymes belonging to the GT2 and GT4 families represent 76.19 % of the total GTs, being responsible for the synthesis of cellulose synthase, chitin synthase, sucrose synthase, galactosyltransferase, glucosyltransferase. GH are the main enzymes involved in carbohydrate metabolism and play an important role in the hydrolysis of the carbohydrate glycosidic bond [43]. The genome of this strain had genes involved in the synthesis of β-glucosidase (GH1), α-glucosidase (GH13_23), β-galactosidase (GH2) enzymes. Enzymes that can play an active role in the metabolism of carbohydrates, such as sucrose, lactose and oligosaccharides, essential for the development of the strain in different environments, such as dairy products [43, 59]. The enzyme lysozyme (GH73 or GH25) was also identified in the genome of the strain, normally associated as a catalyst for the hydrolysis of the β(1-4) bond between N-acetylglucosamine and N-acetylmuramic acid of bacterial cell walls and may also have antimicrobial activity [60]. *P. pentosaceus* ST65ACC genome encodes important CAZimas that are involved in carbohydrate synthesis and hydrolysis during fermentation. The bacteria's carbohydrate utilization capacity is an important indicator of strain functionality and lays a foundation for strain cultivation and selection [3].

Pediocin-like bacteriocins (class IIa) constitute a large and important group of antimicrobial peptides produced by LAB [61, 62]. Common features in class IIa bacteriocin gene clusters are structural genes encoding the core peptide, gene encoding the immunity protein, and transporter genes [63, 64]. In the current study, the pediocin PA-1/AcH operon presented the necessary genes and was considered a potential cluster of biosynthetic genes encoding bacteriocin (Fig. 7A) [2, 65]. A cluster with the main genes related to the encoding of penocin A was presented [57]. However, according to Diep et al. [5] two regulatory genes are also required for an

expressive production of penocin: a gene that encodes an accessory protein that acts together with the ABC transporter to remove GG leaders concomitant with the export of mature peptides and a gene that encodes an inducing peptide (*penI*) required for activation of the two-component regulatory system (*penKR*) that eventually triggers high transcription of all genes at the bacteriocin locus. In this study, as well as in the work carried out by Diep et al. [5], the accessory gene involved in transport and the inductor gene involved in regulation (*penI*) are absent, which demonstrated that the *P. pentosaceus* strain ATCC 25745 is a weak producer of penocin-A. Consequently, the strain ST65ACC may be a poor producer of the bacteriocin penocin-A.

In a previous study by Todorov et al. [66], obtaining and purifying the bacteriocin produced by *P. pentosaceus* ST65ACC, resulted in the identification of a partial sequence KYYGNGVTCGKHSCSVDWGK corresponding to class IIa bacteriocins. The partial sequence showed similarity to the bacteriocin coagulin A produced by *B. coagulans* I4 [67] and pediocin PA-1 [68, 69]. Both bacteriocins are very similar, coagulin A differs from pediocin PA-1/AcH only in the amino acid at position 41, in that a threonine residue was present at position 41 (T41) in coagulin, while pediocin PA-1/AcH contain an asparagine residue (N41) [63, 67]. The sequencing of the ST65ACC genome showed that this partial sequence obtained corresponds to the pediocin PA-1/AcH (Fig.7B). *In silico* analysis of the ST65ACC genome showed that its high antimicrobial capacity of the strain is related to the presence of the identified bacteriocins, pediocin PA-1 and penocin A, showing the potential of this strain for use in food biopreservation.

An evaluation of the metabolic pathways of technological interest present in *P. pentosaceus* ST65ACC through KEGG was performed. In dairy substrates, lactose is the main source of energy for LAB, being essential for fermentation and

characterization of dairy products. *Pediococcus* spp. are generally characterized as incapable of fermenting lactose [70, 71], however research carried out with *P. pentosaceus* has shown that some strains may use lactose as a carbon source [3, 71], which is contradictory to the view that lactose-positive strains within the naturally occurring *Pediococcus* genus are absent [3]. Thus, the presence of lactose utilization genes and Leloir's metabolic pathway in the ST65ACC genome may allow this organism to utilize lactose [58], which needs to be specified during future research.

Diacetyl is an important aroma compound responsible for the flavor of many dairy products such as butter and cheese. Its formation results from the conversion of pyruvate to α-acetolactate, which is later converted to diacetyl and CO_2 by oxidative decarboxylation. Furthermore, acetoin can be produced from α-acetolactate and diacetyl, and eventually converted to 2,3-butanediol [72]. Although the strain ST65ACC does not have the complete citrate metabolic pathway, the main α-acetolactate production pathway, the strain presented the genes necessary for the production of diacetyl and acetoin from pyruvate, and this species has been reported to produce diacetyl [73, 74] using pyruvate derived from carbohydrate or amino acid metabolism.

The presence of many gene sequences responsible for the transport and metabolism of carbohydrates and amino acids is considered an important probiotic and technological feature in LAB. The LAB proteolytic system comprises specific proteinases, peptidases and transport proteins [75]. Although the *P. pentosaceus* ST65ACC genome has many peptidases and transporter genes, genes corresponding to CEPs were not found, which are important for the efficient use of caseins in milk [76]. CEPs were also not found in the chromosome of *P. pentosaceus* strain ATCC25745 [77]. In addition to CEPs, endopeptidase O (*pepO*), found in the

P. pentosaceus ST65ACC genome, plays an important role in the use of casein and milk oligopeptides [78].

Tolerance to acids, bile and associated extrusion mechanisms are desirable characteristics in probiotic strains, as they need to survive transit and the complexity of the gastrointestinal tract, in order to play its functional role as a probiotic [79]. In the *P. pentosaceus* ST65ACC genome, F_0F_1-ATPase synthase complex proteins and Na+/H+ antiporters contribute to pH homeostasis, as they act as channels for proton translocation at the cost of ATP synthesis or hydrolysis [80]. In addition, general stress response genes help bacteria respond and thrive in rapidly changing environmental conditions [81], such as the proteins encoded by the *groEL*, *groES*, *recA*, *dnaJ*/*dnaK* genes and *clp* complex, which can protect bacteria against acid stress, ensuring the proper functioning of DNA repair and recombination pathways [82].

To resist the deleterious action of bile salts, microorganisms have developed specific defense mechanisms, such as bile efflux, induction of general stress proteins, bile salt hydrolysis and reorganization of central metabolic pathways [79]. The presence of multidrug transporters (efflux pump) is a common bacterial mechanism to counteract bile toxicity [83, 84] and the *P. pentosaceus* ST65ACC genome presents an *abcA*/*bmrA* transporter (Supplementary Table 6), which may aid biliary tolerance and/or extrusion activity in this species. Bile salt resistance can also be acquired by selection for other stress conditions such as acidic pH [82].

The general stress response (*htrA*, *dnaK*, *groEL*), protection against oxidative damage can also be used to neutralize some of the cellular damage caused by these compounds and, in addition, F_0F_1-ATPase has been regulated in bile environments acting in maintenance of intracellular pH in a variety of bacteria [79]. In a previous

study by Cavicchioli et al. [12], the exposure of *P. pentosaceus* ST65ACC to the simulated gastric and enteric environment led to a decrease from 7.6 to 6.2 log CFU/mL, demonstrating a good resistance to the simulated gastrointestinal tract, which can be associated with the structures and mechanisms present in the genome of this strain.

The adhesion and aggregation properties are also important characteristics in a probiotic strain, as they determine the bacterial colonization capacity in the intestinal environment. Cell adhesion involves contact between the bacterial cell membrane and the interacting surfaces that line the intestine and, in most cases, the aggregation capacity is related to cell adhesion properties [82, 85]. Lipoteichoic acid, a surface molecule, can mediate host-microbe interactions in the gastrointestinal tract, through the adhesion of the bacterial surface to the mucin and glycans of the intestinal mucosa [86].

The presence of some surface proteins, such as proteins anchored in the cell wall, has been shown to improve hydrophobic interactions and adhesion in some LAB [87]. The enolase present in *P. pentosaceus* ST65ACC genome is known to bind collagen in the extracellular matrix [88]. Multifunctional proteins can also act as adhesion factors, such as heat shock proteins GroEL, elongation factor Tu, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [86]. The percentage of cell surface hydrophobicity of *P. pentosaceus* ST65ACC was 46.5 %, while the percentage of self-aggregation in 1 h of incubation was 71.8 % and the co-aggregation rate of the strain with *L. monocytogenes* L711, L422 and L637 strains were 48.1 %, 71.7 % and 71.0 %, respectively [12]. A strain with good adhesion capacity can compete with pathogens for host binding sites and, in addition to competitive exclusion, co-aggregation and production of antimicrobial metabolites

can favor the elimination of pathogens [82, 89].

The presence of mobile genetic elements is an important factor to be investigated in microorganisms destined for food applications, since the presence of prophages, plasmids and insertion elements can be vehicles of horizontal gene transfer (HGT) of pathogenic genes or AMR to other microorganisms [90, 91]. The genome of *P. pentosaceus* ST65ACC showed an intact prophage region and a questionable region. Prophages are genetic elements often present in several genera of LAB, including *Pediococcus* [3, 92, 93]. Thus, further analysis is needed in these regions to identify the presence of pathogenic genes or AMR genes, not being identified in the *P. pentosaceus* ST65ACC genome. CRISPR-Cas systems act as adaptive immunity in bacterial cells to combat invasive elements such as bacteriophages and, in addition, are involved in the regulation of gene activity, DNA repair and genome reorganization [94, 95]. *P. pentosaceus* ST65ACC genome does not have a CRISPR-Cas system, despite having two questionable isolated CRISPR locus and two isolated *cas* locus. According to Zhang and Ye [96] CRISPR regions without *cas* genes can be non-functional or work with distant *cas* locus in the same genome.

The ability to produce BA should be evaluated for microbial strains with possible use in food, since the unwanted accumulation of BA in food can be harmful to health [97]. In the safety assessment of the *P. pentosaceus* ST65ACC genome, no BA gene were found, confirming results obtained by Cavicchioli et al. [12]; the strain did not produce BA in the phenotypic tests and genes related to the expression of histamine, tyramine and putrescine were absent. Regarding virulence genes, the gene encoding the hemolysin III toxin (*hlyIII*) was detected in the genome using the KEGG database [28]. A manual investigation using the BLASTp search confirmed the

identification of the gene with 100 % identity for “hemolysin III family protein” (WP_011672786.1) of *Pediococcus*. Even with the presence of the *hlyIII* gene, the strain ST65ACC did not induce hemolysis in an in vitro test performed by Cavicchioli et al. [12].

AMR analysis via the KEGG database identified the presence of seven AR genes in the *P*. *pentosaceus* ST65ACC genome. Notably, the presence of a *lsa* macrolide resistance gene in the *P*. *pentosaceus* ST65ACC genome did not confer resistance to erythromycin and clindamycin, macrolide antibiotics tested in a study by Cavicchioli et al. [12]. This can be due to several factors, such as the level of gene expression and the substrate specificity of the expressed product [98]. Likewise, despite having a class A beta-lactamase gene (*penP*), the *P*. *pentosaceus* strain ST65ACC was sensitive to ampicillin, imipenem and penicillin G and showed resistance only to oxacillin [12]. Although no specific vancomycin and trimethoprim/sulfamethoxazole resistance genes were found in the *P*. *pentosaceus* ST65ACC genome, Cavicchioli et al. [12] identified strain resistance to these antibiotics. However, an efflux pump-related gene (*abcA*/*bmrA*), which confers resistance to multiple drugs, and CAMP resistance genes (*dltA*, *dltB*, *dltC*and*dltD*) were also identified in the genome. These systems may contribute to the strain's resistance these antibiotics. The main concern regarding genes that can confer AR in LABs is the possibility of its transfer to other bacteria, mainly pathogens, which can lead to complications, reducing the effectiveness of antibiotic treatment [99]. Analyzing the prophage regions found in the *P. pentosaceus* ST65ACC genome, no AR genes were located in these regions and no plasmids were identified, presenting a low risk of being transferred to other bacteria.

Conclusion

The characterization of the *P. pentosaceus* ST65ACC genome revealed important genes, such as stress adaptation and adhesion genes, milk sugar utilization, some peptidases and genes that encode the machinery involved in the maturation, immunity and export of pediocin PA-1/AcH. The genomic study also revealed the absence of negative traits, resistance to transmissible antibiotic genes and plasmids. Altogether, the results of investigations into genomic information indicate *P. pentosaceus* ST65ACC a promising probiotic candidate to be considered, as well as its possible use in food biopreservation.

Supplementary Information The online version contains supplementary material available.

Author Contribution FS Oliveira: Methodology, Investigation, Data curation, Software, Writing original draft. RS Rodrigues: Investigation, Data curation, Software, Writing-original draft. AF Carvalho: Writing-review and editing, Supervision, Funding acquisition. LA Nero: Conceptualization, Writing-review and editing, Resources, Supervision, Funding acquisition.

Acknowledgements The authors are thankful for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil, Financialcode 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil).

Data Availability Further data that support the findings of this study are available on request from the corresponding author. Sequences were available at GenBank, as described in Material and Methods section.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Cavicchioli VQ, Camargo AC, Todorov SD, Nero LA (2017) Novel bacteriocinogenic *Enterococcus hirae* and *Pediococcus pentosaceus* strains with antilisterial activity isolated from Brazilian artisanal cheese. J Dairy Sci 100:2526-2535. https://doi.org/10.3168/jds.2016-12049
2. Miller KW, Ray P, Steinmetz T, Hanekamp T, Ray B (2005) Gene organization and sequences of pediocin AcH/PA-1 production operons in *Pediococcus* and *Lactobacillus* plasmids. Lett Appl Microbiol 40:56-62. https://doi.org/10.1111/j.1472-765X.2004.01627.x
3. Jiang J, Yang B, Ross RP, Stanton C, Zhao J, Zhang H, Chen W (2020) Comparative genomics of *Pediococcus pentosaceus* isolated from different niches reveals genetic diversity in carbohydrate metabolism and immune system. Front Microbiol 11:253. https://doi.org/10.3389/fmicb.2020.00253
4. Kuniyoshi TM, Mendonça CMN, Vieira VB, Robl D, de Melo Franco BDG, Todorov SD, Tomé E, O'Connor PM, Converti A, Araújo WL, Vasconcellos LPSP, Varani AM, Cotter PD, Rabelo SC, Oliveira RPS (2021) Pediocin PA-1 production by *Pediococcus pentosaceus* ET34 using non-detoxified hemicellulose hydrolysate obtained from hydrothermal pretreatment of sugarcane bagasse. Bioresour Technol 338:125565. https://doi.org/10.1016/j.biortech.2021.125565
5. Diep DB, Godager L, Brede D, Nes IF (2006) Data mining and characterization of a novel pediocin-like bacteriocin system from the genome of *Pediococcus pentosaceus* ATCC25745. Microbiology 152:1649–1659. https://doi.org/10.1099/mic.0.28794-0
6. Umu ÖC, Bäuerl C, Oostindjer M, Pope PB, Hernández PE, Pérez-Martínez G, Diep DB (2016) The potential of class II bacteriocins to modify gut microbiota to improve host health. PLoS One 11:e0164036. https://doi.org/10.1371/journal.pone.0164036
7. Bédard F, Hammami R, Zirah S, Rebuffat S, Fliss I, Biron E (2018) Synthesis, antimicrobial activity and conformational analysis of the class IIa bacteriocin pediocin PA-1 and analogs thereof. Sci Rep 8:9029. https://doi.org/10.1038/s41598-018-27225-3

8. Liu S, Liu Y, Zhang P (2019) Properties of *Listeria monocytogenes* on acquisition of Pediocin resistance. Ann Microbiol 69:123–130. https://doi.org/10.1007/s13213-018-1411-6

9. Bagenda DK., Hayashi K., Yamazaki K, Kawai Y (2008) Characterization of an antibacterial substance produced by *Pediococcus pentosaceus* lz3. 13 isolated from Japanese fermented marine food. Fish Sci 74:439-448. https://doi.org/10.1111/j.1444-2906.2008.01542.x

10. Nieto-Lozano JC, Reguera-Useros JI, Peláez-Martínez MC, Sacristán-Pérez-Minayo G, Gutiérrez-Fernández AJ, Torre AH (2010) The effect of the pediocin PA-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. Food Control 21:679-685. https://doi.org/10.1016/j.foodcont.2009.10.007

11. Ghosh B, Sukumar G, Ghosh AR (2019) Purification and characterization of pediocin from probiotic *Pediococcus pentosaceus* GS4, MTCC 12683. Folia Microbiol 64:765-778. https://doi.org/10.1007/s12223-019-00689-0.

12. Cavicchioli VQ, Camargo AC, Todorov SD, Nero LA (2019) Potential control of *Listeria monocytogenes* by bacteriocinogenic *Enterococcus hirae* ST57ACC and *Pediococcus pentosaceus* ST65ACC strains isolated from artisanal cheese. Probiotics Antimicrob Proteins 11:696–704. https://doi.org/10.1007/s12602-018-9449-0

13. Kuda T, Kawahara M, Nemoto M, Takahashi H, Kimura B (2014) *In vitro* antioxidant and anti-inflammation properties of lactic acid bacteria isolated from fish intestines and fermented fish from the Sanriku Satoumi region in Japan. Food Res Int 64:248-255. https://doi.org/10.1016/j.foodres.2014.06.028

14. Shin JS, Jung JY, Lee SG, Shin KS, Rhee YK, Lee MK, Hong HD, Lee KT (2016) Exopolysaccharide fraction from *Pediococcus pentosaceus* KFT18 induces immune stimulatory activity in macrophages and immune suppressed mice. J Appl Microbiol 120:1390–1402. https://doi.org/10.1111/jam.13099

15. Yin H, Ye P, Lei Q, Cheng Y, Yu H, Du J, Pan H, Cao Z (2020) *In vitro* probiotic properties of *Pediococcus pentosaceus* L1 and its effects on enterotoxigenic *Escherichia coli*-induced inflammatory responses in porcine intestinal epithelial cells. MicrobPathog 144:104163. https://doi.org/10.1016/j.micpath.2020.104163

16. Wang Y, You Y, Tian Y, Sun H, Li X, Wang X, Wang Y, Liu J (2020) *Pediococcus pentosaceus* PP04 ameliorates high-fat diet-induced hyperlipidemia by regulating lipid metabolism in C57BL/6N mice. J Agric Food Chem 68:15154–1563. https://doi.org/10.1021/acs.jafc.0c05060

17. Jiang S, Cai L, Lv L, Li L (2021) *Pediococcus pentosaceus*, a future additive or probiotic candidate. Microb Cell Fact 20:45. https://doi.org/10.1186/s12934-021-01537-y

18. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (2018) Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA J 16:e05206. https://doi.org/10.2903/j.efsa.2018.5206

19. Wu C, Huang J, Zhou R (2017) Genomics of lactic acid bacteria: Current status and potential applications. Crit Rev Microbiol 43:393-404. https://doi.org/10.1080/1040841X.2016.1179623

20. Coton M, Lebreton M, Leyva Salas M, Garnier L, Navarri M, Pawtowski A, Le Blay G, Valence-Bertel F, Coton E, Mounier J (2018) Biogenic amine and antibiotic resistance profiles determined for lactic acid bacteria and a *Propionibacterium* prior to use as antifungal bioprotective cultures. Int Dairy J 85:21–26. https://doi.org/10.1016/j.idairyj.2018.05.001

21. Coil D, Jospin G, Darling AE (2015) A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661

22. Peng Y, Leung HC, Yiu SM, Chin FY (2012) IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28:1420-1428. https://doi.org/10.1093/bioinformatics/bts174

23. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, PevznerPA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455-477. https://doi.org/10.1089/cmb.2012.0021

24. Kosugi S, Hirakawa H, Tabata S (2015) GMcloser: closing gaps in assemblies accurately with a likelihood-based selection of contig or long-read alignments. Bioinformatics 31:3733–3741. https://doi.org/10.1093/bioinformatics/btv465

25. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068-2069. https://doi.org/10.1093/bioinformatics/btu153

26. Grant JR, Stothard P (2008) The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res 36:W181–W184. https://doi.org/10.1093/nar/gkn179

27. Kanehisa M, Sato Y, Morishima K (2016) BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726-731.https://doi.org/10.1016/j.jmb.2015.11.006

28. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182-W185. https://doi.org/10.1093/nar/gkm321

29. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS (2016) PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16-W21. https://doi.org/10.1093/nar/gkw387

30. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C (2018) CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res 46:W246–W251. https://doi.org/10.1093/nar/gky425

31. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M (2006) ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32–D36. https://doi.org/10.1093/nar/gkj014

32. Carattoli A, Hasman H (2020) PlasmidFinder and *In Silico* pMLST: Identification and typing of plasmid replicons in whole-genome sequencing (WGS). Methods Mol Biol 2075:285-294. https://doi.org/10.1007/978-1-4939-9877-7_20

33. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J (2016) JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929-931. https://doi.org/10.1093/bioinformatics/btv681

34. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471-2105-14-60

35. Meier-Kolthoff JP, Goker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3

36. Emms DM, Kelly S (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol 16:157. https://doi.org/10.1186/s13059-015-0721-2

37. Conway JR, Lex A, Gehlenborg N (2017) UpSetR: an R package for the visualization of intersecting sets and their properties. Bioinformatics 33:2938-2940. https://doi.org/10.1093/bioinformatics/btx364

38. Tatusov RL, Galperin MY, Natale DA, Koonin EV (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28:33–36. https://doi.org/10.1093/nar/28.1.33

39. Marchler-Bauer A, Bryant SH (2004) CD-Search: protein domain annotations on the fly. Nucleic Acids Res 32:W327-W331. https://doi.org/10.1093/nar/gkh454

40. Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147

41. Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT (2009) Reordering contigs of draft genomes using the Mauve aligner. *Bioinformatics* 25:2071–2073. https://doi.org/10.1093/bioinformatics/btp356

42. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA (2011) BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402

43. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-activeenzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490-D495. https://doi.org/10.1093/nar/gkt1178

44. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res 46:W95-W101. https://doi.org/10.1093/nar/gky418

45. Finn RD, Clements J, Eddy SR (2011) HMMER web server: interactive sequence similarity searching. Nucleic Acids Res 39:W29-W37. https://doi.org/10.1093/nar/gkr367

46. van Heel AJ, de Jong A, Song C, Viel JH, Kok J, Kuipers OP (2018) BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. Nucleic Acids Res 46:W278–W281. https://doi.org/10.1093/nar/gky383

47. Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14:1188-1190. https://doi.org/10.1101/gr.849004

48. Alcock BP, Raphenya AR, Lau T, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen AV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran HK, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG (2020) CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517-D525. https://doi.org/10.1093/nar/gkz935

49. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B,

50. Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM (2020) ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500. https://doi.org/10.1093/jac/dkaa345

51. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain JM (2014) ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58:212-220. https://doi.org/10.1128/AAC.01310-13

52. Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, Tyson GH, Zhao S, Hsu CH, McDermott PF, Tadesse DA, Morales C, Simmons M, Tillman G, Wasilenko J, Folster JP, Klimke W (2019) Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother63:e00483-19. https://doi.org/10.1128/AAC.00483-19

53. Liu B, Zheng D, Jin Q, Chen L, Yang J (2019) VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/gky1080

54. Cosentino S, Voldby Larsen M, MøllerAarestrup F, Lund O (2013) PathogenFinder--distinguishing friend from foe using bacterial whole genome sequence data. PLoS One 8:e77302. https://doi.org/10.1371/journal.pone.0077302. Erratum in: PLoS One 8. https://doi.org/10.1371/annotation/b84e1af7-c127-45c3-be22-76abd977600f

55. Lefort V, Desper R, Gascuel O (2015) FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798-2800. https://doi.org/10.1093/molbev/msv150

56. Farris JS (1972) Estimating phylogenetic trees from distance matrices. Am Nat 106:645-668. https://www.jstor.org/stable/2459725

57. Marugg JD, Gonzalez CF, Kunka BS, Ledeboer AM, Pucci MJ, Toonen MY, Walker SA, Zoetmulder LC, Vandenbergh PA (1992) Cloning, expression, and nucleotide sequence of genes involved in production of pediocin PA-1, and bacteriocin from *Pediococcus acidilactici* PAC1.0. Appl Environ Microbiol 58:2360-2367. https://doi.org/10.1128/aem.58.8.2360-2367.1992

58. Martino ME, Maifreni M, Marino M, Bartolomeoli I, Carraro L, Fasolato L, Cardazzo B (2013) Genotypic and phenotypic diversity of *Pediococcus pentosaceus* strains isolated from food matrices and characterisation of the penocin operon. Antonie Van Leeuwenhoek 103:1149-1163. https://doi.org/10.1007/s10482-013-9897-1

59. Aleksandrzak-Piekarczyk T (2013) Lactose and β-glucosides metabolism and its regulation in *Lactococcus lactis*: a review. In: Kongo M (ed) Lactic Acid Bacteria–R & D for Food, Health And Livestock Purposes, (Croatia: InTech), 467–486. https://doi.org/10.5772/50889

60. Vera C, Guerrero C, Aburto C, Cordova A, Illanes A (2020) Conventional and non-conventional applications of β-galactosidases. BiochimBiophys Acta Proteins Proteom 1868:140271. https://doi.org/10.1016/j.bbapap.2019.140271.

61. Michlmayr H, Kneifel W (2014) β-Glucosidase activities of lactic acid bacteria: mechanisms, impact on fermented food and human health. FEMS Microbiol Lett 2014 352:1-10. https://doi.org/10.1111/1574-6968.12348.

62. Papagianni M, Anastasiadou S (2009) Pediocins: the bacteriocins of Pediococci. Sources, production, properties and applications. Microb Cell Fact 8:3. https://doi.org/10.1186/1475-2859-8-3

63. Cui Y, Zhang C, Wang Y, Shi J, Zhang L, Ding Z, Qu X, Cui H (2012) Class IIa bacteriocins: diversity and new developments. Int J Mol Sci 13:16668-16707. https://doi.org/10.3390/ijms131216668

64. Collins FWJ, O'Connor PM, O'Sullivan O, Gómez-Sala B, Rea MC, Hill C, Ross RP (2017) Bacteriocin Gene-Trait matching across the complete *Lactobacillus* Pan-genome. Sci Rep 7:3481. https://doi.org/10.1038/s41598-017-03339-y

65. Wang S, Yang B, Ross RP, Stanton C, Zhao J, Zhang H, Chen W (2020) Comparative genomics analysis of *Lactobacillus ruminis* from different niches. Genes (Basel) 11:70. https://doi.org/10.3390/genes11010070

66. Drider D, Fimland G, Héchard Y, McMullen LM, Prévost H (2006) The continuing story of class IIa bacteriocins. Microbiol Mol Biol Rev 70:564–582. https://doi.org/10.1128/MMBR.00016-05

67. Todorov SD, Cavicchioli VQ, Ananieva M, Bivolarski VP, Vasileva TA, Hinkov AV, Todorov DG, Shishkov S, Haertlé T, Iliev IN, Nero LA, Ivanova IV (2020) Expression of coagulin A with low cytotoxic activity by *Pediococcus pentosaceus* ST65ACC isolated from raw milk cheese. J Appl Microbiol 128:458–472. https://doi.org/10.1111/jam.14492

68. Le Marrec C, Hyronimus B, Bressollier P, Verneuil B, Urdaci MC (2000) Biochemical and genetic characterization of coagulin, a new antilisterial bacteriocin in the pediocin family of bacteriocins, produced by *Bacillus coagulans*

l (4). Appl Environ Microbiol 66:5213–5220. https://doi.org/10.1128/AEM.66.12.5213-5220.2000

69. Rodríguez JM, Martínez MI, Kok J (2002) Pediocin PA-1, a wide-spectrum bacteriocin from lactic acid bacteria. Crit Rev Food Sci Nutr 42:91–121. https://doi.org/10.1080/10408690290825475

70. Todorov SD, Holzapfel W, Nero LA (2016) Characterization of a novel bacteriocin produced by *Lactobacillus plantarum* ST8SH and some aspects of its mode of action. Ann Microbiol 66:949–962. https://doi.org/10.1007/s13213-015-1180-4

71. Porto MC, Kuniyoshi TM, Azevedo PO, Vitolo M, Oliveira RP (2017) *Pediococcus* spp.: An important genus of lactic acid bacteria and pediocin producers. Biotechnol Adv 35:361-374. https://doi.org/10.1016/j.biotechadv.2017.03.004.

72. Semjonovs P, Zikmanis P (2008) Evaluation of novel lactose-positive and exopolysaccharide-producing strain of *Pediococcus pentosaceus* for fermented foods. Eur Food Res Technol 227:851–856. https://doi.org/10.1007/s00217-007-0796-4

73. Guo T, Kong J, Zhang L, Zhang C, Hu S (2012) Fine tuning of the lactate and diacetyl production through promoter engineering in *Lactococcus lactis*. PLoS One 7:e36296. https://doi.org/10.1371/journal.pone.0036296

74. Pasteris SE, Strasser de Saad AM (2005) Aerobic glycerol catabolism by *Pediococcus pentosaceus* isolated from wine. Food Microbiol 22:399–407. https://doi.org/10.1016/j.fm.2004.10.001

75. Irmler S, Bavan T, Oberli A, Roetschi A, Badertscher R, Guggenbühl B, Berthoud H (2013) Catabolism of serine by *Pediococcus acidilactici* and *Pediococcus pentosaceus*. Appl Environ Microbiol 79:1309–1315. https://doi.org/10.1128/AEM.03085-12

76. Savijoki K, Ingmer H, Varmanen P (2006) Proteolytic systems of lactic acid bacteria. Appl Microbiol Biotechnol 71:394-406. https://doi.org/10.1007/s00253-006-0427-1

77. Ji D, Ma J, Xu M, Agyei D (2021) Cell-envelope proteinases from lactic acid bacteria: Biochemical features and biotechnological applications. Compr Rev Food Sci Food Saf 20:369–400. https://doi.org/10.1111/1541-4337.12676

78. Liu M, Bayjanov JR, Renckens B, Nauta A, Siezen RJ (2010) The proteolytic system of lactic acid bacteria revisited: a genomic comparison. BMC Genomics 11:36. https://doi.org/10.1186/1471-2164-11-36

79. Toropov V, Demyanova E, Shalaeva O, Sitkin S, Vakhitov T (2020) Whole-genome sequencing of *Lactobacillus helveticus* D75 and D76 confirms safety and probiotic potential. Microorganisms 8:329. https://doi.org/10.3390/microorganisms8030329

80. Ruiz A, Margolles B, Sánchez B (2013) Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. Front Microbiol 4:396. https://doi.org/10.3389/fmicb.2013.00396

81. Cotter PD, Hill C (2003) Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiol Mol Biol Rev 67:429–453. https://doi.org/10.1128/MMBR.67.3.429-453.2003

82. Sanchez B, Ruiz L, de los Reyes-Gavilan CG, Margolles A (2008) Proteomics of stress response in *Bifidobacterium*. Front Biosci 13:6905-6919. https://doi.org/10.2741/3198

83. Soni R, Nanjani S, Keharia H (2021) Genome analysis reveals probiotic propensities of *Paenibacillus polymyxa* HK4. Genomics 113:861-873. https://doi.org/10.1016/j.ygeno.2020.10.017

84. Piddock L (2006) Multidrug-resistance efflux pumps? not just for resistance. Nat Rev Microbiol 4:629–636. https://doi.org/10.1038/nrmicro1464

85. Pfeiler EA, Klaenhammer TR (2009) Role of transporter proteins in bile tolerance of *Lactobacillus acidophilus.* Appl Environ Microbiol 75:6013–6016. https://doi.org/10.1128/AEM.00495-09

86. Collado MC, Meriluoto J, Salminen S (2008) Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol 226:1065–1073. https://doi.org/10.1007/s00217-007-0632-x

87. Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A (2019) Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl Microbiol Biotechnol 103:6463–6472. https://doi.org/10.1007/s00253-019-09978-7

88. Haddaji N, Mahdhi AK, Krifi B, Ismail MB, Bakhrouf A (2015) Change in cell surface properties of *Lactobacillus casei* under heat shock treatment. FEMS Microbiol Lett 362. http://dx.doi.org/10.1093/femsle/fnv047

89. Salzillo M, Vastano V, Capri U, Muscariello L, Sacco M, Marasco R (2015) Identification and characterization of enolase as a collagen-binding protein in *Lactobacillus plantarum*. J Basic Microbiol 55:890–897. https://doi.org/10.1002/jobm.201400942

90. de Melo Pereira GV, de Oliveira Coelho B, Magalhães Júnior AI, Thomaz-Soccol V, Soccol CR (2018) Howtoselect a probiotic? A review and update of methods and criteria. Biotechnol Adv 36:2060–2076.

91. Canchaya C, Proux C, Fournous G, Bruttin A, Brüssow H (2003) Prophage genomics. Microbiol Mol Biol Rev 67:238-276. https://doi.org/10.1128/MMBR.67.2.238-276.2003. Erratum in: Microbiol Mol Biol Rev 67:473

92. Ferri M, Ranucci E, Romagnoli P, Giaccone V (2017) Antimicrobial resistance: a global emerging threat to public health systems. Crit Rev Food Sci Nutr 57:2857–2876. https://doi.org/10.1080/10408398.2015.1077192

93. Snauwaert I, Stragier P, De Vuyst L, Vandamme P (2015) Comparative genome analysis of *Pediococcus damnosus* LMG 28219, a strain well-adapted to the beer environment. BMC Genomics 16:267. https://doi.org/10.1186/s12864-015-1438-z

94. Surachat K, Kantachote D, Deachamag P, Wonglapsuwan M (2021) Genomic insight into *Pediococcus acidilactici* HN9, a potential probiotic strain isolated from the traditional thai-style fermented beef nhang. Microorganisms 9:50. https://doi.org/10.3390/microorganisms9010050

95. Westra E, Buckling A, Fineran P (2014) CRISPR–Cas systems: beyond adaptive immunity. Nat Rev Microbiol 12:317–326. https://doi.org/10.1038/nrmicro3241

96. Lander ES (2016) The heroes of CRISPR. Cell 164:18–28. https://doi.org/10.1016/j.cell.2015.12.041

97. Zhang Q, Ye Y (2017) Not all predicted CRISPR–Cas systems are equal: isolated *cas* genes and classes of CRISPR like elements. BMC Bioinformatics 18:92. https://doi.org/10.1186/s12859-017-1512-4

98. EFSA Panel on Biological Hazards (BIOHAZ) (2011) Scientific Opinion on risk based control of biogenic amine formation in fermented foods. EFSA J 9:2393. https://doi.org/10.2903/j.efsa.2011.2393

99. Chokesajjawatee N, Santiyanont P, Chantarasakha K, Kocharin K, Thammarongtham C, Lertampaiporn S, Vorapreeda T, Srisuk T, Wongsurawat T, Jenjaroenpun P, Nookaew I, Visessanguan W (2020) Safety assessment of a *Nham* starter culture *Lactobacillus plantarum* BCC9546 via Whole-genome analysis. Sci Rep 10:10241. https://doi.org/10.1038/s41598-020-66857-2

100. Mathur S, Singh R (2005) Antibiotic resistance in food lactic acid bacteria--a review. Int J Food Microbiol 105:281-295. https://doi.org/10.1016/j.ijfoodmicro.2005.03.008.

Supplementary Material

Supplementary Table 1 Genome information of *Pedicococcus pentosaceus* strains used for genetic analysis.

Strains	Source	Genome size (Mb)	GC (%)	CDS (n°)	Accession number[a]
P. pentosaceus ST65ACC	Raw-milk cheese	1.93	37.00	1,950	JAJHSK000000000
P.pentosaceus ATCC25745	Plant	1.83	37.40	1,814	NC_008525.1
P. pentosaceus SL4	Kimchi	1.79	37.30	1,784	NC_022780.1
P. pentosaceus GDIAS 001	Plant feed material	1.83	37.10	1,744	NZ_CP046938.1
P. pentosaceus wikim20	Kimchi	1.83	37.20	1,779	NZ_CP015918.1
P. pentosaceus SRCM100194	South Korea Food	1.87	37.38	1,725	NZ_CP021927.1
P. pentosaceus SRCM100892	South Korea Food	2.00	37.27	2,282	NZ_CP021474.1
P. pentosaceus SRCM102734	Soybean paste	1.71	37.40	1,595	NZ_CP028254.1
P. pentosaceus SRCM102736	Soybean paste	1.81	37.39	1,709	NZ_CP028259.1
P. pentosaceus SRCM102738	Soybean paste	1.87	37.41	1,749	NZ_CP028264.1
P. pentosaceus SRCM102739	Soybean paste	1.89	37.14	1,774	NZ_CP028268.1
P. pentosaceus SRCM102740	Soybean paste	1.87	37.41	1,749	NZ_CP028269.1
P. pentosaceus KCCM40703	Sake mash	1.76	37.20	1,664	NZ_CP020018.1
P. pentosaceus SS1-3	Korean adult feces	1.84	37.28	1,730	NZ_CP023008.1
P. pentosaceus JQI-7	Fermented dairy	1.73	37.20	1,654	NZ_CP023655.1

[a]NCBI accession number (GenBank).

Supplementary Table 2 Relatedness of the sequenced genomes of the *P. pentosaceus* ST65ACC to whole genome sequences of species type strains based on ANIb, ANIm, TETRA and GGDC.

Strains	**ANIb**	**ANIm**	**TETRA**	**GGDC**
ST65ACC	100.00	100.00	1.00000	100.00
P. pentosaceus ATCC25745	98.37	98.97	0.99770	90.70
P. pentosaceus GDIAS001	98.68	99.00	0.99827	91.40
P. pentosaceus JQI-7	99.27	99.71	0.99867	97.20
P. pentosaceus KCCM40703T	98.61	99.00	0.99806	91.40
P. pentosaceus SRCM100194	98.47	98.94	0.99860	90.60
P. pentosaceus SL4	98.51	98.93	0.99798	90.20
P. pentosaceus SRCM100892	98.19	98.76	0.99802	89.00
P. pentosaceus SRCM102734	98.37	99.01	0.99746	91.20
P. pentosaceus SRCM102736	99.31	99.71	0.99878	97.20
P. pentosaceus SRCM102738	98.43	98.94	0.99822	90.10
P. pentosaceus SRCM102739	98.41	98.94	0.99832	90.10
P. pentosaceus SRCM102740	98.43	98.94	0.99822	90.10
P. pentosaceus SS1-3	98.32	98.92	0.99814	90.10
P. pentosaceus WIKIM20	98.55	98.96	0.99821	91.10

Supplementary Table 3 Genes of *P. pentosaceus* ST65ACC that are encoding proteins, involved in sugar utilization, via pentose phosphate pathway, pyruvate metabolism and butanoate metabolism.

Gene	Enzyme (EC number)	KEGG-ID	ST65ACC gene ID (location)
Glycolysis / Embden-Meyerhof-Parnaspathway			
gapA	glyceraldehyde 3-phosphatedehydrogenase (EC:1.2.1.12)	K00143	PDHAGCLH_00144 (142449..143471)
pfkA	6-phosphofructokinase 1 (EC:2.7.1.11)	K00850	PDHAGCLH_01467 (2062..3030)
pyk	pyruvatekinase [EC:2.7.1.40]	K00873	PDHAGCLH_01468 (3103..4866)
pgk	phosphoglyceratekinase (EC:2.7.2.3)	K00927	PDHAGCLH_00143 (141124..142326)
fbaA	fructose-bisphosphate aldolase, class II (EC:4.1.2.13)	K01624	PDHAGCLH_01388 (15030..15893)
eno	enolase (EC:4.2.1.11)	K01689	PDHAGCLH_00086 (84726..86012), PDHAGCLH_00141 (138891..140213)
tpiA	triosephosphate isomerase (TIM) (EC:5.3.1.1)	K01803	PDHAGCLH_00142 (140289..141044)
pgi	glucose-6-phosphate isomerase (EC:5.3.1.9)	K01810	PDHAGCLH_01390 (17413..18762)
gpmA	2,3-bisphosphoglycerate-dependent phosphoglyceratemutase (EC:5.4.2.11)	K01834	PDHAGCLH_00489 (100686..101375)
pfkB	6-phosphofructokinase 2 (EC:2.7.1.11)	K16370	PDHAGCLH_01158 (30556..31491)
glk	glucokinase (EC:2.7.1.2)	K25026	PDHAGCLH_00898 (93047..94009)
Lactose utilization			
lacZ	beta-galactosidase (EC.3.2.1.23)	K01190	PDHAGCLH_01218
lacY	MFS transporter, OHS family lactose permease	K02532	PDHAGCLH_00716
lacI	LacI family transcriptional regulator	K02529	PDHAGCLH_00479, PDHAGCLH_01136, PDHAGCLH_01214, PDHAGCLH_01912
Leloir pathway - Galactose degradation			
galK	galactokinase (EC:2.7.1.6)	K00849	PDHAGCLH_01133 (2655..3827), PDHAGCLH_01219 (100075..101238)
galT	UDPglucose--hexose-1-phosphate uridylyltransferase (EC:2.7.7.12)	K00965	PDHAGCLH_01221 (102299..103759)
galE	UDP-glucose 4-epimerase (EC:5.1.3.2)	K01784	PDHAGCLH_01220 (101295..102299), PDHAGCLH_01756 (18377..19375)
galM	aldose 1-epimerase (EC:5.1.3.3)	K01785	PDHAGCLH_01215 (93595..94623),PDHAGCLH_01234 (114164..115168)
Pentose phosphate pathway			
pgd	6-phosphogluconate dehydrogenase (EC:1.1.1.44 1.1.1.343)	K00033	PDHAGCLH_00917 (112804..114222),

			PDHAGCLH_01378 (4495..5394)
g6pd, zwf	glucose-6-phosphate 1-dehydrogenase (EC:1.1.1.49 1.1.1.363)	K00036	PDHAGCLH_01929 (43675..45162)
pgl	6-phosphogluconolactonase (EC:3.1.1.31)	K07404	PDHAGCLH_01271 (10717..11727)
rpiA	ribose 5-phosphate isomerase A (EC:5.3.1.6)	K01807	PDHAGCLH_01787 (43507..44193)
hxlA	3-hexulose-6-phosphate synthase (EC:4.1.2.43)	K08093	PDHAGCLH_00081 (80571..81194)
hxlB	6-phospho-3-hexuloisomerase (EC:5.3.1.27)	K08094	PDHAGCLH_00080(80029 ..80571)
prsA	ribose-phosphatepyrophosphokinase (EC:2.7.6.1)	K00948	PDHAGCLH_00310 (314891..315871), PDHAGCLH_01300 (41895..42872)
Pyruvate metabolism			
adh	alcoholdehydrogenase (EC:1.1.1.1)	K00001	PDHAGCLH_00697 (315377..316420)
ldh	L-lactatedehydrogenase (EC:1.1.1.27)	K00016	PDHAGCLH_01725 (57316..58278)
adhC, frmA	S-(hydroxymethyl)glutathionedehydrogenase/alcoholdehydrogenase (EC:1.1.1.284 1.1.1.1)	K00121	PDHAGCLH_01195 (71785..72906)
poxB	pyruvatedehydrogenase (quinone) (EC:1.2.5.1)	K00156	PDHAGCLH_01914 (27974..29722)
poxL	pyruvate oxidase (EC:1.2.3.3)	K00158	PDHAGCLH_00077 (76484..78232), PDHAGCLH_00990 (184067..185800)
pdhA	pyruvatedehydrogenase E1 component alpha subunit (EC:1.2.4.1)	K00161	PDHAGCLH_00557 (166644..167762)
pdhB	pyruvatedehydrogenase E1 component beta subunit (EC:1.2.4.1)	K00162	PDHAGCLH_00556 (165661..166641)
frdA	fumaratereductaseflavoproteinsubunit (EC:1.3.5.4)	K00244	PDHAGCLH_00287 (289929..291317)
pdhD	dihydrolipoamidedehydrogenase (EC:1.8.1.4)	K00382	PDHAGCLH_00554 (162946..164352)
pta	phosphateacetyltransferase (EC:2.3.1.8)	K00625	PDHAGCLH_00267 (269591..270562)
pdhC	pyruvatedehydrogenase E2 component(dihydrolipoamideacetyltransferase) (EC:2.3.1.12)	K00627	PDHAGCLH_00555 (164355..165668)
pyk	pyruvatekinase (EC:2.7.1.40)	K00873	PDHAGCLH_01468 (3103..4866)
ackA	acetatekinase (EC:2.7.2.1)	K00925	PDHAGCLH_00268 (270566..271765), PDHAGCLH_00320 (325954..327141), PDHAGCLH_00606 (221827..223014)
acyP	acylphosphatase (EC:3.6.1.7)	K01512	PDHAGCLH_00913 (108842..109114)
fumC	fumarate hydratase, class II (EC:4.2.1.2)	K01679	PDHAGCLH_01222 (103951..105333)
pyc	pyruvatecarboxylase (EC:6.4.1.1)	K01958	PDHAGCLH_00383 (382812..386237)
accC	acetyl-CoAcarboxylase, biotincarboxylasesubunit (EC:6.4.1.2 6.3.4.14)	K01961	PDHAGCLH_00832 (36839..38206)

accA	acetyl-CoAcarboxylasecarboxyl transferase subunit alpha (EC:6.4.1.2 2.1.3.15)	K01962	PDHAGCLH_00830 (35238..36005)
accD	acetyl-CoAcarboxylasecarboxyl transferase subunitbeta (EC:6.4.1.2 2.1.3.15)	K01963	PDHAGCLH_00831 (36019..36849)
accB	acetyl-CoAcarboxylasebiotincarboxylcarrierprotein	K02160	PDHAGCLH_00834 (38637..39077)
ldhA	D-lactatedehydrogenase(EC:1.1.1.28)	K03778	PDHAGCLH_01114 (124712..125707)
yiaY	alcoholdehydrogenase (EC:1.1.1.1)	K13954	PDHAGCLH_00358 (358557..359726)
mleA, mleS	malolacticenzyme (EC:4.1.1.101)	K22212	PDHAGCLH_00398 (8352..9980)
larA	lactateracemase (EC:5.1.2.1)	K22373	PDHAGCLH_00473 (82671..83945)
yvgN	methylglyoxal/glyoxalreductase (EC:1.1.1.283 1.1.1.-)	K23257	PDHAGCLH_00656 (276684..277316)
Butanoate metabolism			
alsD	α-acetolactatedecarboxylase (EC:4.1.1.5)	K01575	PDHAGCLH_00196 (201197..201901)
als	acetolactate synthase I/II/III large subunit (EC:2.2.1.6)	K01652	PDHAGCLH_00197 (201913..203589)
budC, butA	meso-butanedioldehydrogenase/(S,S)-butanedioldehydrogenase/diacetylreductase (EC:1.1.1.- 1.1.1.76 1.1.1.304)	K03366	PDHAGCLH_00348 (349251..350030)

Supplementary Table 4 Genes of *P. pentosaceus* ST65ACC that are encoding proteins involved in proteolytic system and amino acid conversion pathways.

Gene	Enzyme (EC number)	KEGG-ID	ST65ACC gene ID (location)
glnH, glnP/glnM, metQIN, trp/tyr	amino acid transporter	K10036/K10039, K10040, K17077, K02073/K02072/K02071, K01989/K05832/K05833	PDHAGCLH_01492 (25770..27221)/PDHAGCLH_00212 (219147..219983), PDHAGCLH_00210 ((217844..218503))/PDHAGCLH_00211 (218500..219150), PDHAGCLH_01331 (72185..73651), PDHAGCLH_01430 (53260..54075)/PDHAGCLH_01429 (52559..53242)/PDHAGCLH_01428 (51532..52569), PDHAGCLH_00355 (355703..356698)/ PDHAGCLH_00356 (356695..357606)/PDHAGCLH_00357 (357608..358366)
oppABCDF	oligopeptide transporter	K15580, K15581, K15582, K15583, K10823	PDHAGCLH_00206 (213284..214906), PDHAGCLH_01695 (28617..30257), PDHAGCLH_00205 (212265..213194), PDHAGCLH_00204 (211235..212260), PDHAGCLH_00203 (210147..211220), PDHAGCLH_00202 (209187..210134)
pepN, pepT, map, pepS	amimopeptidases (EC:3.4.11.2), (EC:3.4.11.4), (EC:3.4.11.18), (EC:3.4.11.-)	K01256, K01258, K01265, K19689	PDHAGCLH_00185 (188197..190731), PDHAGCLH_00437 (56766..58007), PDHAGCLH_00227 (234066..234848), PDHAGCLH_00019 (19719..20957)
pepQ	dipeptidases (EC:3.4.13.9)	K01271	PDHAGCLH_01913 (26823..27917)
pepX	dipeptidyl-peptidases and tripeptidyl-peptidases (EC:3.4.14.11)	K01281	PDHAGCLH_00222 (227452..229890)
mrcA, dacA, pbp2A, pbp4b	serine-type carboxypeptidases (EC:2.4.1.129 3.4.16.4), (EC:3.4.16.4)	K05366 K07258 K12555 K21469	PDHAGCLH_01045 (54557..56866), PDHAGCLH_00500 (110541..111797), PDHAGCLH_00958 (151502..153655), PDHAGCLH_01752 (14890..15867)
lexA, lepB, clpP, ctpA, gluP, degP	Serine endopeptidases(EC:3.4.21.88), (EC:3.4.21.89), (EC:3.4.21.92), (EC:3.4.21.102), (EC:3.4.21.105), (EC:3.4.21.107)	K01356, K03100, K01358, K03797, K19225, K04771	PDHAGCLH_01120 (128256..128885), PDHAGCLH_00625 (244252..244830), PDHAGCLH_00148 (146454..147047), PDHAGCLH_01498 (30854..32272), PDHAGCLH_00900 (94272..94961), PDHAGCLH_00577 (189292..190542)
pepC, srtA, comA	Cysteine endopeptidases (EC:3.4.22.40), (EC:3.4.22.70),(EC:3.4.22.-)	K01372, K07284, K12292	PDHAGCLH_01788 (44263..45609), PDHAGCLH_01713 (45924..46613), PDHAGCLH_01971 (8653..10827)
lspA, comC	Aspartic endopeptidases (EC:3.4.23.36), (EC:3.4.23.43 2.1.1.-)	K03101, K02236	PDHAGCLH_01037 (47562..48014), PDHAGCLH_01641 (45093..45773)
ftsH, htpX, pepO, pepF, rseP	Metallo endopeptidases (EC:3.4.24.-)	K03798, K03799, K07386, K0862, K11749	PDHAGCLH_01734 (67539..69620), PDHAGCLH_01715 (47301..48197), PDHAGCLH_00303 (307336..309249), PDHAGCLH_00414 (30027..31826), PDHAGCLH_01107 (118256..119518)
hslV	threonine endopeptidases (EC:3.4.25.2)	K01419	PDHAGCLH_01014 (22578..23132)
pepDA, pedDB, cwlO	peptidases (EC:3.4.-.-)	K08659, K21471	PDHAGCLH_01673 (76204..77610), PDHAGCLH_01305 (48091..49581)

Supplementary Table 5 Genes of *P. pentosaceus* ST65ACC that are encoding stress resistance and surface adhesive proteins.

Gene	Enzyme (EC number)	KEGG-ID	ST65ACC gene ID (location)
atpF1A, atpA	F-type H+/Na+-transporting ATPase subunit alpha (EC:7.1.2.2 7.2.2.1)	K02111	PDHAGCLH_01420 (44732..46249)
atpF1B, atpD	F-type H+/Na+-transporting ATPase subunit beta (EC:7.1.2.2 7.2.2.1)	K02112	PDHAGCLH_01422 (47232..48641)
atpF1G, atpG	F-type H+-transporting ATPase subunit gamma	K02115	PDHAGCLH_01421 (46287..47207)
atpF1D, atpH	F-type H+-transporting ATPase subunit delta	K02113	PDHAGCLH_01419 (44160..44702)
atpF1E, atpC	F-type H+-transporting ATPase subunit epsilon	K02114	PDHAGCLH_01423 (48653..49072)
atpF0A, atpB	F-type H+-transporting ATPase subunit a	K02108	PDHAGCLH_01416 (42636..43352)
atpF0B, atpF	F-type H+-transporting ATPase subunit b	K02109	PDHAGCLH_01418 (43642..44163)
nhaC	Na+:H+ antiporter, NhaCfamily	K03315	PDHAGCLH_00622 (240208..241575)
nhaK	Monovalent cation/hydrogen antiporter	K24163	PDHAGCLH_00238 (244851..246827), PDHAGCLH_00594 (207422..209521)
recA	Recombination protein RecA	K03553	PDHAGCLH_01463 (87025..88083)
arcA	Arginine deiminase (EC:3.5.3.6)	K01478	PDHAGCLH_00410 (25429..26652)
arcC	carbamate kinase (EC:2.7.2.2)	K00926	PDHAGCLH_00411 (26705..27634)
arcD, lysI,lysP	arginine:ornithine antiporter / lysine permease	K03758	PDHAGCLH_00408 (22331..23752)
hsp20	HSP20 family protein	K13993	PDHAGCLH_00424 (40792..41214)
hslO	molecular chaperone Hsp33	K04083	PDHAGCLH_01735 (69643..70557)
groEL	Chaperonin GroEL	K04077	PDHAGCLH_00181 (184435..186054)
grpE	molecular chaperone GrpE	K03687	PDHAGCLH_01095 (102940..103509)
groES	Chaperonin GroES	K04078	PDHAGCLH_00182 (186082..186366)
dnaK	molecular chaperone DnaK	K04043	PDHAGCLH_01094 (101055..102914)
dnaJ	molecular chaperone DnaJ	K03686	PDHAGCLH_01093 (99834..100958)
clpP	ATP-dependent Clp protease, protease subunit (EC:3.4.21.92)	K01358	PDHAGCLH_00148 (146454..147047)
clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	K03544	PDHAGCLH_01327 (69574..70830)
clpE	ATP-dependent Clp protease ATP-binding subunit ClpE	K03697	PDHAGCLH_00008 (7273..9492)
clpL	ATP-dependent Clp protease ATP-binding subunit ClpL	K04086	PDHAGCLH_01004 (8724..10838)
clpC	ATP-dependent Clp protease ATP-binding subunit ClpC	K03696	PDHAGCLH_01646 (54420..56885)

hslV, clpQ	ATP-dependent HslUV protease, peptidase subunit HslV (EC:3.4.25.2)	K01419	PDHAGCLH_01014 (22578..23132)
srtA	sortase A (EC:3.4.22.70)	K07284	PDHAGCLH_01713 (45924..46613)
ppiB	peptidyl-prolyl cis-trans isomerase B (cyclophilin B) (EC:5.2.1.8)	K03768	PDHAGCLH_01224 (105601..106185)
ltaS	lipoteichoic acid synthase (EC:2.7.8.20)	K19005	PDHAGCLH_00111 (109581..110111), PDHAGCLH_00235 (240445..242601)
eno	enolase (EC:4.2.1.11)	K01689	PDHAGCLH_00086 (84726..86012), PDHAGCLH_00141 (138891..140213)
gapA	glyceraldehyde 3-phosphate dehydrogenase (EC:1.2.1.12)	K00134	PDHAGCLH_00144 (142449..143471)
tuf	elongation factor Tu	K02358	PDHAGCLH_01325 (66799..67986)
tsf	elongation factor Ts	K02357	PDHAGCLH_01112 (122784..123662)
fusA	elongation factor G	K02355	PDHAGCLH_01638 (41702..43795)
talA, talB	transaldolase (EC:2.2.1.2)	K00616	PDHAGCLH_01787 (43507..44193)

Supplementary Table 6 Antimicrobial resistance genes and their locations in the *P. pentosaceus* ST65ACC genome.

N°	Resistance	Gene Name	KEGG-ID	ST65ACC gene ID (location)
1	Macrolideresistance	*lsa*; lincosamide and streptogramin A transport system ATP-binding/permease protein	K19350	PDHAGCLH_00647 (268698..270182)
2	beta-Lactam resistance	*penP*; beta-lactamaseclass A (EC:3.5.2.6)	K17836	PDHAGCLH_00687 (301638..302813)
3	CAMP resistance	*dltA*; D-alanine--poly(phosphoribitol) ligase subunit 1 (EC:6.1.1.13)	K03367	PDHAGCLH_01689 (21044..22570)
4	CAMP resistance	*dltB*; membrane protein involved in D-alanine export	K03739	PDHAGCLH_01690 (22570..23763)
5	CAMP resistance	*dltC*; D-alanine--poly(phosphoribitol) ligase subunit 2 (EC:6.1.1.13)	K14188	PDHAGCLH_01691 (23786..24019)
6	CAMP resistance	*dltD*; D-alanine transfer protein	K03740	PDHAGCLH_01692 (24022..25308)
7	Multi drug resistance	*abcA*, *bmrA*; ATP-binding cassette, subfamily B (EC:7.6.2.2)	K18104	PDHAGCLH_00439 (59193..60971)

Annex 1

Probiotics and Antimicrobial Proteins (2023) 15:630–645
https://doi.org/10.1007/s12602-021-09894-1

Genomic Analyses of *Pediococcus pentosaceus* ST65ACC, a Bacteriocinogenic Strain Isolated from Artisanal Raw-Milk Cheese

Francielly Soares Oliveira[1,2] · Rafaela da Silva Rodrigues[1,2] · Antônio Fernandes de Carvalho[2] · Luís Augusto Nero[1]

Accepted: 16 December 2021 / Published online: 4 January 2022

Abstract
Pediococcus pentosaceus ST65ACC was obtained from a Brazilian artisanal cheese (BAC) and characterized as bacteriocinogenic. This strain presented beneficial properties in previous studies, indicating its potential as a probiotic candidate. In this study, we aimed to carry out a genetic characterization based on whole-genome sequencing (WGS), including taxonomy, biotechnological properties, bacteriocin clusters and safety-related genes. WGS was performed using the Illumina MiSeq platform and the genome was annotated with the Prokaryotic Genome Annotation (Prokka). *P. pentosaceus* ST65ACC taxonomy was investigated and bacteriocin genes clusters were identified by BAGEL4, metabolic pathways were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) and safety-related genes were checked. *P. pentosaceus* ST65ACC had a total draft genome size of 1,933,194 bp with a GC content of 37.00%, and encoded 1950 protein coding sequences (CDSs), 6 rRNA, 55 tRNA, 1 tmRNA and no plasmids were detected. The analysis revealed absence of a CRISPR/Cas system, bacteriocin gene clusters for pediocin PA-1/AcH and penocin-A were identified. Genes related to beneficial properties, such as stress adaptation genes and adhesion genes, were identified. Furthermore, genes related to biogenic amines and virulence-related genes were not detected. Genes related to antibiotic resistance were identified, but not in prophage regions. Based on the obtained results, the beneficial potential of *P. pentosaceus* ST65ACC was confirmed, allowing its characterization as a potential probiotic candidate.

Keywords *Pediococcus pentosaceus* · Whole-genome sequencing · Bacteriocin · Comparative genomics

Introduction

Pediococcus pentosaceus ST65ACC was isolated from a Brazilian artisanal cheese (BAC) and characterized as bacteriocinogenic in a previous study carried out by Cavicchioli et al. [1]. Many strains of *P. pentosaceus* are described as producing pediocin PA-1/AcH [2–4] and may also harbor operons of other bacteriocins of the class IIa, such as penicocin-A and enterolysin A, class III [3, 5]. Class IIa bacteriocins are particularly attractive because they have an efficient bactericidal effect against *Listeria monocytogenes* [6–8] and can inhibit other foodborne pathogenic such as *Clostridium botulinum* [9], *Clostridium perfringens* [10], and *Staphylococcus aureus* [11], making them potentially useful for use as bio-preservatives.

A previous study also demonstrated that the *P. pentosaceus* strain ST65ACC has desired beneficial properties, such as resistance to simulated gastrointestinal conditions, auto-aggregation capacity and aggregation with *L. monocytogenes* [12], which may facilitate the elimination of this pathogen and adhesion to intestinal mucosa. Also, some *P. pentosaceus* strains have been associated with anti-inflammatory capacity, antioxidant properties and lipid-lowering effect [13–16]. *P. pentosaceus* strains and their bacteriocins are being increasingly considered as supplements in the food industry and in the gut health [17].

Advances in genome sequencing technologies and the increasing availability of tools for sequence analysis have enabled the genomic characterization of microorganisms and has become mandatory for defining the exact taxonomy of new strains and for evaluating all technological/beneficial

✉ Luís Augusto Nero
nero@ufv.br

[1] InsPOA - Laboratório de Inspeção de Produtos de Origem Animal, Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, MG 36570 900, Brazil

[2] Inovaleite - Laboratório de Pesquisa Em Leite E Derivados, Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Viçosa, MG 36570 900, Brazil

CHAPTER 3: Influence of different culture media on the antimicrobial activity of *Pediococcus pentosaceus* ST65ACC against *Listeria monocytogenes*

Francielly Soares Oliveira et al.

Manuscript prepared for submission to BMC Microbiology

Titlepage

Francielly Soares Oliveira[1,2], Rafaela da Silva Rodrigues[1,2], Valéria Quintana Cavicchioli[3], Antônio Fernandes de Carvalho[2], Luís Augusto Nero[1*]

Influence of different culture media on the antimicrobial activity of *Pediococcus pentosaceus* ST65ACC against *Listeria monocytogenes*

[1]InsPOA – Laboratório de Inspeção de Produtos de Origem Animal, Departamento de Veterinária, Universidade Federal de Viçosa, 36570 900, Viçosa MG, Brazil

[2]InovaLeite - Laboratório de Pesquisa em Leites e Derivados, Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, 36570 900, Viçosa MG, Brazil

[3]Centro de Pesquisa em Alimentos, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, 74690 900, Goiânia, GO, Brazil

*Corresponding author: Luís Augusto Nero (nero@ufv.br)

Abstract

Pediococcus pentosaceus ST65ACC is a bacteriocinogenic lactic acid bacteria (LAB) isolated from Brazilian artisanal cheese, capable of inhibiting different food pathogens, mainly *L. monocytogenes*. The production of bacteriocins can be influenced by several growth conditions, such as temperature, pH and medium composition. This study aimed to evaluate the effect of different culture media on the production of bacteriocins and antimicrobial activity of *P. pentosaceus* ST65ACC on *L. monocytogenes* Scott A. The strains were inoculated alone and in co-culture in four different media: MRS broth, BHI broth, reconstituted skim milk, and meat broth.The broths were then incubated at 37 °C for 96 h, and count analysis, pH measurement, and bacteriocin production were performed at the times 0, 24, 48, 72 and 96 h. *L. monocytogenes* was inhibited to non-detectable levels in co-culture with *P. pentosaceus* ST65ACC in MRS broth within 96h, consistent with the high production of bacteriocin throughout the analysis period (3,200 – 12,800 AU/ml). However, lower inhibitory activities of *P. pentosaceus* ST65ACC on *L. monocytogenes* Scott A were recorded in BHI, milk and meat broth, with low or no production of bacteriocins at the analyzed times. The composition of these culture media may have repressed the production and the activity of bacteriocins and, consequently, the antagonist activity of *P. pentosaceus* ST65ACC on *L. monocytogenes* Scott A. The results showed that the antimicrobial activity was more effective in MRS broth, presenting greater production of bacteriocins and less variability when compared to the other media analyzed.

Keywords: *Pediococcus pentosaceus,* bacteriocin, pediocin, antimicrobial activity, *Listeria monocytogenes*

Introduction

Bacteriocins are antimicrobial proteins or peptides ribosomally encoded by different bacterial species, capable of controlling the growth of pathogens and spoilage bacteria. Bacteriocins synthesized by lactic acid bacteria (LAB) have great potential as biopreservative agents in food (Papagianni, 2003; Cotter et al., 2013: Garsa et al., 2014). Pediocins, class IIa of the bacteriocin group, produced by *Pediococcus* strains (Papagianni, 2003; Deegan et al., 2006), are extensively studied and characterized by their bactericidal activity at low concentrations against different pathogenic bacteria, including *Listeria monocytogenes*, *Clostridium perfringens* and *Staphylococcus aureus* (Nieto-Lozano et al., 2010; Bédard et al., 2018; Ghosh et al., 2019; Khorshidian et al., 2021). In addition, they are thermotolerant, maintain their activity in a wide pH range and their use as a food preservative is allowed in some countries (Papagianni & Anastasiadou, 2009).

Pediococcus pentosaceus ST65ACC was isolated from Brazilian artisanal cheese (Cavicchioli et al., 2017), presenting bactericidal and/or bacteriostatic action against different pathogens, mainly *L. monocytogenes* (Cavicchioli et al., 2017; 2019), and can produce the bacteriocins pediocin PA-1 and penocin A (Oliveira et al., 2023).In a previous study carried out by Todorov et al. (2019), *P. pentosaceus* ST65ACC was able to grow in MRS supplemented with xylo-oligosaccharide (XOS) as the sole carbon source and produce bacteriocins, but at lower levels than in MRS broth.

Optimal bacterial growth and bacteriocin production is strongly dependent on severalfactors such as medium composition, pH, temperature, incubation time, carbon and nitrogen sources (Todorov et al., 2012; Zhang et al. 2012), and are specific for each producing strain (Kaur & Tiwari, 2017). Thus, for the application of a

bacteriocinogenic strain in food, studies that evaluate the effective production of its bacteriocins are very important, since the composition of the medium can interfere with its production and/or activity, and the synthesis of low concentrations may be insufficient to inhibit pathogenic and spoilagebacteria (Gänzle et al. 1999; Zhang et al. 2012; Perin et al., 2015).

The present study aimed to evaluate the effect of different culture media on bacteriocin production and antimicrobial activity of *P. pentosaceus* ST65ACC on *L. monocytogenes* Scott A.

Material and methods

Bacterial strains and culture conditions

P. pentosaceus ST65ACC was originally isolated from artisanal raw milk cheese, characterized as bacteriocinogenic (Cavicchioli et al., 2017) and through whole-genome sequencing the presence of the operons of the bacteriocins pediocin PA-1 and penocin-A was identified (Oliveira et al., 2023). *P. pentosaceus* MLEV8 (Colombo et al., 2018) was selected as a non-bacteriocinogenic strain and used as a negative control. *L. monocytogenes* Scott A was used as the target. The strains were kept in Tryptone Soya Broth (TSB, Oxoid Ltd., Basingstoke, UK) for *L. monocytogenes* and Man, Rogosa and Sharpe broth (MRS, Oxoid) for lactic acid bacteria (LAB) with 20 % (v/v) glycerol and stored at -20 °C. At the time of use, the strains were recovered in their respective culture media, incubated at 37 °C for 24h and, subsequently, the cultures were diluted in NaCl 0.85 % (w/v) at a turbidity similar to the McFarland scale 0.5, which corresponds approximately to $1.5x10^8$ colony-forming units per milliliter (CFU/ml).

P. pentosaceus ST65ACC and *L. monocytogenes* interaction

The antimicrobial activity of *P. pentosaceus* ST65ACC against *L. monocytogenes* Scott A was performed in meat broth (Freney et al., 1999), brain heart infusion (BHI) broth (Oxoid), MRS broth (Oxoid) and reconstituted skim milk (10 %, w/v, Molico, Nestlé Brasil Ltd., São Paulo, SP, Brazil) in different treatments (T): T1, only *P. pentosaceus* ST65ACC (10^6 CFU/ml); T2, only *P. pentosaceus* MLEV8 (non-bacteriocinogenic strain); T3, co-culture of ST65ACC (10^6 CFU/ml) with *L. monocytogenes* Scott A (10^3 CFU/ml); T4, co-culture of MLEV8 (10^6 CFU/ml) with *L. monocytogenes* Scott A (10^3 CFU/ml); T5, only *L. monocytogenes* Scott A (10^3 CFU/ml); Control, without strain inoculation. Initially, 250 ml of each broth were prepared, sterilized and the LAB strains and *L. monocytogenes* Scott A were inoculated alone and in co-culture in the broths, incubated at 37 °C for 96 h. The selectionofthe media incubation temperature was based on previous results obtained by Cavicchioli et al., (2017), in which bacteriocin production was higher at 37 °C. Every 24 h (0, 24, 48, 72 and 96 h) count analysis, pH measurement, and antimicrobial activity were performed. The experiment was conducted in three independent repetitions.

Bacterial count and pH measurement

For enumeration of populations, aliquots of 25 ml of each treatment were obtained under sterile conditions and serial dilutions were performed in saline solution (NaCl 0.85 %, w/v). LAB strains were enumerated on MRS agar (Oxoid, Frank; Yousef, 2004) and *L. monocytogenes* Scott A on Oxford agar (Oxoid, ISO, 2017), both by surface plating, in triplicate, and incubated at 37 °C for 24 h. All results

obtained were expressed in log CFU/ml. The pH of the samples was determined with a FiveEasy™ bench type 20 pH meter (Mettler Toledo, Greifensee, Switzerland).

Antimicrobial activity

The antimicrobial activity of *P. pentosaceus* strains against *L. monocytogenes* Scott A was determined by the critical dilution technique (Todorov et al., 2008), with modifications. Samples of 5 ml of all treatments, at the times of analysis were collected and cell-free supernatants (CFS) were obtained. The samples were centrifuged at 6,800 × *g* for 20 min at 4 °C and then the pH of the supernatants was adjusted to 6.5 with 1M NaOH and heated at 80 °C for 10 min. Subsequently, the CFS were filter sterilized (0.22 µm pore size filter units, Merck Millipore Ltd., Cork, Ireland) and the supernatants obtained were subjected to the critical dilution method in 10 mM phosphate buffered saline (PBS) pH 6.5. Aliquots of 10 µl were applied to the surface of TSA agar (Oxoid) containing 10^6 CFU/ml of *L. monocytogenes* Scott A and the plates were incubated at 37 °C for 24 h. The antimicrobial activity was expressed in arbitrary units per milliliter (AU/ml), calculated as 2^n x 100, where “2” corresponds to the dilution factor and “n” corresponds to the last dilution that produced an inhibition zone greater than 2 mm of diameter (Cavicchioli et al., 2015).

Data analysis

The experiment was carried out in three replications and data were expressed as averages ± standard deviation.

Results

Figure 1 shows populations of *P. pentosaceus* ST65ACC and *P. pentosaceus* MLEV8 inoculated alone and in co-culture with *L. monocytogenes* Scott A in MRS broth, BHI broth, reconstituted skim milk and meat broth at 37 °C for 96 h. In all culture media it was possible to observe an exponential increase in the population of *P. pentosaceus* ST65ACC and *P. pentosaceus* MLEV8 between 0 and 24 hours, reaching values from 7.84 to 9.62 log CFU/ml and remaining constant or with a slight decline in the 96 h of analysis. The strains showed similar growth in all treatments, except when grown in meat broth. The greatest population increase of 9.62 log CFU/ml was obtained in the MRS broth, with a greater reduction in the pH of the medium also being observed (Table 1).

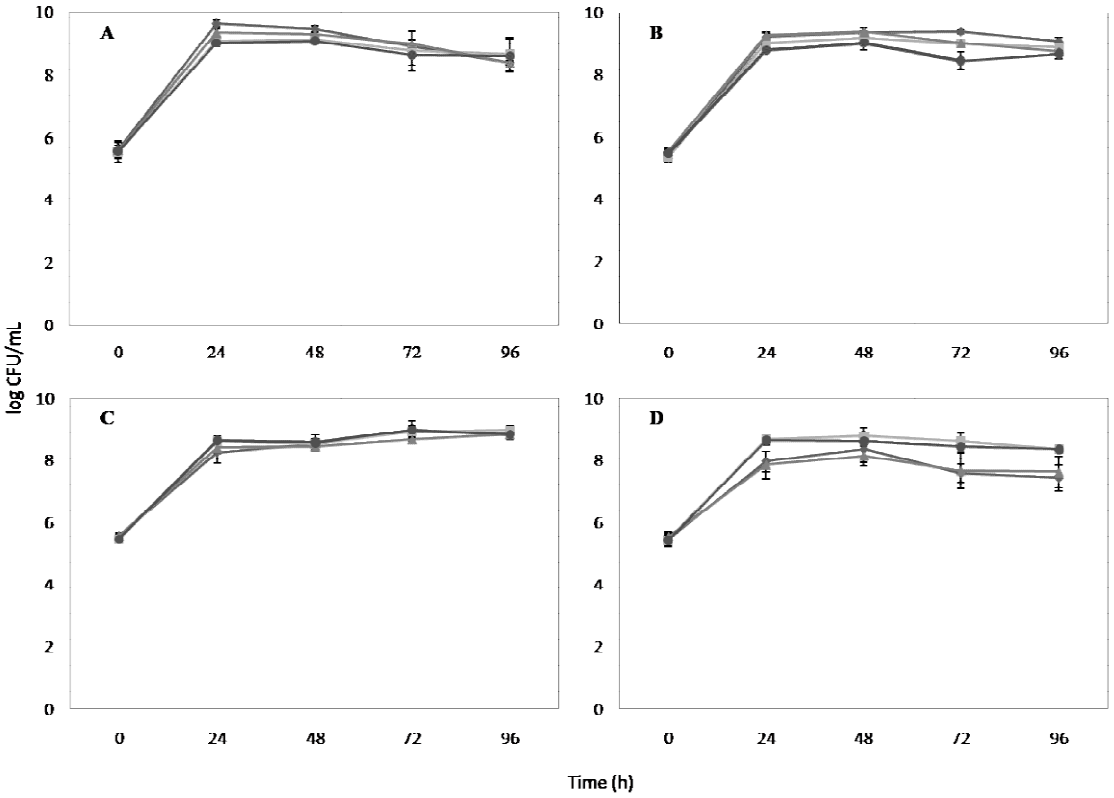

Fig. 1. Mean counts (± standard error) of *P. pentosaceus* ST65ACC and *P. pentosaceus* MLEV8 inoculated in MRS broth (A), BHI broth (B), reconstituted skim milk (C) and meat broth (D) (♦:

ST65ACC alone; ■: MLEV8 alone; ▲: ST65ACC in co-culture with *L. monocytogenes* Scott A; ●: MLEV8 in co-culture with *L. monocytogenes* Scott A).

Table 1. Average pH values obtained during bacterial growth in MRS broth, BHI broth, reconstituted skim milk and meat broth.

Culture mediums	Time (h)	T1	T2	T3	T4	T5	Control
	0	6.06±0.09	6.06±0.09	6.06±0.09	6.06±0.24	6.06±0.09	6.06±0.09
	24	3.79±0.12	4.18±0.36	3.76±0.10	4.18±0.36	5.61±0.28	6.04±0.08
MRS broth	48	3.67±0.07	4.02±0.30	3.66±0.08	4.07±0.33	4.76±0.40	6.02±0.07
	72	3.62±0.06	3.99±0.25	3.63±0.04	4.08±0.26	4.59±0.41	6.06±0.11
	96	3.61±0.03	3.94±0.26	3.61±0.03	4.03±0.29	4.48±0.42	5.96±0.12
	0	7.47±0.08	7.47±0.08	7.47±0.08	7.47±0.08	7.47±0.08	7.47±0.08
	24	6.20±0.07	5.51±0.12	5.84±0.24	5.58±0.05	5.41±0.11	7.47±0.06
BHI broth	48	6.22±0.06	5.54±0.13	5.83±0.18	5.51±0.13	5.38±0.13	7.41±0.06
	72	6.23±0.03	5.51±0.13	5.79±0.17	5.50±0.12	5.38±0.13	7.40±0.07
	96	6.21±0.08	5.69±0.30	5.84±0.21	5.57±0.01	5.49±0.10	7.38±0.09
	0	7.21±0.08	7.21±0.08	7,21±0.08	7.21±0.08	7.21±0.08	7.21±0.08
	24	6.50±0.35	6.57±0.06	6,52±0.19	6.57±0.13	6.82±0.17	6.92±0.28
Milk	48	5.60±0.34	5.93±0.22	6,03±0.31	5.82±0.57	5.98±0.44	6.70±0.62
	72	5.31±0.09	5.21±0.34	5,38±0.52	5.37±0.30	5.63±0.61	6.70±0.62
	96	5.13±0.02	5.03±0.37	5.17±0.19	4.92±0.38	5.60±0.85	6.50±0.50
	0	6.32±0.21	6.32±0.21	6.32±0.21	6.32±0.21	6.32±0.21	6.32±0.21
	24	6.02±0.15	6.01±0.24	6.04±0.08	6.03±0.30	5.58±0.40	6.08±0.20
Meat broth	48	6.15±0.06	6.00±0.18	6.08±0.12	6.00±0.20	5.36±0.38	6.03±0.27
	72	6.02±0.16	6.21±0.54	6.09±0.14	6.05±0.27	5.40±0.41	6.03±0.28
	96	6.16±0.07	6.07±0.15	6.08±0.12	6.08±0.26	5.43±0.53	6.00±0.22

T1: *P. pentosaceus* ST65ACC (Lac +); T2: *P. pentosaceus* MLEV8 (Lac -); T3: *P. pentosaceus* ST65ACC with *L. monocytogenes* Scott A; T4: *P. pentosaceus* MLEV8 with *L. monocytogenes* Scott A; T5: *L. monocytogenes* Scott A alone.

Regarding the antimicrobial activity of *P. pentosaceus* ST65ACC and *P. pentosaceus* MLEV8 against *L. monocytogenes* Scott A in MRS broth, BHI broth, reconstituted skim milk and meat broth at 37 °C for 96 h, populations of *L. monocytogenes* Scott A inoculated alone and in co-culture are shown in Figure 2.

L. monocytogenes Scott A population was inhibited to non-detectable levels in co-culture with *P. pentosaceus* ST65ACC and *P. pentosaceus* MLEV8 in MRS broth within 96 h (Figure 2). Inhibition of *L. monocytogenes* Scott A by the *P. pentosaceus* ST65ACC strain is consistent with high bacteriocin production throughout the analysis period, in all replicates of the experiment, with values ranging from 3,200 and 12,800 AU/ml (data not shown). However, *P. pentosaceus* MLEV8 (non-bacteriocinogenic strain) also inhibited the population of *L. monocytogenes* Scott A in MRS broth, a result that can be attributed to other antimicrobial compounds produced by the strain, such as organic acids, which can also be observed by the low pH values from the 24 h analysis time (Table 1).

In the BHI broth, a similar behavior was verified in populations of *L. monocytogenes* Scott A cultivated alone and in co-culture with *P. pentosaceus* ST65ACC (treatments 3 and 5), with no decrease in the population during the period of analysis. *L. monocytogenes* Scott A counts in BHI broth were lower when co-cultured with *P. pentosaceus* MLEV8 (non-bacteriocinogenic strain). The production of bacteriocins by *P. pentosaceus* in BHI broth was not constant between repetitions during the analysis period, with the production of 100 AU/ml being observed in the first repetition only within 24 hours (data not shown). In the other repetitions, bacteriocin production was verified at times of 24, 48 and 72 h, with values varying between 100 and 200 AU/ml. In milk, populations of *L. monocytogenes* Scott A showed similar counts when inoculated alone and in co-culture with *P. pentosaceus*

ST65ACC at 24 and 48 h, with a reduction of only 1 log cycle at 72 h and 96 h (in co-culture with *P. pentosaceus* ST65ACC). The production of bacteriocins by the strain *P. pentosaceus* ST65ACC was verified in only one of the experiment repetitions, with the values of 200 AU/ml at 24 h and 100 AU/ml in the other periods of analysis. *L. monocytogenes* Scott A counts in co-culture with *P. pentosaceus* MLEV8 in milk increased exponentially up to 24 h and remained constant throughout the analysis period. In meat broth, *L. monocytogenes* Scott A showed similar counts in co-culture with *P. pentosaceus* ST65ACC and *P. pentosaceus* MLEV8 (treatment 3 and 4), with a reduction of 2 log cycles in the *L. monocytogene*s population with in 24 h. However, the counts increased at 48 and 72 h, equaling the strain inoculated alone at 96 h, with no bacteriocin production being observed at any of the analysis times.

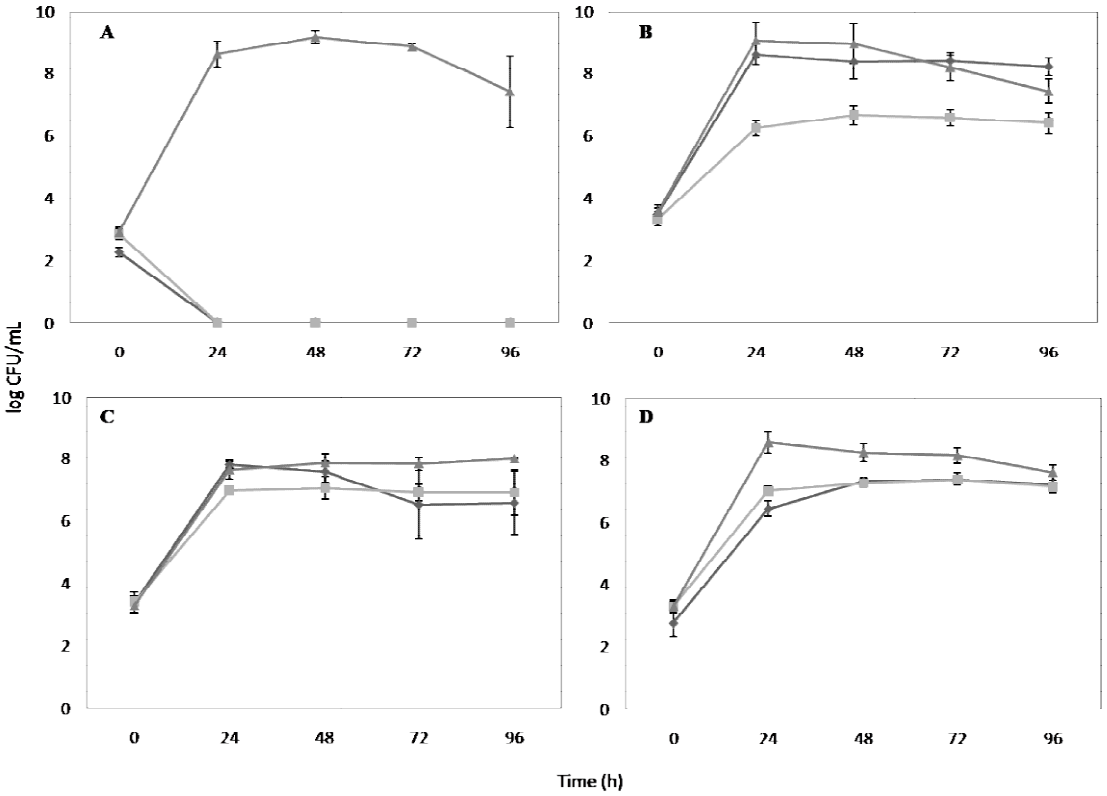

Fig. 2. Mean counts (± standard error) of *L. monocytogenes* Scott A inoculated in MRS broth (A), BHI broth (B), reconstituted skim milk (C) and meat broth (D) (♦: in co-culture with ST65ACC; ■: in co-culture with MLEV8; ▲: inoculated alone).

Discussion

The variation in the results obtained in this study is directly related to the composition of the media, including the presence of amino acids, carbon/nitrogen ratio, type of carbohydrate, as they play an essential role in bacterial growth and can also affect the production of enzymes, compounds necessary for the synthesis and expression of bacteriocins (Gänzle et al. 1999; Kaur; Tiwari, 2017; Lajis et al., 2020). In the MRS broth, a high activity of bacteriocins from *P. pentosaceus* ST65ACC inoculated alone and in co-culture with *L. monocytogenes* Scott A was observed at all analysis times, despite some variation between replicates. However, lower inhibitory activities of *P. pentosaceus* ST65ACC were recorded in BHI broth, milk and meat broth, with low and/or no bacteriocin production despite relatively good strain growth. Todorov and Dicks (2004) also found that MRS broth was the best medium for bacteriocin activity produced by *Lactobacillus pentosus* ST151BR (6,400 AU/ml) compared to BHI broth, M17 broth, soy milk and molasses (200 AU/ml). Khalil et al. (2009) reported a greater inhibitory effect of the bacteriocin produced by the *Bacillus megaterium* 19 strains in MRS broth than in BHI medium, which was not suitable for bacteriocin production.

The MRS medium is a complex medium, rich in different sources of carbon and nitrogen (Yang et al., 2018), which favors the growth of bacteria and the production of bacteriocins. The amount of glucose in the medium can also regulate the production of bacteriocins (Malheiros et al., 2015). Simha et al. (2012) observed greater growth and pediocin production when *P. pentosaceous* NCDC 273 was inoculated in MRS medium supplemented with glucose than supplemented with lactose. They also verified that the initial pH of 6.0 and 7.0 of the MRS supplemented with 20 g/l of glucose or lactose, respectively, was ideal for the maximum level of

pediocin production. A similar observation was reported by Kaur and Tiwari (2017) where the growth and activity of *P. pentosaceus* LB44 was greater in the presence of glucose than in the presence of lactose. Todorov et al. (2008) also observed an increase in bacteriocin production by *Lactobacillus plantarum* AMA-K in the presence of glucose. This may explain the greater production of bacteriocins in the MRS medium in this study, since it has a higher concentration of glucose than other media. In previous studies, Cavicchioli et al. (2017) also verified the low production of bacteriocins by the strain of *P. pentosaceus* ST65ACC in milk, showing no inhibitory effect on *L. monocytogenes*, a result attributed to the limited metabolism of the strain to ferment lactose, the main sugar in milk (Papagianni & Anastasiadou, 2009; Cavicchioli et al., 2017). These oscillating results may also be associated with the interaction of bacteriocins with the components of the medium and/or degradation by proteases (Aasen et al., 2000; Lajis et al., 2020).

Biswas et al. (1991) reported that a low final pH and a high cell density were required for a high level of pediocin AcH production by *Pediococcus acidilactici* H in TGE broth (glucose tryptone extract), since bacteriocin production was negligible when the medium pH was maintained at 5.0 or above, even in the presence of high cell mass. Similar result was reported by Guerra and Pastrana (2003), in which greater pH drops increased nisin and pediocin production by *Lactococcus lactis* and *Pediococcus acidilactici*, respectively. This may also justify the result obtained in this study, since a sharp drop in pH was not observed in BHI broth, milk and meat broth.

Conclusion

The results obtained showed that the antimicrobial activity of *P. pentosaceus* ST65ACC on *L. monocytogenes* Scott A was more effective in MRS broth, showing

greater production of bacteriocins and less variability when compared to the other media under analysis. The composition of the BHI broth, milk and meat broth may have repressed the production and/or activity of bacteriocins and, consequently, the antimicrobial activity of *P. pentosaceus* ST65ACC on *L. monocytogenes* Scott A.

Thus, verifying that the composition of the culture medium plays an important role in the production of bacteriocins, and some media may not be suitable for this purpose. The evaluation of this parameter, as well as other environmental factors, are important for optimizing the growth and production of bacteriocins, and essential for the use of a strain or its bacteriocins as biopreservation agents in food.

References

Aasen, I. M., Møretrø, T., Katla, T., Axelsson, L., &Storrø, I. (2000). Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei*CCUG 42687. Applied microbiology and biotechnology, 53(2), 159–166. https://doi.org/10.1007/s002530050003

Bédard, F., Hammami, R., Zirah, S., Rebuffat, S., Fliss, I., & Biron, E. (2018). Synthesis, antimicrobial activity and conformational analysis of the class IIa bacteriocin pediocin PA-1 and analogs there of. Scientific reports, 8(1), 9029. https://doi.org/10.1038/s41598-018-27225-3

Biswas, S. R., Ray, P., Johnson, M. C., & Ray, B. (1991). Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. Applied and environmental microbiology, 57(4), 1265–1267. https://doi.org/10.1128/aem.57.4.1265-1267.1991

Cavicchioli, V. Q., Camargo, A. C., Todorov, S. D., & Nero, L. A. (2017). Novel bacteriocinogenic *Enterococcus hirae* and *Pediococcus pentosaceus* strains with antilisterial activity isolated from Brazilian artisanal cheese. Journal of dairy science, 100(4), 2526–2535. https://doi.org/10.3168/jds.2016-12049

Cavicchioli, V.Q., Camargo, A.C., Todorov, S.D., & Nero, L. A. (2019). Potential control of *Listeria monocytogenes* by bacteriocinogenic *Enterococcus hirae* ST57ACC and *Pediococcus pentosaceus* ST65ACC strains isolated from artisanal cheese. Probiotics and Antimicrobial Proteins, 11, 696–704.https://doi.org/10.1007/s12602-018-9449-0

Cavicchioli, V. Q., Dornellas, W.dosS., Perin, L. M., Pieri, F. A., Franco, B. D., Todorov, S. D., & Nero, L. A. (2015). Genetic diversity and some aspects of antimicrobial activity of lactic acid bacteria isolated from goat milk. Applied biochemistry and biotechnology, 175(6), 2806–2822. https://doi.org/10.1007/s12010-015-1511-8

Colombo, M., Castilho, N.P.A., Todorov, S.D., Nero, L.A. (2018). Beneficial properties of lactic acid bacteria naturally present in dairy production. BMC Microbiology, 18(219). https://doi.org/10.1186/s12866-018-1356-8

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins - a viable alternative to antibiotics?. Nature reviews. Microbiology, 11(2), 95–105. https://doi.org/10.1038/nrmicro2937

Deegan, L.H., Cotter, P.D., Hilla, C., Ross, P. (2006) Bacteriocins: biological tools for bio-preservation and shelf-life extension. International Dairy Journal, 16(9), 1058–1071. https://doi.org/10.1016/j.idairyj.2005.10.026

Frank, J.F., Yousef, A.E. Tests for groups of microorganisms. In: Wehr, H.M., Frank, J.F. (Eds.), Standard Methods for the Examination of Dairy Products. Washington: American Public Health Association. 2004. p.227-248. https://doi.org/10.2105/9780875530024ch08

Freney, J., Kloos, W. E., Hajek, V., Webster, J. A., Bes, M., Brun, Y., &Vernozy-Rozand, C. (1999). Recommended minimal standards for description of new staphylococcal species. Subcommittee on the taxonomy of staphylococci and streptococci of the International Committee on Systematic Bacteriology. International journal of systematic bacteriology, 49(2), 489–502. https://doi.org/10.1099/00207713-49-2-489

Gänzle, M. G., Weber, S., & Hammes, W. P. (1999). Effect of ecological factors on the inhibitory spectrum and activity of bacteriocins. International journal of food microbiology, 46(3), 207–217. https://doi.org/10.1016/s0168-1605(98)00205-0

Garsa, A.K., Kumariya, R., Sood, S.K. Kumar, A., Kapila, S. (2014). Bacteriocin production and different strategies for their recovery and purification. Probiotics and Antimicrobial Proteins, 6, 47–58. https://doi.org/10.1007/s12602-013-9153-z

Ghosh, B., Sukumar, G., Ghosh, A.R. (2019). Purification and characterization of pediocin from probiotic *Pediococcus pentosaceus* GS4, MTCC 12683. Folia Microbiologica, 64, 765–778. https://doi.org/10.1007/s12223-019-00689-0

Guerra, N. P., Pastrana, L. (2003). Influence of pH drop on both nisin and pediocin production by *Lactococcus lactis* and *Pediococcus acidilactici*. Letters in applied microbiology, 37(1), 51–55. https://doi.org/10.1046/j.1472-765x.2003.01346.x

ISO. 11290-1:2017. (2017). Microbiology of the food chain- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. - Part 1: Detection method. International Organization for Standardization, Geneva, 36p.

Kaur, R., Tiwari, S.K. (2017). Optimization of culture conditions for bacteriocin production by soil isolates *Pediococcus pentosaceus* LB44 and *Weissella confusa* LM85. International Journal of Infection., 4(3), e15842. https://doi.org/10.5812/iji.15842

Khalil, R., Elbahloul, Y., Djadouni, F., Omar, S. (2009). Isolation and partial characterization of a bacteriocin produced by a newly isolated *Bacillus megaterium* 19 Strain. Pakistan Journal of Nutrition, 8(3), 242-250. https://scialert.net/abstract/?doi=pjn.2009.242.250

Khorshidian, N., Khanniri, E., Mohammadi, M., Mortazavian, A. M., Yousefi, M. (2021). Antibacterial activity of Pediocin and Pediocin-producing bacteria against *Listeria monocytogenes* in meat products. Frontiers in microbiology, 12, 709959. https://doi.org/10.3389/fmicb.2021.709959

Lajis, A.F.B. (2020). Biomanufacturing process for the production of bacteriocins from *Bacillaceae* family. Bioresour Bioprocess, 7(8). https://doi.org/10.1186/s40643-020-0295-z

Malheiros, P.S., Sant'Anna, V., Todorov, S.D., Franco, B.D.G.M. (2015). Optimization of growth and bacteriocin production by *Lactobacillus sake*i subsp. *Sakei* 2a. Brazilian Journal of Microbiology, 46(1), 825-834. https://doi.org/10.1590/S1517-838246320140279

Nieto-Lozano, J.C., Reguera-Useros, J.I., Peláez-Martínez, M.C., Sacristán-Pérez-Minayo, G., Gutiérrez-Fernández, A.J., Torre, A.H. (2010). The effect of the pediocin PA-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. Food Control, 21(5), 679–685. https://doi.org/10.1016/j.foodcont.2009.10.007

Papagianni M. (2003). Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function, and applications. Biotechnology advances, 21(6), 465–499. https://doi.org/10.1016/s0734-9750(03)00077-6

Papagianni, M., Anastasiadou, S. (2009). Pediocins: The bacteriocins of Pediococci. Sources, production, properties and applications. Microbial cell factories, 8(3). https://doi.org/10.1186/1475-2859-8-3

Perin, L. M., Dal Bello, B., Belviso, S., Zeppa, G., Carvalho, A. F., Cocolin, L., & Nero, L. A. (2015). Microbiota of Minas cheese as influenced by the nisin producer *Lactococcus lactis* subsp. *lactis* GLc05. International journal of food microbiology, 214, 159–167. https://doi.org/10.1016/j.ijfoodmicro.2015.08.006

Oliveira, F. S., da Silva Rodrigues, R., de Carvalho, A. F., Nero, L. A. (2023). Genomic analyses of *Pediococcus pentosaceus* ST65ACC, a bacteriocinogenic strain isolated from artisanal raw-milk cheese. Probiotics and Antimicrobial Proteins, 15(3), 630–645. https://doi.org/10.1007/s12602-021-09894-1

Simha, B. V., Sood, S.K.,Kumariya, R., Garsa, A.K. (2012). Simple and rapid purification of pediocin PA-1 from *Pediococcus pentosaceous* NCDC 273 suitable for

industrial application. Microbiological Research, 167(9), 544-549. https://doi.org/10.1016/j.micres.2012.01.001

Todorov, S. D. (2008). Bacteriocin production by *Lactobacillus plantarum* AMA-K isolated from Amasi, a Zimbabwean fermented milk product and study of the adsorption of bacteriocin AMA-K to *Listeria* sp. Brazilian Journal of Microbiology, 39(1), 178–187. https://doi.org/10.1590/S1517-838220080001000035

Todorov SD, Cavicchioli VQ, Ananieva M, Bivolarski VP, Vasileva TA, Hinkov AV, Todorov DG, Shishkov S, Haertlé T, Iliev IN, Nero LA, Ivanova IV (2020) Expression of coagulin A with low cytotoxic activity by *Pediococcus pentosaceus* ST65ACC isolated from raw milk cheese. J Appl Microbiol 128:458–472. https://doi.org/10.1111/jam.14492

Todorov, S., Dicks, L. (2004). Effect of medium components on bacteriocin production by *Lactobacillus Pentosus* ST151BR, a strain isolated from beer produced by the fermentation of maize, barley and soy flour. World Journal of Microbiology and Biotechnology, 20, 643–650. https://doi.org/10.1023/B:WIBI.0000043196.09610.de

Todorov, S.D., Oliveira, R.P.S., Vaz-Velho, M. (2012). Media optimization of bacteriocin ST22CH production by *Lactobacillus sakei* ST22CH isolated from salpicao, a traditional meat-product from Portugal. Chemical Engineering Transactions, 27, 283–288. https://doi.org/10.3303/CET1227048

Yang, E., Fan, L., Yan, J.,Jiang, Y., Doucette, C., Fillmore, S., Walker, B. (2018). Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. AMB Express, 8(10). https://doi.org/10.1186/s13568-018-0536-0

Zhang, J., Zhang, Y., Liu, S. N., Han, Y., Zhou, Z. J. (2012). Modelling growth and bacteriocin production by *Pediococcus acidilactici* PA003 as a function of temperature and pH value. Applied biochemistry and biotechnology, 166(6), 1388–1400. https://doi.org/10.1007/s12010-011-9532-4

GENERAL CONCLUSIONS AND PERSPECTIVES

The present work genomically characterized the strain *P. pentosaceus* ST65ACC mainly regarding its beneficial and safety characteristics through comparative analyses. The results showed the presence of two operons that can encode the bacteriocins pediocin PA-1/AcH and penocin-A, and these clusters showed the structural gene of bacteriocins and the machinery involved in their maturation, immunity and export. The study also revealed the presence of important genes, such as stress adaptation and adhesion genes, confirming previously obtained results, in which the strain showed the ability to survive gastrointestinal conditions, displayed hydrophobic potential and exhibited self-aggregation, suggesting its potential as a beneficial strain. In addition, the research identified absence of genes that can encode biogenic amines and low frequency of virulence factors. The presence of the *hlyIII* gene, which encodes the hemolysin III toxin, was identified, but this toxin was not expressed in phenotypic tests. Some antibiotic resistance genes were idenfied in the genome, but with a low risk of being transferred to other bacteria, since they were not located in prophage regions and no plasmids were identified. These findings demonstrate that the strain can be considered safe for application in food.

The results obtained from the evaluating the antimicrobial activity of *P. pentosaceus* ST65ACC against *L. monocytogenes* Scott A in MRS broth, BHI, milk, and meat broth showed that the antibacterial activity was more effective in the MRS broth, with greater production of bacteriocins and lower variability when compared to the other media analyzed. The composition of the medium plays an important role in the activity of these bacteria, being able to repress the production and/or activity of

bacteriocins and, consequently, their antimicrobial activity.

Taking into account the results obtained, we can conclude that *P. pentosaceus* ST65ACC can be considered as a potential antimicrobial agent in food biopreservation and other segments. Additionally, the strain presents interesting beneficial properties, showing itself as a potential probiotic strain to be considered. Further studies involving the expression of bacteriocins and optimal conditions for production and activity need to be investigated, to provide a better understanding of their activity and interaction with foodborne pathogens.