UNIVERSIDADE DE SÃO PAULO

Faculdade de Ciências Farmacêuticas

Programa de Tecnologia Bioquímico-Farmacêutica

Área de Tecnologia de Fermentações

Cultivo de bactérias ácido-láticas em meio contendo resíduos de café e obtenção de compostos antimicrobianos de interesse alimentar e farmacêutico

Anna Carolina Meireles Piazentin

São Paulo

2022

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Anna Carolina Meireles Piazentin

Versão original

Tese para a defesa do Doutorado em Ciências.

Orientador:
Prof. Dr. Ricardo Pinheiro de Souza Oliveira

São Paulo

2022

UNIVERSITY OF SÃO PAULO

School of Pharmaceutical Sciences

Program in Pharmaceutical and Biochemical Technology

Fermentation Technology Area

Cultive of lactic acid bacteria in coffee residues elaborated media and obtention of antimicrobial compounds of food and pharmaceutical interest

Anna Carolina Meireles Piazentin

Original version

Thesis presented for the Degree of Doctor in Sciences.

Advisor:
Prof. Dr. Ricardo Pinheiro de Souza Oliveira

São Paulo

2022

Anna Carolina Meireles Piazentin

Cultive of lactic acid bacteria in coffee residues elaborated media and obtention of antimicrobial compounds of food and pharmaceutical interest

Commission of Thesis for the degree of Doctor in Science

Prof. Dr. Ricardo Pinheiro de Souza Oliveira
Advisor/President

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1st Examiner

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2nd Examiner

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3rd Examiner

São Paulo, , 2022.

DEDICATION

I would like to dedicate this work to my partner Eduardo, my parents, my siblings, and my aunt. Also, I would like to dedicate this work to Titi, you always be in my heart.

ACKNOWLEDGMENT

I would like to express my thanks:

To professor Ricardo Pinheiro de Souza Oliveira, for all the orientation, supervision, advice and support, not only for the doctorate, but also for all the years we've been working together.

To professor Solange Ines Mussatto, for the supervision and orientation, and the excellent reception in her research group during my internship at DTU. To DTU (Denmark Technical University) in special for the Novo Nordisk Center for Biossustainability.

To my husband Eduardo and family for all the support and love during this journey.

For all the friends and colleagues, I have made through the years. In special to Thamires and Marcela "senhoras", who I have a special place in my heart, for all the tears, laughs and special moments that we were and will share together. To my lab friends Pamela, Vivi, Well and Carlos for all the support and friendship.

To the friendships I've made during my internship at DTU, in special Celina, Larissa and Regiane.

To all the professors, technicians, and secretaries from Biochemical and Pharmaceutical Program, you were an important part of my journey.

To CNPQ (Conselho Nacional de Pesquisa) for the PhD fellowship (Process: 169012/2017-4), to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) Finance Code - 001 for the PRInt program and the fellowship (CAPES-PRINT - 88887.371211/2019-00) who permitted me to do my internship at DTU, to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the financial support (grant #2018/25511–1), and to the Novo Nordisk Foundation, Denmark (grant number NNF20SA0066233).

EPÍGRAFE

O que tiver que se ser, será! Mas um café te ajuda a lidar.

Autor desconhecido

ABSTRACT

PIAZENTIN, A. C. M. **Cultive of lactic acid bacteria in coffee residues elaborated media and obtention of antimicrobial compounds of food and pharmaceutical interest.** 2022. 64 p. Thesis (PhD) – Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2022.

The objective of this study was to evaluate the potential of lactic acid bacteria (LAB) to produce BLIS (Bacteriocin Like Inhibitory Substances) with activity against pathogenic bacteria of food interest and clinical importance, in addition to developing an alternative culture media based on waste from the coffee industry, such as coffee silverskin (CS) and spent coffee grounds (SCG) where BAL could be able to grow and produce BLIS. *Enterococcus faecium* 135, which was isolated from the intestine of a starfish (Order Forcipulatida), stood out as a producer of BLIS with anti-listeric activity. However, its activity was reduced when cultured with the bacteria *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri*. For the elaboration of the alternative media containing the CS and SCG residues, they were subjected to an acid pretreatment with 120 and 100 mg of H_2SO_4/g, for 75 and 45 minutes at 140 ºC in an autoclave. For CS and SCG, the hydrolysates passed through a post-hydrolysis with 4% (v/v) H_2SO_4 at 121ºC for 60 min, after which the hydrolysates were detoxified using a C-18 silica column and the pH was adjusted to 6. The media was prepared based on the commercial MRS medium (by Man, Rogosa and Sharp), and the hydrolyzate concentrations used for the preparation of the media were 0, 25, 50 and 100 % (v/v) in addition to the detoxified hydrolysate. The media was supplemented with sources of nitrogen and salts equal to the commercial MRS, and also sugars for the diluted media, so the concentration of sugars was equal to the 100% hydrolysate. The supplemented detoxified SCG and CS 25% medium stood out from the others, because *E. faecium* 135 obtained a growth of ~ 1.9 log CFU/mL. In addition the antimicrobial activity was superior to the control, being 480 AU/mL for CS 25 % and 428 AU/mL for supplemented detoxified SCG, in addition to lactic acid production, which was 10.51 g/L for 25% CS. The probiotic potential of *E. faecium* 135 was also tested and it showed resistance to low pH (2.5 and 3.0). Apart from resistance to 3% (w/v) bile salts, the strain was able to adhere to Caco-2 cells, and presented negative results for virulence factors. The presence of some genes responsible for the production of enterocins was also observed in the DNA of the bacterium. *E. faecium* 135 was a lactic acid bacterium that could be good candidate as a probiotic, and it can make BLIS, even in an alternative media made with CS and SCG coffee residues. These residues were interesting carbon sources for the growth of the bacterium, and they could be used as an alternative to traditional culture medium.

Keywords: lactic acid bacteria, *Enterococcus faecium*, BLIS, *coffee silverskin*, *spent coffee grounds*, probiotic potential.

RESUMO

PIAZENTIN, A. C. M. **Cultivo de bactérias ácido-láticas em meio contendo resíduos de café e obtenção de compostos antimicrobianos de interesse alimentar e farmacêutico.** 2022. 64 f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2022.

O objetivo desse trabalho foi avaliar o potencial de bactérias ácido-láticas (BAL) produzirem BLIS (*Bacteriocin Like Inhibitory Substances*) com atividade antimicrobiana contra cepas patogênicas de interesse alimentar e importância farmacêutica, além de elaborar um meio de cultivo alternativo baseado nos resíduos da indústria do café, como película prateada (*coffee silverskin* – CS) e a borra do café (*spent coffee grounds* – SCG) onde as BAL pudessem crescer e produzir o BLIS. *Enterococcus faecium* 135, que foi isolada do intestino de uma estrela do mar (Ordem Forcipulatida), se destacou como produtora de BLIS com atividade anti-listérica, entretanto teve sua atividade reduzida quando cultivada com as bactérias *Ligilactobacillus salivarius* e *Limosilactobacillus reuteri*. Para a elaboração do meio alternativo contendo os resíduos CS e SCG, os mesmos foram submetidos um pré-tratamento ácido 120 e 100 mg de H_2SO_4/g, durante 75 e 45 minutos a 140 ºC em autoclave, para CS e SCG respectivamente, os hidrolisados passaram por uma pós-hidrólise com 4% (v/v) H_2SO_4 a 121ºC durante 60 min, após esse período uma parte dos hidrolisados foi detoxificada utilizando uma coluna de sílica C-18 e o pH foi ajustado para 6. Posteriormente, os meios foram elaborados com base no meio comercial MRS (de Man, Rogosa and Sharp), e as concentrações de hidrolisado utilizadas para elaboração do meio foram 0, 25, 50 e 100 % (v/v) além do hidrolisado detoxificado. Os meios tiveram suplementação com fontes de nitrogênio e sais iguais ao meio comercial, e de açúcares para os meios diluídos, a fim de que a concentração de açúcares fosse igual ao meio 100%. Os meios SCG detoxificado suplementado e CS 25% destacaram-se dos demais pois, *E. faecium* 135 obteve um crescimento de ~ 1,9 log UFC/mL, além da atividade antimicrobiana superior ao controle, sendo de 480 AU/mL para CS 25% e 428 AU/mL para SCG detoxificado suplementado, além da produção de ácido lático que foi de 10,51 g/L para CS 25 %. O potencial probiótico de *E. faecium* 135 também foi testado e o mesmo apresentou resistência a pH baixos (2.5 e 3.0), além de resistência a sais de bile 3% (w/v), a cepa foi capaz de se aderir a células Caco-2, e apresentou resultados negativos para fatores de virulência, também foi observada a presença de alguns genes referentes a produção de enterocinas no DNA da cepa. Em conclusão *E. faecium* 135 foi uma bactéria ácido-lática que apresentou potencial probiótico, e produtor de BLIS, mesmo em meio alternativo elaborado com os resíduos do café CS e SCG. Resíduos esses que se mostraram interessantes fontes de carbono para o crescimento da cepa, podendo ser utilizados com alternativa aos meios de cultivo convencionais.

Palavras-chave: bactérias ácido-láticas, *Enterococcus faecium*, BLIS, *coffee silverskin*, *spent coffee grounds*, potencial probiótico

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ABREVIATIONS

AU: arbitrary units

BLIS: bacteriocin like substances

CFU: colony forming unity

CFS: cell-free supernatant

CS: coffee silverskin

GRAS: Generally Recognized As Safe

HPLC: high performance liquid chromatography

LAB: lactic acid bacteria

O.D.: optical density

PAN: primary aminoacids

rpm: rotations per minute

SCG: spent coffee grounds

g: centrifugal force

v/v: volume per volume

w/v: weight per volume

5-HMF: hydroxymethylfurfural

Summary

GENERAL INTRODUCTION

BLIS (bacteriocin like inhibitory substances) are peptides or proteins produced by ribosomes and present antimicrobial activity against several microorganisms (COTTER; HILL; ROSS, 2005). The most common producers of these molecules are the lactic acid bacteria (LAB), mostly because they are usually considered GRAS (Generally Recognized As Safe) strains and a larger number of them are isolated from fermented foods (QIN et al., 2017). The research about BLIS has increased over the years. The applications vary, but in the food industry is where they could have more usage in food preservation against foodborne pathogens (COTTER; ROSS; HILL, 2013; DOBSON et al., 2012; SOLTANI et al., 2021). Recently, BLIS has been studied and applied as an alternative to the use of antibiotics in animal production due to the increasing cases of antibiotic resistance (CROTTA; GEORGIEV; GUITIAN, 2017; POPOVA, 2017). Alternatives have started to be required, so LAB or probiotic strains the are capable of producing BLIS are applied as a supplement to the animal feeding (JAHROMI et al., 2016; MESSAOUDI et al., 2011).

The use of agro-industrial waste is increasing as an alternative carbon source for the growth of probiotic and lactic acid bacteria. Among these residues are the ones from the coffee industry, which increase each year due to the high demand for the beverage (ICO-International Coffee Organization, 2021). Coffee silverskin (CS) and spent coffee grounds (SCG) are two of the principal residues generated in the coffee industry. Both are usually used as fertilizer and for the elaboration of biofuels (MCNUTT; HE, 2019; NARITA; INOUYE, 2014). Although they are sources for cellulose and hemicellulose (BALLESTEROS; TEIXEIRA; MUSSATTO, 2014), CS and SCG show potential as prebiotics (JIMÉNEZ-ZAMORA; PASTORIZA; RUFIN-HENARES, 2015) and alternative growth medium for fungi and yeast (MACHADO et al., 2012). However, using these residues for the production of BLIS is not approached by literature.

Due to the potential of LAB to produce BLIS and the availability of sugar sources from CS and SCG, the aim of this study was to evaluate the capability of LAB to produce BLIS with activity against some pathogens from the food industry and clinical interests, and the elaboration of an alternative media with CS and SCG that a LAB would grow and produce BLIS.

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OBJECTIVES

GENERAL

Optimization and production of BLIS by *Enterococcus faecium* 135, elaboration of media containing coffee residues and evaluation of the antimicrobial activity against foodborne and clinically important pathogens.

SPECIFICS

- Evaluation of the production of bacteriocin like inhibitory substances (BLIS) by monoculture of *E. faecium* 135 and in co-culture with *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri.*

- Optimization of BLIS production by *E. faecium* 135, and evaluation of the antimicrobial activity against *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Choleraesius, *Staphylococcus aureus* and *Streptococcus agalactiae*.

- Elaboration of an alternative growth media using spent coffee grounds and coffee silverskin, and evaluation of the growth and BLIS production by *E. faecium* 135.

- Pre-purification of the BLIS produced by *E. faecium* 135.

- Determination of the probiotic nature and presence of bacteriocin genes in *E. faecium* 135.

PRESENTATION

This thesis is organized in the form of scientific articles (published, and to be submitted for publication, and is divided into the following chapters.

Chapter 1: "*Bacteriocin-like inhibitory substances production by Enterococcus faecium 135 in co-culture with Ligilactobacillus salivarius and Limosilactobacillus reuteri*" - This chapter aimed to evaluate the best conditions for growth and production of antimicrobial compounds produced by the monoculture of *E. faecium* and the ternary culture with *L. salivarius* and *L. reuteri.* The following published scientific article resulted in this chapter: **Piazentin, A.C.M**, Mendonça, C.M.N., Vallejo, M., Mussatto, S.I., Oliveira, R.P.S. (2022) Bacteriocin-like inhibitory substances production by *Enterococcus faecium* 135 in co-culture with *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri*. *Brazilian Journal of Microbiology*, v. 53, p. 131-141. https://doi.org/10.1007/s42770-021-00661-6

Chapter 2: "*Use of coffee residues as alternative media for growth and production of antimicrobial compounds from Enterococcus faecium 135*" - The work presented in this chapter was performed during the internship performed at Denmark Technical University. The aim of the work was to recover sugars from spent coffee grounds and coffee silverskin. Both residues were hydrolysate, and from this hydrolysate it was elaborate an alternative media similar to a commercial medium used in lactic acid growth. It was evaluated the capability of *E. faecium* 135 to grow in this new media and produce antimicrobial compounds. - This content resulted in a manuscript, which will be submitted: **Piazentin, A.C.M**, Yamakawa, C.K., Vallejo, M., Oliveira, R.P.S., Mussatto, S.I. (2022) Use of coffee residues as alternative meda for growth and production of antimicrobial compounds from *Enterococcus faecium* 135. *Bioresource Technology*, **to be submitted.**

Chapter 3: "*Bacteriocinogenic probiotic bacteria isolated from an aquatic environment inhibit the growth of food and fish pathogens*" - This chapter evaluates the probiotic potential of *E. faecium* 135 and other lactic acid bacteria isolated from aquatic environments, besides the presence of bacteriocin production genes and the production of

bacteriocin like substances. The first authorship of this article was divided by the first to authors who equally contributed to the work: **Pereira, W.A., Piazentin, A.C.M.**, Oliveira, R.C., Mendonça, C.M.N., Tabata, Y.A., Mendes, M.A., Fock, R.A., Makiyama. E.N., Corrêa, B., Vallejo, M., Villalobos, E.F., Oliveira, R.P.S. (2022) Bacteriocinogenic probiotic bacteria isolated from an aquatic environment inhibit the growth of food and fish pathogens. *Scientific Reports*, v. 12:5530. https://doi.org/10.1038/s41598-022-09263-0

CHAPTER
.1.

Bacteriocin-like inhibitory substances production by *Enterococcus faecium* 135 in co-culture with *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri*

Available in: https://doi.org/10.1007/s42770-021-00661-6

CHAPTER
.2.

CHAPTER .3.

Bacteriocinogenic probiotic bacteria isolated from an aquatic environment inhibit the growth of food and fish pathogens

Available in: https://doi.org/10.1038/s41598-022-09263-0

GENERAL CONCLUSIONS

In this study was observed that the BLIS produced by *E. faecium* 135 have a good antimicrobial activity against *L. monocytogenes*, an important food pathogen. It was also observed that *E. faecium* 135 was capable to grow and produce BLIS when an alternative growth media elaborated with the detoxified hydrolysate of SCG and 25% (w/w) diluted CS hydrolysate, bout supplemented with nitrogen sources was used. A characterization study was carried out and revealed that *E. faecium* 135 was resistant to low pH conditions (from 2.5 to 3.0) and different concentrations of bile salts (3%), the strain was able to adhere to Caco-2 cells and in the tests of expression of virulence/resistance factors, such as hemolysin and resistance to antibiotics, had negative results, an important demonstration of its safety and probiotic potential. In conclusion, *E. faecium* 135 is a promising LAB as it can grow in an alternative growth media based on coffee residues, produce BLIS with antimicrobial activity against *L. monocytogenes* and had good results and the study of probiotic characterization. However, a further investigation is necessary to confirm the presence of bacteriocins on the BLIS produced by *E. faecium* 135.

ATTACHMENTS

ATTACHAMENT 1

Brazilian Journal of Microbiology (2022) 53:131–141
https://doi.org/10.1007/s42770-021-00661-6

BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - RESEARCH PAPER

Bacteriocin-like inhibitory substances production by *Enterococcus faecium* 135 in co-culture with *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri*

Anna Carolina Meireles Piazentin[1] · Carlos Miguel Nóbrega Mendonça[1] · Marisol Vallejo[2] · Solange I. Mussatto[3] · Ricardo Pinheiro de Souza Oliveira[1]

Received: 19 April 2021 / Accepted: 16 November 2021 / Published online: 6 January 2022
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Abstract
The use of lactic acid bacteria (LAB) and probiotic cultures in the breeding of animals such as poultry and swine are quite common. It is known that those strains can produce bacteriocins when grown in pure culture. However, the production of bacteriocin using co-culture of microorganisms has not been much studied so far. The present study contributes with innovation in this area by embracing the production of bacteriocin-like inhibitory substances (BLIS) by a newly isolated strain of *Enterococcus faecium* 135. Additionally, the co-cultivation of this strain with *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri* was also investigated. The antimicrobial activity of the produced BLIS was evaluated against *Listeria monocytogenes*, *Listeria innocua*, *Salmonella enterica*, and *Salmonella enterica* serovar Typhimurium using two methods: turbidimetric and agar diffusion. In addition, the presence of enterocin genes was also evaluated. The BLIS produced showed a bacteriostatic effect against the bio-indicator strains, and the highest antimicrobial activities expressed by arbitrary units per mL (AU/mL) were obtained against *L. monocytogenes* in monoculture (12,800 AU/mL), followed by the co-culture of *E. faecium* with *Limosilactobacillus reuteri* (400 AU/mL). After concentration with ammonium sulfate, the antimicrobial activity raised to 25,600 AU/mL. Assays to determine the proteinaceous nature of the BLIS showed susceptibility to trypsin and antimicrobial activity until 90 °C. Finally, analysis of the presence of structural genes of enterocins revealed that four enterocin genes were present in *E. faecium* 135. These results suggest that BLIS produced by *E. faecium* 135 has potential to be a bacteriocin and, after purification, could potentially be used as an antimicrobial agent in animal breeding.

Keywords *Enterococcus faecium* · Antimicrobial Activity · Co-culture · Foodborne pathogens

ATTACHAMENT 2

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OPEN

Bacteriocinogenic probiotic bacteria isolated from an aquatic environment inhibit the growth of food and fish pathogens

Wellison Amorim Pereira[1,8], Anna Carolina M. Piazentin[1,8], Rodrigo Cardoso de Oliveira[1,8], Carlos Miguel N. Mendonça[1,8], Yara Aiko Tabata[2], Maria Anita Mendes[3], Ricardo Ambrósio Fock[4], Edson Naoto Makiyama[4], Benedito Corrêa[5], Marisol Vallejo[6], Elias Figueroa Villalobos[7] & Ricardo Pinheiro de S. Oliveira[1]✉

The conditions of aquatic environments have a great influence on the microbiota of several animals, many of which are a potential source of microorganisms of biotechnological interest. In this study, bacterial strains isolated from aquatic environments were bioprospected to determine their probiotic profile and antimicrobial effect against fish and food pathogens. Two isolates, identified via 16S rRNA sequencing as *Lactococcus lactis* (L1 and L2) and one as *Enterococcus faecium* 135 (EF), produced a bacteriocin-like antimicrobial substance (BLIS), active against *Listeria monocytogenes*, *Salmonella* Choleraesuis and *Salmonella* Typhimurium. Antimicrobial activity of BLIS was reduced when exposed to high temperatures and proteolytic enzymes (trypsin, pepsin, papain and pancreatin). All strains were sensitive to 7 types of antibiotics (vancomycin, clindamycin, streptomycin, gentamicin, chloramphenicol, rifampicin and ampicillin), exhibited a high rate of adherence to Caco-2 cells and expressed no hemolysin and gelatinase virulence factors. EF showed some resistance at pH 2.5 and 3.0, and L2/EF showed higher resistance to the action of bile salts. Finally, the presence of bacteriocin

ATTACHAMENT 3

Library of Congress Cataloging-in-Publication Data

Names: Albuquerque, Marcela Albuquerque Cavalcanti de, 1984- editor. | LeBlanc, Alejandra de Moreno de, editor. | LeBlanc, Jean Guy (Jean Guy Joseph), editor. | Bedani, Raquel, editor.
Title: Lactic acid bacteria : a functional approach / editors, Marcela Albuquerque Cavalcanti de Albuquerque, Alejandra de Moreno de LeBlanc, Jean Guy LeBlanc, and Raquel Bedani.
Other titles: Lactic acid bacteria (Albuquerque)
Description: Boca Raton : CRC Press, Taylor & Francis Group, [2020] | Includes bibliographical references and index. | Summary: "The book deals with advances made in the functionalities of lactic acid bacteria (LAB) such as, their effect on vitamin D receptor expression, impact on neurodegeneratives pathologies, production of B-vitamins for food bio-enrichment, production of bacteriocins to improve gut microbiota dysbiosis, production of metabolites from polyphenols and their effects on human health, effect on reducing the immunoreaction of food allergens, as biological system using time-temperature to improve food safety, and the use of probiotic in animal feed. The book also reviews the use of LAB and probiotics technologies to develop new functional foods and functional pharmaceutical"-- Provided by publisher.
Identifiers: LCCN 2019056212 | ISBN 9781138391635 (hardback)
Subjects: MESH: Lactobacillales--physiology | Probiotics--therapeutic use | Nutritive Value | Lactobacillales--metabolism | Functional Food--microbiology
Classification: LCC QR82.L3 | NLM QU 145.5 | DDC 579.3/7--dc23
LC record available at https://lccn.loc.gov/2019056212

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Impact of Probiotics on Animal Health

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Introduction

The concept of probiotics is very well known and their effects on human's health have been extensively reported along the last years. Meanwhile, the application of probiotics in feed nutrition are far less explored and documented. Probiotics started to be described in 1974, when Parker stated that probiotics are "organisms and substances which contribute to intestinal microbial balance" thus including both living organisms and non-living substances. Later, Fuller (1989) defined probiotics as "a live microbial

ATTACHAMENT 4

J. Dairy Sci. 103
https://doi.org/10.3168/jds.2019-18078
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Buffalo milk increases viability and resistance of probiotic bacteria in dairy beverages under in vitro simulated gastrointestinal conditions

Thamires Maria Simões da Silva,[1] Anna Carolina Meirelles Piazentin,[1] Carlos Miguel Nóbrega Mendonça,[1] Attilio Converti,[2] Cristina Stewart Bittencourt Bogsan,[1] Diego Mora,[3] and Ricardo Pinheiro de Souza Oliveira[1]*

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ATTACHAMENT 5

International Journal of Biological Macromolecules 186 (2021) 788–799

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Characterization of levan produced by a *Paenibacillus* sp. isolated from Brazilian crude oil

Carlos M.N. Mendonça [a,b], Rodrigo C. Oliveira [a], Rominne K.B. Freire [a], Anna C.M. Piazentin [a], Wellison A. Pereira [a], Eduardo J. Gudiña [c], Dmitry V. Evtuguin [b], Attilio Converti [d], João H.P. M. Santos [a], Cláudia Nunes [b], Lígia R. Rodrigues [c,1], Ricardo P.S. Oliveira [a,*,1]

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ATTACHAMENT 6

Veterinary Microbiology 269 (2022) 109431

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Beneficial effects of probiotics on the pig production cycle: An overview of clinical impacts and performance

Wellison A. Pereira [a,1], Sara M. Franco [a,1], Iara L. Reis [a], Carlos M.N. Mendonça [a], Anna C.M. Piazentin [a], Pamela O.S. Azevedo [a], Marcos L.P. Tse [b], Elaine C.P. De Martinis [c], Martin Gierus [d], Ricardo P.S. Oliveira [a,*]

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ATTACHAMENT 7

Supplementary material from chapter 3

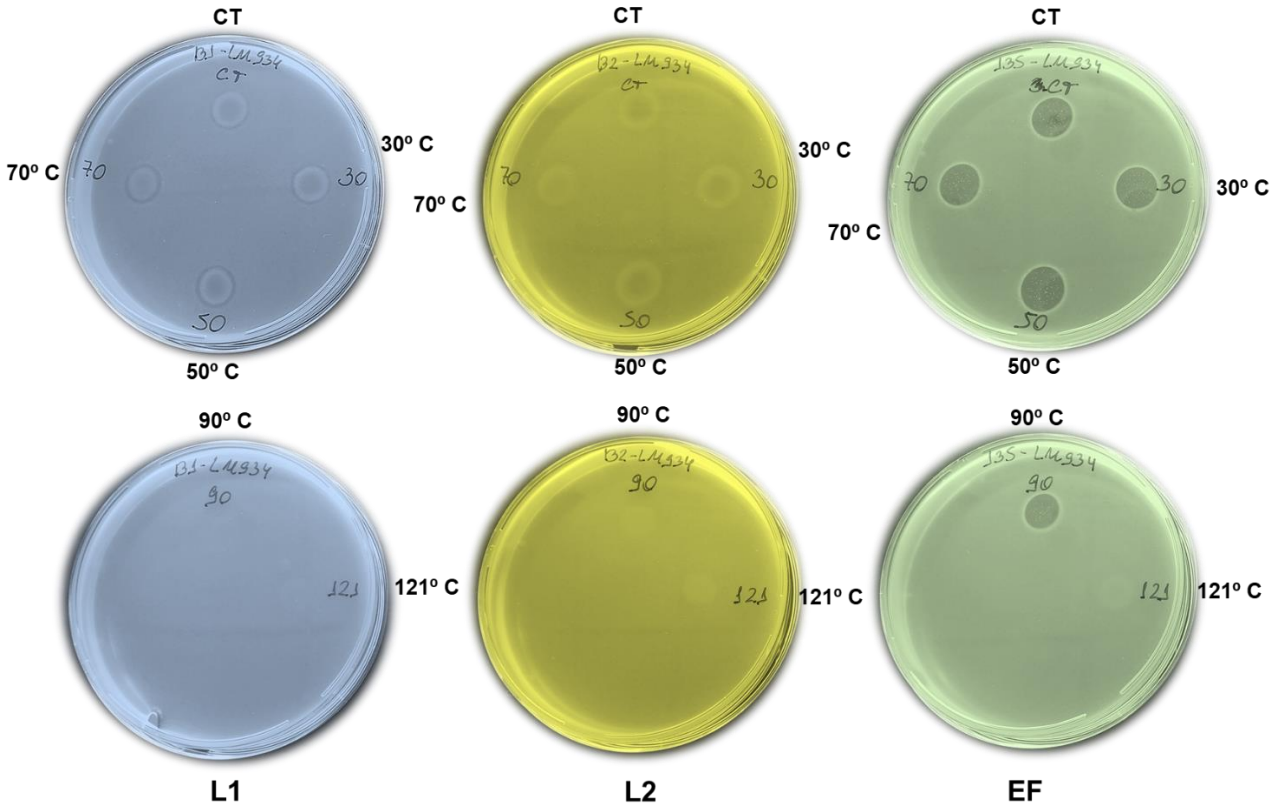


Figure S1. Exposure of BLIS produced by isolates to different temperatures. CT= control (BLIS without the temperature treatment applied in the other samples).

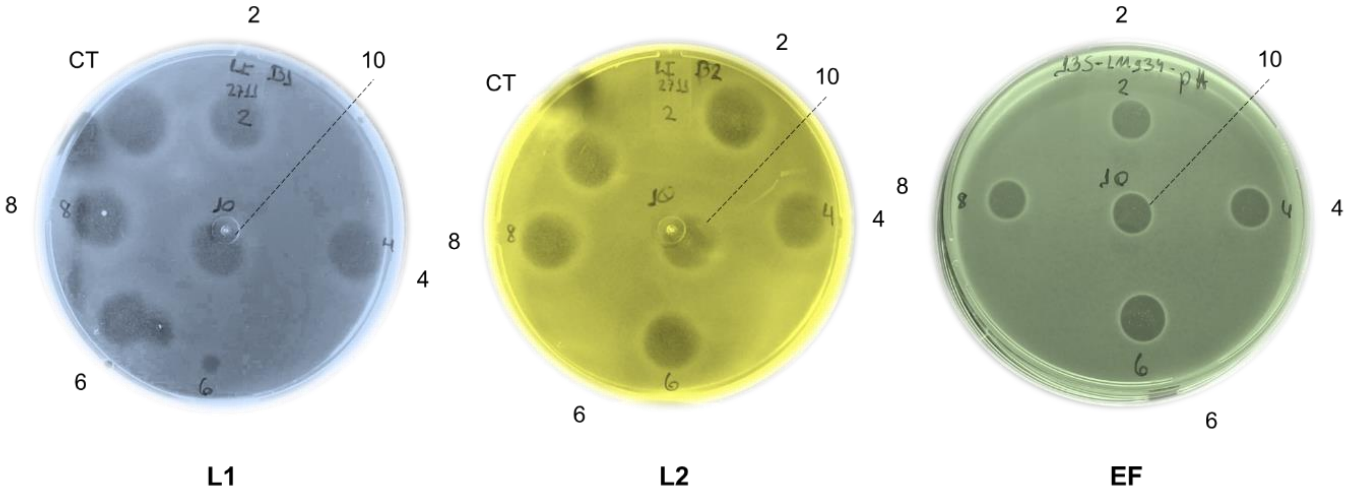


Figure S2. Exposure of BLIS produced by isolates at different pHs. CT= control (BLIS without the pH treatment).

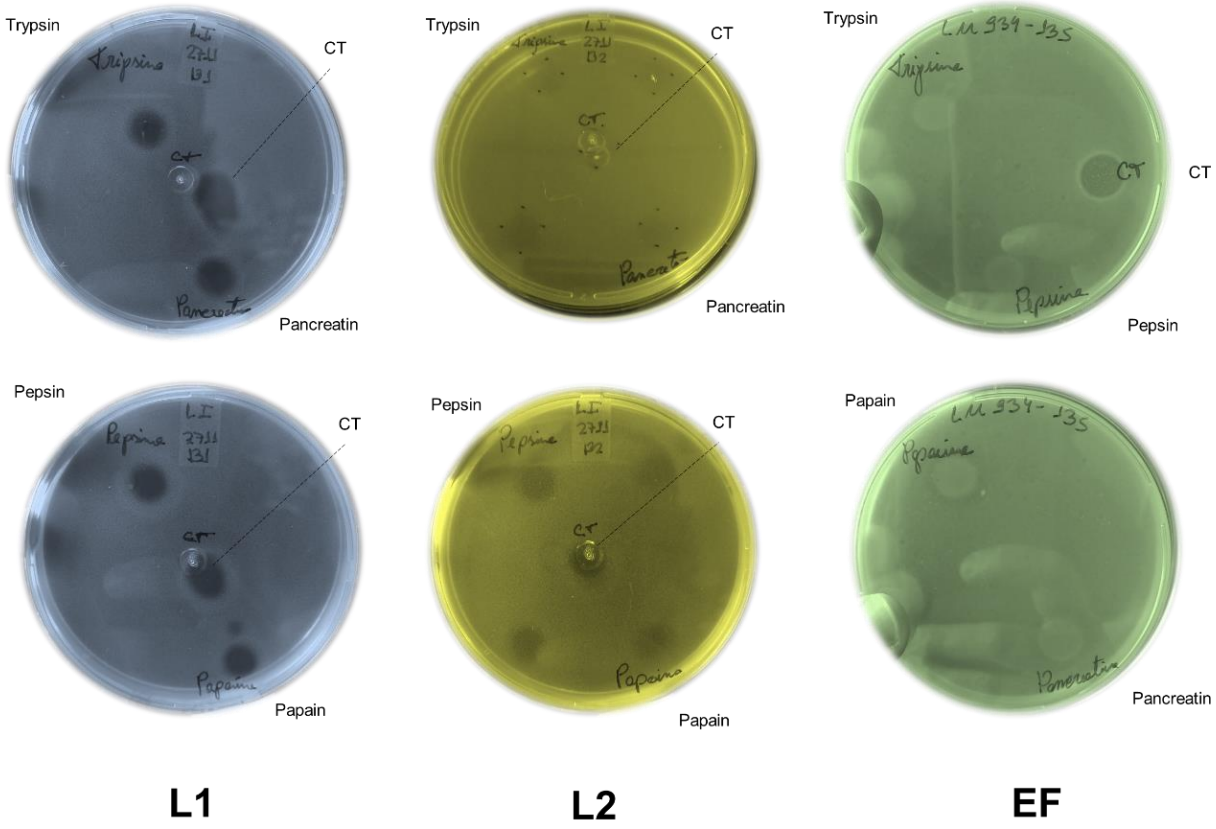


Figure S3. Exposure of BLIS produced by isolates to different proteolytic enzymes. CT= control (BLIS without the enzymatic treatment).

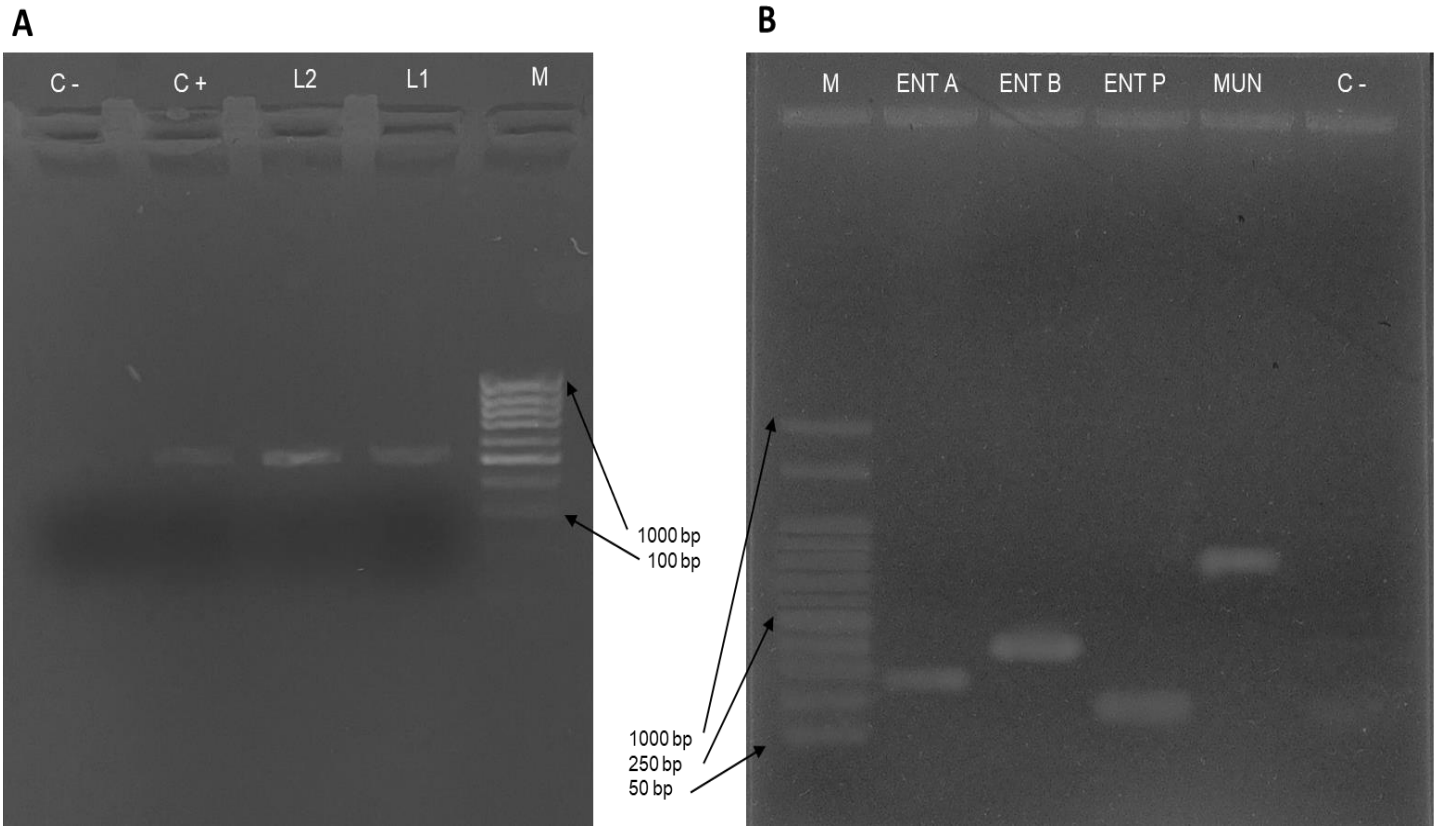


Figure S4. Screening for presence of bacteriocin genes in *L. lactis* L1 – L2 (A) and *E, faecium* strains (B). Lane M: 1 kb DNA markers; Lane C + and C -: products of positive and negative control PCR reactions, respectively. Lane L2 and L1: PCR amplification products of the nisin gene of *L. lactis* L1 and L2; Lane ENT A, ENT B, ENT P, and MUN: PCR amplification products of bacteriocin genes of *E. faecium*.

Table S1. Effect of acids (pH 2, 2.5 and 3) on the viability (log CFU/mL) of *L. lactis* (L1 and L2) and *E. faecium* (EF).

Strain	pH	Time			
		0h	1h	2h	3h
L1	Control	9.27 ± 0.01 ^{Aa}	9.26 ± 0.22 ^{Aa}	9.15 ± 0.03 ^{Aa}	9.34 ± 0.00 ^{Aa}
	pH 2	7.45 ± 0.15 ^{D}	-	-	-
	pH 2.5	8.78 ± 0.18 ^{C}	-	-	-
	pH 3	9.47 ± 0.04 ^{A}	-	-	-
L2	Control	9.15 ± 0.11 ^{Aa}	9.09 ± 0.09 ^{Aa}	9.12 ± 0.16 ^{Aa}	8.99 ± 0.09 ^{Ba}
	pH 2	7.95 ± 0.05 ^{E}	-	-	-
	pH 2.5	9.14 ± 0.06 ^{A}	-	-	-
	pH 3	9.31 ± 0.14 ^{Aa}	9.14 ± 0.06 ^{Aa}	-	-
EF	Control	8.45 ± 0.08 ^{Ba}	8.25 ± 0.05 ^{Bb}	8.46 ± 0.10 ^{Ba}	8.59 ± 0.04 ^{Ca}
	pH 2	8.59 ± 0.19 ^{BC}	-	-	-
	pH 2.5	8.69 ± 0.09 ^{BCa}	6.78 ± 0.35 ^{Cb}	5.03 ± 0.10 ^{Dc}	3.69 ± 0.20 ^{Ed}
	pH 3	8.41 ± 0.08 ^{Ba}	8.31 ± 0.14 ^{Ba}	8.09 ± 0.05 ^{Cb}	8.06 ± 0.03 ^{Db}

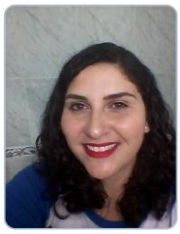
The results are expressed as means ± standard deviations, n = 3; (-) indicates that the counts were < 100 CFU/mL. Different uppercase letters in the same column mean statistically different values according to the Tukey's test ($P < 0.05$). Different lowercase letters in the same row mean statistically different values according to the Tukey's test ($P < 0.05$).

Table S2. Effect of bile salts (0.1, 0.2, and 0.3%) on the viability (log CFU/mL) of *L. lactis* (L1 and L2) and *E. faecium* (EF).

Strain	Bile (%)	Time			
		0h	2h	4h	6h
L1	Control	9.48 ± 0.08 ^{ABa}	9.37 ± 0.16 ^{Aa}	8.00 ± 0.00 ^{Cb}	7.54 ± 0.06 ^{Dc}
	0.1	9.27 ± 0.27 ^{ABC}	-	-	-
	0.2	6.78 ± 0.18 ^{E}	-	-	-
	0.3	6.00 ± 0.00 ^{F}	-	-	-
L2	Control	9.15 ± 0.11 ^{ABCa}	9.09 ± 0.09 ^{ABa}	9.10 ± 0.02 ^{ABa}	8.19 ± 0.04 ^{Cb}
	0.1	8.95 ± 0.05 ^{Ca}	8.59 ± 0.59 ^{Ba}	9.09 ± 0.09 ^{ABa}	9.05 ± 0.10 ^{ABa}
	0.2	7.14 ± 0.06 ^{DEb}	9.02 ± 0.06 ^{ABa}	8.99 ± 0.09 ^{ABa}	9.08 ± 0.04 ^{ABa}
	0.3	7.31 ± 0.14 ^{Dc}	9.14 ± 0.06 ^{Aa}	8.93 ± 0.03 ^{Bb}	9.19 ± 0.01 ^{Aa}
EF	Control	9.65 ± 0.29 ^{Aa}	9.08 ± 0.04 ^{ABb}	9.02 ± 0.10 ^{ABb}	8.98 ± 0.05 ^{ABb}
	0.1	9.08 ± 0.07 ^{BCa}	9.10 ± 0.07 ^{ABa}	9.13 ± 0.05 ^{Aa}	8.90 ± 0.04 ^{Bb}
	0.2	9.00 ± 0.08 ^{Ca}	8.95 ± 0.07 ^{ABa}	9.00 ± 0.08 ^{ABa}	8.83 ± 0.20 ^{Ba}
	0.3	9.02 ± 0.05 ^{Ca}	8.92 ± 0.18 ^{ABa}	8.90 ± 0.10 ^{Ba}	9.00 ± 0.09 ^{ABa}

The results are expressed as means ± standard deviations n = 3; (-) indicates that the counts were < 100 CFU/mL. Different uppercase letters in the same column mean statistically different values according to the Tukey's test ($p < 0.05$). Different lowercase letters in the same row mean statistically different values according to the Tukey's test ($p < 0.05$).

ATTACHAMENT 8



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2017 Doutorado em andamento em Programa de Pós-Graduação em Tecnologia Bioquímico-Farmacêutica.
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Título: Cultivo de bactérias ácido-láticas em meio contendo resíduos de café e obtenção de compostos antimicrobianos de interesse alimentar e farmacêutico,
Orientador: Ricardo Pinheiro de Souza Oliveira.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.
Palavras-chave: BLIs; Probióticos; Resíduos agroindustriais do café; Campylobacter jejuni; Listeria monocytogenes; Salmonella enterica.
Grande área: Ciências da Saúde

2014 - 2016 Mestrado em Tecnologia Bioquímico-Farmacêutica.
Universidade de São Paulo, USP, Brasil.
Título: Efeito de culturas probióticas em produto a base de soja: resistência ao armazenamento refrigerado, ao estresse in vitro gastrointestinal e atividade antimicrobiana.,Ano de Obtenção: 2016.
Orientador: Ricardo Pinheiro de Souza Oliveira.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.

2009 - 2012 Graduação em Farmácia.
Universidade Cruzeiro do Sul, UNICSUL, Brasil.
Título: O uso de Synadenium grantii Hook f. no tratamento de Diabetes Mellitus.
Orientador: Roberto Adati Tsuyoshi.

2008 - 2009 Curso técnico/profissionalizante em Técnico em Química.
ETEC Getúlio Vargas, ETEC GV, Brasil.

Formação Complementar

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2018 - 2018 Vínculo: Estágiário, Enquadramento Funcional: Monitor, Carga horária: 6

Atividades

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Estágio realizado
Programa de Aperfeiçoamento de Ensino (PAE), realizado na Faculdade de Ciências Farmacêuticas/ USP, para o curso de Farmácia dentro da disciplina de Biotecnologia-Farmacêutica.

Hospital das Clínicas da Faculdade de Medicina da USP, HCFMUSP, Brasil.

Vínculo institucional

2012 - 2014 Vínculo: Servidor Público, Enquadramento Funcional: Técnico de Laboratório, Carga horária: 20

Outras informações Atuação na divisão de farmácia, unidade de farmacotécnica, setor de acabamento, relativo à unitarização. Utilização de maquinário industrial, para a emblistamento de medicamentos que são produzidos pelo hospital, como os que são comprados pelo mesmo. Controle microbiológico dos principais produtos produzidos na unidade.

Bem Emergências Médicas, BEM, Brasil.

Vínculo institucional

2012 - 2012 Vínculo: Funcionário, Enquadramento Funcional: Auxiliar de almoxarifado, Carga horária: 40

Outras informações Organização de almoxarifado médico-hospitalar, montagem de kits para as viaturas, assim como a verificação de aparelhos médicos, tais, como oxímetros, desfibriladores, respiradores e incubadores.

Associação Paulista para o Desenvolvimento da Medicina - Hospital São Paulo, SPDM/HSP, Brasil.

Vínculo institucional

2010 - 2011 Vínculo: Funcionário, Enquadramento Funcional: Auxiliar de farmácia, Carga horária: 30

Outras informações Dispensação de medicamentos e materiais médico-hospitalares, organização de almoxarifado médico e atendimento ao público.

Revisor de periódico

2015 - 2015 Periódico: International Journal of Food Sciences and Nutrition

Áreas de atuação

1. Grande área: Ciências da Saúde / Área: Farmácia.
2. Grande área: Ciências Biológicas / Área: Biotecnologia.
3. Grande área: Ciências Biológicas / Área: Microbiologia / Subárea: Fermentações láticas.

Idiomas

Inglês Compreende Bem, Fala Razoavelmente, Lê Bem, Escreve Razoavelmente.

Prêmios e títulos

2021 Menção Honrosa pelo trabalho "Use of spent coffee grounds as alternative media for growth and production of antimicrobial compounds by Enterococcus faecium 135", XXIV

Semana Farmacêutica de Ciência e, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo.

Produções

Produção bibliográfica

Artigos completos publicados em periódicos

Ordenar por

Ordem Cronológica

1. **PIAZENTIN, ANNA CAROLINA MEIRELES**; MENDONÇA, CARLOS MIGUEL NÓBREGA ; VALLEJO, MARISOL ; MUSSATTO, SOLANGE I. ; DE SOUZA OLIVEIRA, RICARDO PINHEIRO . Bacteriocin-like inhibitory substances production by Enterococcus faecium 135 in co-culture with Ligilactobacillus salivarius and Limosilactobacillus reuteri. BRAZILIAN JOURNAL OF MICROBIOLOGY (ONLINE) JCR, v. 1, p. 1, 2022.
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   Citações: WEB OF SCIENCE ™ 1
5. SIMÕES DA SILVA, THAMIRES MARIA ; **PIAZENTIN, ANNA CAROLINA MEIRELLES** ; MENDONÇA, CARLOS MIGUEL NÓBREGA ; CONVERTI, ATTILIO ; BOGSAN, CRISTINA STEWART BITTENCOURT ; MORA, DIEGO ; DE SOUZA OLIVEIRA, RICARDO PINHEIRO . Buffalo milk increases viability and resistance of probiotic bacteria in dairy beverages under in vitro simulated gastrointestinal conditions. JOURNAL OF DAIRY SCIENCE JCR, v. 103, p. 7890-7897, 2020.

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6. **PIAZENTIN, ANNA CAROLINA MEIRELES**; DA SILVA, THAMIRES MARIA SIMÕES ; FLORENCE-FRANCO, ANA CAROLINA ; BEDANI, RAQUEL ; CONVERTI, ATTILIO ; DE SOUZA OLIVEIRA, RICARDO PINHEIRO . Soymilk fermentation: effect of cooling protocol on cell viability during storage and in vitro gastrointestinal stress. BRAZILIAN JOURNAL OF MICROBIOLOGY (ONLINE) JCR, v. 51, p. 1645-1654, 2020.

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Resumos publicados em anais de congressos

1. **PIAZENTIN, A. C. M.**; Mussatto, S. I. ; OLIVEIRA, R. P. S. . Use of spent coffee grounds as alternative media for growth and production of antimicrobial compounds by Enterococcus faecium 135. In: 31º Congresso Brasileiro de Microbiologia, 2021. Anais do 31º Congreso Brasileiro de Microbiologia 2021, 2021.
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5. FARINHA, L. R. L. ; **PIAZENTIN, A. C. M.** ; AZEVEDO, P. O. S. ; OLIVEIRA, R. P. S. . Acidification kinectic and growth of Streptococcus thermophilus TA040 and Lactococcus lactis CECT4434 from whey. In: 28º Congresso Brasileiro de Microbiologia, 2015, Florianópolis. ANAIS DO 28º CBM 2015, 2015.
6. PORTO, M. C. W. ; AZEVEDO, P. O. S. ; **PIAZENTIN, A. C. M.** ; OLIVEIRA, R. P. S. . EVALUATION OF Pediococcus pentosaceus ATCC 43200 GROWTH AND BIOPRODUCTION OF ANTIMICROBIAL COMPOUND IN DIFFERENTS GROWTHS SUPPLEMENTED BY POLYDEXTROSE, CARBON SOURCE SYNTHETIC WITH PREBIOTIC EFFECT. In: 28º Congresso Brasileiro de Microbiologia, 2015, Florianópolis. ANAIS DO 28º CBM 2015, 2015.

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1. **PIAZENTIN, A. C. M.**; Mussatto, S. I. ; OLIVEIRA, R. P. S. . Use of spent coffee grounds as alternative media for growth and production of antimicrobial compounds by Enterococcus faecium 135. 2021. (Apresentação de Trabalho/Outra).
2. **PIAZENTIN, A. C. M.**; OLIVEIRA, R. P. S. . Produção de biomoléculas antimicrobianas por bactérias ácidos láticas contra Salmonella spp. Listeria monocytogenes. 2019. (Apresentação de Trabalho/Congresso).
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8. **PIAZENTIN, A. C. M.**; SILVA, T. M. S. ; OLIVEIRA, R. P. S. . Influence of fermentation temperature on viability of yoghurt cultures and Lactobacillus paracasei Lpc-37 using soy exctract medium. 2015. (Apresentação de Trabalho/Congresso).
9. FARINHA, L. R. L. ; **PIAZENTIN, A. C. M.** ; AZEVEDO, P. O. S. ; OLIVEIRA, R. P. S. . Acidificaton kinetic and growth of Streptococcus thermophilus TA 040 and Lactococcus lactis CECT 4434 from whey. 2015. (Apresentação de Trabalho/Congresso).
10. PORTO, M. C. W. ; AZEVEDO, P. O. S. ; **PIAZENTIN, A. C. M.** ; OLIVEIRA, R. P. S. . Evaluation of Pediococcus pentosaceus ATCC 43200 growth and bioproduction of antimicrobial compound in diferents growths supplemented by polydextrose, carbon source synthetic with prebiotic effect. 2015. (Apresentação de Trabalho/Congresso).
11. **PIAZENTIN, A. C. M.**; ALBUQUERQUE, M. A. A. ; FREITAS, T. ; SILVA, T. M. S. ; PESANHA, M. ; AUAD, I. ; OLIVEIRA, M. N. . Effect of inulin suplementation in the growth of Streptococcus thermephilus and Bifidobacterium animalis subsp. lactis BB-12 in fermented milks. 2015. (Apresentação de Trabalho/Outra).
12. **PIAZENTIN, A. C. M.**; SILVA, T. M. S. ; ALBUQUERQUE, M. A. A. ; FREITAS, T. ; OLIVEIRA, M. N. . Efeito da adição de inulina sobre o crescimento de Streptococcus thermpphilus e Bifidobacterium animalis subsp. lactis BB-12 em leites fermentados simbióticos. 2015. (Apresentação de Trabalho/Congresso).

Demais tipos de produção técnica

1. **PIAZENTIN, A. C. M.**. Biprocessos, Controle de Qualidade e Atividade Prática de Antimicrobianos. 2019. (Curso de curta duração ministrado/Outra).
2. **PIAZENTIN, A. C. M.**. Tecnologia de Iogurtes e elaboração de novos produtos. 2015. (Curso de curta duração ministrado/Outra).
3. SILVA, T. M. S. ; **PIAZENTIN, A. C. M.** . Tecnologia de iogurtes e elaboração de novos produtos. 2015. (Curso de curta duração ministrado/Outra).

Bancas

Participação em bancas de comissões julgadoras

Outras participações

1. **PIAZENTIN, A. C. M.**. Participou como membro da Comissão de Avaliação dos trabalhos apresentados na 19ª Feira Brasileira de Ciência e Engenharia. 2021. Universidade de São Paulo.
2. **PIAZENTIN, A. C. M.**. Participou como membro da Comissão de Avaliação dos trabalhos apresentados na 18ª Feira Brasileira de Ciência e Engenharia. 2020. Universidade de São Paulo.
3. **PIAZENTIN, A. C. M.**. Participou como membro da Comissão de Avaliação dos trabalhos apresentados na 17ª Feira Brasileira de Ciência e Engenharia. 2019. Universidade de São Paulo.

Apresentações de Trabalho

1. **PIAZENTIN, A. C. M.**; OLIVEIRA, R. P. S. . Produção de biomoléculas antimicrobianas por bactérias ácidos láticas contra Salmonella spp. Listeria monocytogenes. 2019. (Apresentação de Trabalho/Congresso).
2. **PIAZENTIN, A. C. M.**; SILVA, T. M. S. ; OLIVEIRA, R. P. S. . Antimicrobial activity of two biomolecules produced by two lactic acid bacteria and their action against Listeria monocytogenes and Salmonella spp.. 2019. (Apresentação de Trabalho/Congresso).

Cursos de curta duração ministrados

1. **PIAZENTIN, A. C. M.**. Tecnologia de Iogurtes e elaboração de novos produtos. 2015. (Curso de curta duração ministrado/Outra).
2. **PIAZENTIN, A. C. M.**. Biprocessos, Controle de Qualidade e Atividade Prática de Antimicrobianos. 2019. (Curso de curta duração ministrado/Outra).

Organização de eventos, congressos, exposições e feiras

1. MONTEIRO, G. ; TAVARES, L. C. ; OROZCO, A. ; SILVA, A. R. S. ; **PIAZENTIN, A. C. M.** ; MENDONCA, C. M. N. ; KLEINGESINDS, E. K. ; FREITAS, F. P. P. ; CHAVES, F. S. ; SILVA, R. R. O. . IV Curso de Inverno. 2019. (Outro).