Bio-Detoxification of Mycotoxins by Lactic Acid Bacteria from Different Food Matrices

Authors : António Inês, Ana Guimarães, José Maria, Vânia Laranjo, Armando Venâncio, Luís Abrunhosa

Abstract : Lactic acid bacteria (LAB) play a key role in the biopreservation of a wide range of fermented food products, such as yogurt, cheese, fermented milks, meat, fish, vegetables (sauerkraut, olives and pickles), certain beer brands, wines and silage, allowing their safe consumption, which gave to these bacteria a GRAS (Generally Recognised as Safe) status. Besides that, the use of LAB in food and feed is a promising strategy to reduce the exposure to dietary mycotoxins, improving their shelf life and reducing health risks, given the unique mycotoxin decontaminating characteristic of some LAB. Mycotoxins present carcinogenic, mutagenic, teratogenic, neurotoxic and immunosuppressive effects over animals and Humans, being the most important ochratoxin A (OTA), aflatoxins (AFB1), trichothecenes, zearalenone (ZEA), fumonisin (FUM) and patulin. In a previous work of our group it was observed OTA biodegradation by some strains of Pediococcus parvulus isolated from Douro wines. So, the aim of this study was to enlarge the screening of the biodetoxification over more mycotoxins besides OTA, including AFB1, and ZEA. This ability was checked in a collection of LAB isolated from vegetable (wine, olives, fruits and silage) and animal (milk and dairy products, sausages) sources. All LAB strains were characterized phenotypically (Gram, catalase) and genotypically. Molecular characterisation of all LAB strains was performed using genomic fingerprinting by MSP-PCR with (GTG)5 and csM13 primers. The identification of the isolates was confirmed by 16S rDNA sequencing. To study the ability of LAB strains to degrade OTA, AFB1 and ZEA, a MRS broth medium was supplemented with 2.0 µg/mL of each mycotoxin. For each strain, 2 mL of MRS supplemented with the mycotoxins was inoculated in triplicate with 109 CFU/mL. The culture media and bacterial cells were extracted by the addition of an equal volume of acetonitrile/methanol/acetic acid (78:20:2 v/v/v) to the culture tubes. A 2 mL sample was then collected and filtered into a clean 2 mL vial using PP filters with 0.45 µm pores. The samples were preserved at 4 °C until HPLC analysis. Among LAB tested, 10 strains isolated from milk were able to eliminate AFB1, belonging to Lactobacillus casei (7), Lb. paracasei (1), Lb. plantarum (1) and 1 to Leuconostoc mesenteroides. Two strains of Enterococcus faecium and one of Ec. faecalis from sausage eliminated ZEA. Concerning to strains of vegetal origin, one Lb. plantarum isolated from elderberry fruit, one Lb. buchnerii and one Lb. parafarraginis both isolated from silage eliminated ZEA. Other 2 strains of Lb. plantarum from silage were able to degrade both ZEA and OTA, and 1 Lb. buchnerii showed activity over AFB1. These enzymatic activities were also verified genotypically through specific gene PCR and posteriorly confirmed by sequencing analysis. In conclusion, due the ability of some strains of LAB isolated from different sources to eliminate OTA, AFB1 and ZEA one can recognize their potential biotechnological application to reduce the health hazards associated with these mycotoxins. They may be suitable as silage inoculants or as feed additives or even in food industry.

Keywords : bio-detoxification, lactic acid bacteria, mycotoxins, food and feed
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BIODETOXIFICATION OF MYCOTOXINS BY LACTIC ACID BACTERIA FROM DIFFERENT FOOD MATRICES

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Lactic acid bacteria LAB play a key role in the biopreservation of a wide range of fermented food products, such as yogurt, cheese, fermented milks, meat, fish, vegetables (sauerkraut, olives and pickles), certain beer brands, wines and silage [1], allowing their safe consumption, which gave to these bacteria a GRAS (Generally Recognised as Safe) status. Besides that, the use of LAB in food and feed is a promising strategy to reduce the exposure to dietary mycotoxins, improving their shelf life and reducing health risks, given the unique mycotoxin decontaminating characteristic of some LAB. Mycotoxins present carcinogenic, mutagenic, teratogenic, neurotoxic and immunosuppressive effects over animals and Humans [2], being the most important ochratoxin A (OTA), aflatoxins (AFB1), trichothecenes, zearalenone (ZEA), fumonisin and patulin. In a previous work of our group it was observed OTA biodegradation by some strains of *Pediococcus parvulus* isolated from Douro wines [3].

So, the aim of this study was to enlarge the screening of the biodetoxification over more mycotoxins besides OTA, including AFB1, and ZEA. This ability was checked in a collection of LAB isolated from vegetable (wine, olives, fruits and silage) and animal (milk and dairy products, sausages) sources. All LAB strains were characterized phenotipically (Gram, catalase) and genotipically. Molecular characterisation of all LAB strains was performed using genomic fingerprinting by MSP-PCR with $(GTG)_5$ and csM13 primers. The identification of the isolates was confirmed by 16S rDNA sequencing.

To study the ability of LAB strains to degrade OTA, AFB1 and ZEA, a MRS broth medium that was supplemented with 2.0 µg/mL of each mycotoxin was prepared. For each strain, 2 mL of MRS supplemented with the mycotoxins was inoculated in triplicate with 10^9 CFU/mL. The culture media and bacterial cells were extracted by the addition of an equal volume of acetonitrile/methanol/acetic acid (78:20:2 v/v/v)to the culture tubes. A 2 mL sample was then collected and filtered into a clean

2 mL vial using PP filters with 0.45 µm pores. The samples were preserved at 4 °C until HPLC analysis performed according to Abrunhosa e Venâncio [4]. Among LAB tested, 10 strains isolated from milk were able to eliminated AFB1, belonging to *Lactobacillus casei* (7), *Lb. paracasei (*1), *Lb. plantarum* (1) and 1 to *Leuconostoc mesenteroides*. Two strains of *Enterococcus faecium* and one of *Ec. faecalis* from sausage eliminated ZEA. Concerning to strains of vegetal origin, one *Lb. plantarum* isolated from elderberry fruit, one *Lb. buchnerii* and one *Lb. parafarraginis* both isolated from silage eliminated ZEA. Other 2 strains of *Lb. plantarum* from silage were able to degrade both ZEA and OTA, and 1 *Lb. buchnerii* showed activity over AFB1.

In conclusion, due the ability of some strains of LAB isolated from different sources to eliminate OTA, AFLA and ZEA one can recognize their potential biotechnological application to reduce the health hazards associated with these mycotoxins. They may be suitable as silage inoculants or as feed additives or even in food industry.

References

[1] M.E. Stiles, 1996. Biopreservation by lactic acid bacteria. Antonie Van Leeuwenhoek, 70 (1996), pp. 331–345

[2] J.W. Bennett, M. Klich 2003. Mycotoxins. Clin. Microbiol. Rev., 16 (2003), pp. 497–516.

[3] Abrunhosa L, Inês A, Rodrigues AI, Guimarães A, Pereira VL, Parpot P, Mendes-Faia A, Venâncio A. 2014. Intenational Journal of Food Microbiololgy, 188, 1: 45-52.

[4] L. Abrunhosa, A. Venâncio, 2007. Isolation and purification of an enzyme hydrolyzing ochratoxin A from *Aspergillus niger*. Biotechnology Letters, 2007, 29, 1909-1914.

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INTRODUCTION

Lactic acid bacteria (LAB) play a key role in the biopreservation of a wide range of fermented food products, such as yogurt, cheese, fermented milks, meat, fish, vegetables (sauerkraut, olives and pickles), certain beer brands, wines and silage

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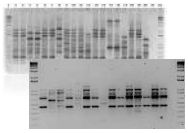
In a previous work of our group it was observed OTA biodegradation by some strains of *Pediococcus parvulus* isolated from Douro wines

OBJECTIVE

So, the aim of this study was to enlarge the screening of the biodetoxification over more mycotoxins besides OTA, including AFB1, and ZEA. This ability was checked in a collection of LAB isolated from vegetable (wine, olives, fruits and silage) and animal (milk and dairy products, sausages) sources

MATERIAL AND METHODS

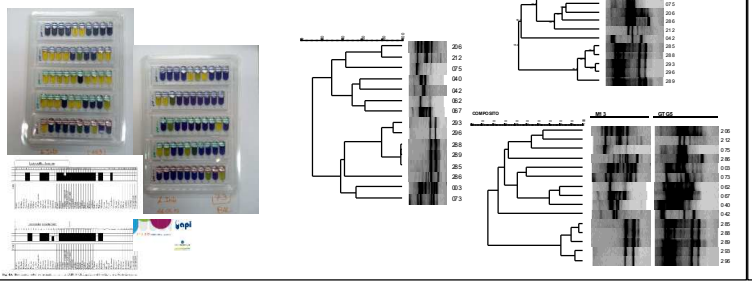
Global strategy for species identification and characterization of the LAB collection



Identification to species level by genotypic methods

RESULTS AND CONCLUSION

Identification at species level and strain characterization of the lactic acid bacteria collection by phenotypic and genotypic approach



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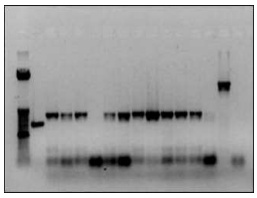
RESULTS AND CONCLUSION

Source	*Species*	Mycotoxins
Milk	*Lactobacillus casei* (7)	AFB1
	Lb. paracasei (1)	
	Lb. plantarum (1)	
	Leuconostoc mesenteroides (1)	
Sausage	*Enterococcus faecium* (1)	ZEA
	En. faecalis (1)	
Elderberry fruit	*Lb. plantarum*(1)	ZEA
Silage	*Lb. buchneril* (1)	
	Lb. parafarraginis (1)	
	Lb. plantarum (2)	ZEA and OTA
	Lb. buchnerii (1)	AFB1

OTA degradation:
Carboxypeptidase



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