

New Bio-Strategies for Ochratoxin a Detoxification Using Lactic Acid Bacteria

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Abstract : The occurrence of mycotoxigenic moulds such as *Aspergillus*, *Penicillium* and *Fusarium* in food and feed has an important impact on public health, by the appearance of acute and chronic mycotoxicoses in humans and animals, which is more severe in the developing countries due to lack of food security, poverty and malnutrition. This mould contamination also constitutes a major economic problem due the lost of crop production. A great variety of filamentous fungi is able to produce highly toxic secondary metabolites known as mycotoxins. Most of the mycotoxins are carcinogenic, mutagenic, neurotoxic and immunosuppressive, being ochratoxin A (OTA) one of the most important. OTA is toxic to animals and humans, mainly due to its nephrotoxic properties. Several approaches have been developed for decontamination of mycotoxins in foods, such as, prevention of contamination, biodegradation of mycotoxins-containing food and feed with microorganisms or enzymes and inhibition or absorption of mycotoxin content of consumed food into the digestive tract. Some group of Gram-positive bacteria named lactic acid bacteria (LAB) are able to release some molecules that can influence the mould growth, improving the shelf life of many fermented products and reducing health risks due to exposure to mycotoxins. Some LAB are capable of mycotoxin detoxification. Recently our group was the first to describe the ability of LAB strains to biodegrade OTA, more specifically, *Pediococcus parvulus* strains isolated from Douro wines. The pathway of this biodegradation was identified previously in other microorganisms. OTA can be degraded through the hydrolysis of the amide bond that links the L- β -phenylalanine molecule to the ochratoxin alpha (OT α) a non toxic compound. It is known that some peptidases from different origins can mediate the hydrolysis reaction like, carboxypeptidase A an enzyme from the bovine pancreas, a commercial lipase and several commercial proteases. So, we wanted to have a better understanding of this OTA degradation process when LAB are involved and identify which molecules were present in this process. For achieving our aim we used some bioinformatics tools (BLAST, CLUSTALX2, CLC Sequence Viewer 7, Finch TV). We also designed specific primers and realized gene specific PCR. The template DNA used came from LAB strains samples of our previous work, and other DNA LAB strains isolated from elderberry fruit, silage, milk and sausages. Through the employment of bioinformatics tools it was possible to identify several proteins belonging to the carboxypeptidase family that participate in the process of OTA degradation, such as serine type D-Ala-D-Ala carboxypeptidase and membrane carboxypeptidase. In conclusions, this work has identified carboxypeptidase proteins being one of the molecules present in the OTA degradation process when LAB are involved.

Keywords : carboxypeptidase, lactic acid bacteria, mycotoxins, ochratoxin a.

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NEW BIO-STRATEGIES FOR OCHRATOXIN A DETOXIFICATION USING LACTIC ACID BACTERIA

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KEYWORDS: Mycotoxins, lactic acid bacteria and carboxypeptidase

The occurrence of mycotoxigenic moulds such as *Aspergillus*, *Penicillium* and *Fusarium* in food and feed has an important impact on public health, by the appearance of acute and chronic mycotoxicoses in humans and animals, which is more severe in the developing countries due to lack of food security, poverty and malnutrition. This mould contamination also constitutes a major economic problem due the lost of crop production. A great variety of filamentous fungi is able to produce highly toxic secondary metabolites known as mycotoxins. Most of the mycotoxins are carcinogenic, mutagenic, neurotoxic and immunosuppressive, being ochratoxin A (OTA) one of the most important. OTA is toxic to animals and humans, mainly due to its nephrotoxic properties. Several approaches have been developed for decontamination of mycotoxins in foods, such as, prevention of contamination, biodegradation of mycotoxins-containing food and feed with microorganisms or enzymes and inhibition or absorption of mycotoxin content of consumed food into the digestive tract. Some group of Gram-positive bacteria named lactic acid bacteria (LAB) are able to release some molecules that can influence the mould growth, improving the shelf life of many fermented products and reducing health risks due to exposure to mycotoxins. Some LAB are capable of mycotoxin detoxification. Recently our group was the first to describe the ability of LAB strains to biodegrade OTA, more specifically, *Pediococcus parvulus* strains isolated from Douro wines [1]. The pathway of this biodegradation was identified previously in other microorganisms [2]. OTA can be degraded through the hydrolysis of the amide bond that links the L- β -phenylalanine molecule to the ochratoxin alpha (OT α) a non toxic compound. It is known that some peptidases from different origins can mediate the hydrolysis reaction like, carboxypeptidase A an enzyme from the bovine pancreas, a commercial lipase and several commercial

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References

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[2] Abrunhosa L, Paterson RRM, Venâncio A 2010. *Toxins*. 2: 1078-1099.

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Introduction

- Mould contamination constitutes a major economic problem due the lost of crop production
- The occurrence of mycotoxigenic moulds in food and feed has an important impact on public health



Aspergillus



Penicillium



Fusarium

- Most of the mycotoxins are carcinogenic, mutagenic, neurotoxic and immunosuppressive, being ochratoxin A (OTA) one of the most important

Introduction

- Several approaches have been developed for decontamination of mycotoxins in foods:
 - microorganisms or enzymes
 - inhibition or absorption of mycotoxins
 - biodegradation of mycotoxins



Objective

To have a better understanding of OTA degradation process when LAB is involved and identify which molecules were present in this process

Methods

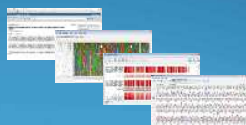
MOLECULAR BIOLOGY TOOLS

- DNA EXTRACTION
- PCR
- ELECTROPHORESIS GEL

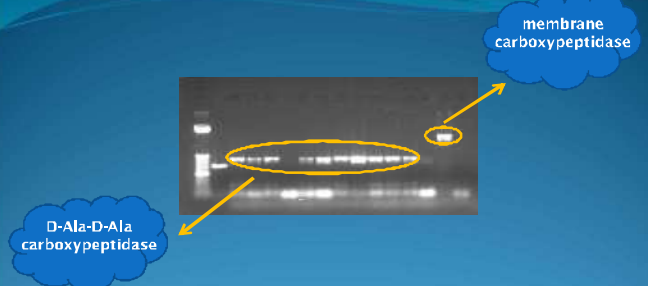


BIOINFORMATIC TOOLS

- BLAST
- CLUSTALX2
- CLC SEQUENCE VIEWER 7
- FINCH TV



Results and Conclusions



Carboxypeptidase proteins were identified being one of the molecules present in the OTA degradation process when LAB is involved



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