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Degradation of Ochratoxin A by *Brevibacterium* Species

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ABSTRACT: The ability to degrade ochratoxin A was studied in different bacteria with a well-known capacity to transform aromatic compounds. Strains belonging to *Rhodococcus*, *Pseudomonas*, and *Brevibacterium* genera were grown in liquid synthetic culture medium containing ochratoxin A. *Brevibacterium* spp. strains showed 100% degradation of ochratoxin A. Ochratoxin α was detected and identified by high-performance liquid chromatography−mass spectrometry (HPLC-MS) as a degradation product in the cell-free supernatants. The degradation of ochratoxin A is of public concern for food and environmental safety, because it could contribute to the development of new biological ochratoxin A detoxification systems in foodstuffs. In this study, the degradation of ochratoxin A by bacteria belonging to the food chain was demonstrated for the first time.

KEYWORDS: Ochratoxin A, ochratoxin α, *Brevibacterium*, degradation, mycotoxin

INTRODUCTION

Mycotoxins are well-known natural contaminants in foods. Among the more than 300 mycotoxins isolated and described until now, ochratoxin A is one of the most important not only because of its toxicological potential but also because of its widespread distribution that leads to common exposure. Ochratoxin A, an isocoumarin derivative linked via the carboxyl group to L-β-phenylalanine, is produced by several species of fungi belonging principally to the genera *Aspergillus* and *Penicillium*. These fungi are capable of growing under a wide range of conditions, including moisture, pH, and temperature, and on a variety of foods, such as cereals, coffee, fruits, cocoa, nuts, spices, and grapes.[1,2] Cereals and cereal derivatives remain the major contributors to ochratoxin A human and animal exposure.[3]

Ochratoxin A is nephrotoxic, teratogenic, hepatotoxic, and carcinogenic (IARC group 2B) in animals, and it is suspected that these effects can also occur in humans (Joint FAO/WHO Expert Committee on Food Additives, JECFA). Ochratoxin A has been detected in human blood after the consumption of contaminated foods,[4] and there is evidence supporting its involvement in the pathogenesis of Balkan endemic nephropathy.[5]

Aside from the health and toxicological perspective, ochratoxin A exposure also has an impact on livestock economics, where the associated decrease in productivity (milk, eggs, and loss of weight) and the increase of mortality rate are of concern.[3] In fact, levels of ochratoxin A found in food products or beverages have been strictly fixed (ranging from 2 to 10 μg/kg) by the European Union in cereals, dried vine fruits, roasted coffee beans, ground coffee, soluble coffee, wine, and grape juice (European Commission Regulation (EC) No. 1881/2006 of 19 December 2006), and the World Health Organization (WHO) has proposed a maximum limit of 5 μg/kg of ochratoxin A in cereals (Fifty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives).

To protect consumers from the risk of exposure to this mycotoxin, reliable methods are needed for reducing the levels of ochratoxin A in foodstuffs. In this regard, it has been shown that decaffeination with solvents significantly reduced ochratoxin A levels in coffee,[6] and it has also been suggested that contaminated grains can be detoxified of ochratoxin A by ozone treatment.[7] Likewise, several studies have focused on the reduction of ochratoxin A in musts and wines in the winery, and different decontamination procedures based on either physical, chemical, or biological removal have been proposed.[8,9]

With regard to the biological degradation of ochratoxin A, enzymes with carboxypeptidase A activity (CPA), such as that obtained from bovine pancreas, can hydrolyze the amide bond in the ochratoxin A molecule with the production of L-β-phenylalanine and ochratoxin α (Figure 1), which is less toxic than ochratoxin A.[10,11] Likewise, degradation of ochratoxin A by *Phenylobacterium immobile*,[12] by *Acinetobacter calcoaceticus*,[13] and by *Aspergillus* species[14] by a mechanism of action similar to that of CPA have been reported. More recently, a protein produced by the fungus *Aspergillus niger* able to hydrolyze ochratoxin A to L-β-phenylalanine and ochratoxin α has been isolated.[15] Furthermore, this mechanism also has been implicated in the capability of *Trichosporon mycotoxinivorans* to degrade ochratoxin A.[16] Therefore, biological degradation of toxins is a very promising approach for the decontamination of foodstuffs, as use of chemical or physical tools in food may also remove, along with the mycotoxin, other organoleptic important substances and nutrients.

On the other hand, soil bacteria, such as *Pseudomonas* spp. and actinobacteria, are able to transform a wide range of aromatic compounds, and thus, they are considered to play a crucial role in the biodegradation of toxic pollutants in soil.[17,18] For instance, among actinobacteria, *Rhodococcus* strains are able to degrade a large variety of organic compounds. In fact, over 200 genes that encode for oxygenases and more than 30 metabolic pathways

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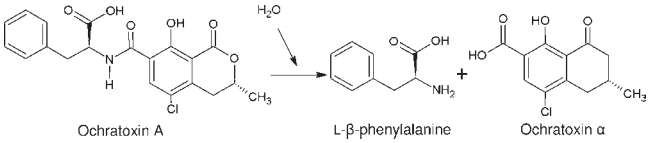

Figure 1. Conversion of ochratoxin A in L-β-phenylalanine and ochratoxin α.

involved in the catabolism of aromatic compounds have been identified in the complete genome sequence of *Rhodococcus jostii* RHA1.[19] Moreover, it has recently been reported that cell extracts of *Rhodococcus erythropolis* can degrade aflatoxin B1, a mycotoxin that also possesses an aromatic ring in its structure.[20] Furthermore, some species from another actinobacteria genus, *Brevibacterium*, that are usually found in milk and cheese curd, such as *Brevibacterium casei*, *Brevibacterium iodinum*, and *Brevibacterium linens* (the usual habitat of which is the exterior of surface-ripened cheeses such as Limburger, Romadour, Munster, Tilsiter, Appenzeller, Gruyère, Brick, and others) have also been isolated from soil. In addition to their versatility, another important characteristic of the actinobacteria from the genus *Brevibacterium* is their extracellular proteinase production. Intracellular and extracellular cell wall-associated proteinases have been reported for *B. linens*, but most studies focused on the latter largely because of their high activity and importance with respect to cheese ripening.[21]

As mentioned above, given its potential health hazard, the presence of ochratoxin A in foods is of considerable public concern for the food industry and regulatory agencies, and consequently, there is a growing demand from control authorities to reduce the allowable limits of ochratoxin A in foods and beverages. Therefore, on the basis of the aforementioned characteristics of some microorganisms, in this study, we screened bacteria with the ability to degrade aromatic compounds, such as *Pseudomonas putida* and several actinobacteria from the genera *Rhodococcus* and *Brevibacterium*, for their ability to degrade ochratoxin A.

MATERIALS AND METHODS

Microorganisms and Growth Conditions. Cultures of the strains *R. erythropolis* CECT 3008, *R. erythropolis* IGTS8, *P. putida* DSM 291, *P. putida* KT2442, and seven strains of *Brevibacterium* species were screened for their ability to degrade ochratoxin A. *R. erythropolis* CECT 3008 (DSM 43060) was purchased from the Spanish Type Culture Collection (CECT). *P. putida* DSM 291^T and the six *Brevibacterium* strains included in the study (*B. epidermidis* DSM 20660^T, *B. iodinum* DSM 20626^T, *B. linens* DSM 20425^T, *B. casei* DSM 20657^T, *B. casei* DSM 9657, and *B. casei* DSM 20658) were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). A *B. casei* strain, RM101, molecularly identified by 16S rDNA sequencing, was isolated at the Instituto de Fermentaciones Industriales IFI-CSIC. *R. erythropolis* IGTS8 and *P. putida* KT2442 strains were kindly provided by Dr. Eduardo Diaz, from the Centro de Investigaciones Biológicas, CSIC, Spain.

All bacteria assayed were routinely grown in Luria−Bertani broth (LB) supplemented with 0.5% glucose and incubated at 30 °C under aerobic conditions. For the ochratoxin A degradation assay, bacteria were grown in a basal salts medium (BSM) that contained 0.2% glycerol, 4 g of $NaH_2PO_4 \cdot H_2O$, 4 g of $K_2HPO_4 \cdot 3H_2O$, 2 g of NH_4Cl, 0.2 g of $MgCl_2 \cdot 6H_2O$, 0.001 g of $CaCl_2 \cdot 2H_2O$, and 0.001 g of $FeCl_3 \cdot 6H_2O$.[22] Glycerol was omitted from experiments designed to determine the potential use of ochratoxin A as a carbon source by the bacteria assayed.

Standards. Ochratoxin A (Sigma Chemical Co., Poole, Dorset, United Kingdom) was suspended in 99% methanol under sterile conditions to make a stock solution of 500 mg/L. A working solution of 1 mg/L was prepared by dilution with water high-performance liquid chromatography (HPLC) grade/methanol 99% (95:5 by volume). A standard solution of 11.9 mg/L of ochratoxin α (LGC Standards, Wesel, Germany) was diluted with acetonitrile (1:1, v/v) to make a working solution of 5.9 mg/L.

L-Phenylalanine (VWR, Milano, Italy) was suspended in 0.1 M HCl to make a stock solution of 1 g/L. The standard solution was made in 75% methanol and contained 15 mg/L of L-phenylalanine. Norvaline (Sigma Chemical Co.) was suspended in 0.1 M HCl to make a stock solution of 500 mg/L.

Ochratoxin A Degradation Assay. Actinobacteria (*R. erythropolis* CECT 3008, *R. erythropolis* IGTS8, and *B. casei* RM101) and *Pseudomonas* spp. (*P. putida* DSM 291^T and *P. putida* KT2442) strains were grown in 25 mL of BSM containing ochratoxin A (about 11 μg/L) under aerobic conditions at 30 °C until the late exponential growth phase, a time at which the supernatants were collected for the analysis of ochratoxin A by HPLC. The medium used for this assay was prepared by diluting 100 times the ochratoxin A working solution (1 mg/L), using 297 mL of BSM and 3.0 mL of ochratoxin A working solution. To confirm ochratoxin A degradation by *Brevibacterium* spp. strains, the bacterial strains were grown in BSM containing a 4-fold increase on the OTA concentration (about 40 μg/L) under aerobic conditions on a rotary shaker at 150 rpm for 10 days. The medium used for this assay was prepared by diluting 25 times the ochratoxin A working solution (1 mg/L), using 576 mL of BSM and 24 mL of ochratoxin A working solution. To determine the ability of *Brevibacterium* spp. (*B. casei* RM101 and *B. linens* DSM 20425^T strains) to degrade ochratoxin A at higher concentrations (40 mg/L, 1000 times greater than previously assayed) and to assess the use of ochratoxin A as a carbon source, bacteria were grown in BSM with or without glycerol (0.2%) and ochratoxin A (40 mg/L), and ochratoxin A degradation was verified after 10 days of incubation at 30 °C. In addition, to determine the time course of ochratoxin A degradation and to quantitate ochratoxin α production, samples from *B. casei* RM101 strain grown in BSM containing ochratoxin A (25 mg/L), with or without glycerol (0.2%), were collected twice daily and analyzed by HPLC. In all of these degradation assays, culture supernatants were separated by centrifugation at 3000*g* for 10 min at 4 °C and further analyzed by HPLC. The *Brevibacterium* spp. cell pellets were stored at −80 °C for subsequent analysis. BSM controls with ochratoxin A and without bacteria were always prepared.

Ochratoxin A, Ochratoxin α, and L-Phenylalanine Quantitation by HPLC. The concentration of ochratoxin A in supernatants, pellets, and pellet's washing solutions was quantified by HPLC as described[23] using a Hewlett-Packard I model 1100 (Hewlett-Packard, Palo Alto, CA), equipped with a degasser, quaternary pump, autosampler, UV/vis (DAD), and fluorescence (FLD) detector. The column used was a 200 mm × 4.6 mm i.d., 5 μm, Alltima C18, with a 7.5 mm × 4.6 mm i.d. guard column of the same material (Alltech, Deerfield, IL). The mobile phase was as follows: solvent A:B = 37:63 (v/v) (isocratic method); solvent (A), acetonitrile; solvent (B), water (HPLC grade)/acetonitrile/glacial acetic acid (89:10:1 by volume); flow, 1.3 mL/min; analysis temperature, 30 °C; analysis time, 20 min; FLD detector (λ_{ex} = 330 nm, λ_{em} = 460 nm); and injection volume, 100 μL. The limit of detection for ochratoxin A in the aforementioned conditions is 0.02 μg/L. Quantitation of ochratoxin A was carried out by a calibration curve, which was constructed by injecting five standard solutions containing ochratoxin A in mobile phase at concentrations from 10 to 200 μg/L. Ochratoxin α was determined with the same chromatographic conditions by using a response factor for quantitation. The limit of detection for ochratoxin α is 0.03 μg/L. Samples were appropriated, diluted with solvent A:B, and filtered through a 0.45 μm glass microfiber filter

Table 1. Reduction in Concentration of Ochratoxin A in BSM by Actinobacteria and *Pseudomonas* spp.

strain	ochratoxin A (μg/L)[a]	ochratoxin A reduction (%)
BSM + OTA (control)	11.01	0
R. erythropolis CECT 3008	7.88	28.47
R. erythropolis IGTS8	8.81	19.98
B. casei RM101	ND[b]	100
P. putida DSM 291^T	10.07	8.54
P. putida KT2442	8.18	25.70

[a] Results are expressed as mean values of two experimental replications for each strain. [b] ND, not detected.

Table 2. Reduction in Concentration of Ochratoxin A in BSM by *Brevibacterium* spp.

strain	ochratoxin A (μg/L)[a]	ochratoxin A reduction (%)
BSM + OTA (control)	39.81	0
B. casei DSM 20657^T	ND[b]	100
B. casei DSM 9657	ND	100
B. casei DSM 20658	ND	100
B. casei RM101	ND	100
B. linens DSM 20425^T	ND	100
B. iodinum DSM20626^T	ND	100
Brevibacterium epidermidis DSM 20660^T	ND	100

[a] Determined from supernatants of three independent cultures. [b] ND, not detected.

(Whatman, Maidstone Kent, United Kingdom) before their analysis by HPLC.

The *Brevibacterium* spp. bacterial pellets were resuspended twice in 2 mL of absolute methanol for 1 h to extract the ochratoxin A. After centrifugation at 3000*g* for 15 min at 20 °C, the methanolic supernatants were separated, collected in 5 mL vials, and evaporated to dryness with a stream of dry nitrogen gas. The dry residues were reconstituted with the mobile phase immediately before analysis for the determination of ochratoxin A concentration.

The concentration of L-phenylalanine in supernatants was quantitated by using the same instrument and column mentioned for ochratoxin A analysis. Briefly, samples were subjected to automatic precolumn derivatization using *o*-phthalaldehyde reagent (Agilent Technologies, Palo Alto, CA). Two eluents were used as mobile phases: eluent A (1.224 g of $NaOAc \cdot 3H_2O$, 500 mL of water, 0.09 mL of triethylamine, and 1.5 mL of tetrahydrofuran) and eluent B (1.088 g of $NaOAc \cdot 3H_2O$, 100 mL of water, 200 mL of acetonitrile, and 200 mL of methanol). A 65 min gradient program started with an initial concentration of 10% eluent B at a flow rate of 0.45 mL/min and finished with 100% eluent B at a flow rate of 0.70 mL/min. The fluorescence wavelengths were 340 nm for excitation and 450 nm for emission. Norvaline (15 mg/L) was added to supernatant samples as an internal standard. Samples were diluted 1:1 with methanol and filtered through a 0.2 μm pore-size filter (VWR International LLC, West Chester, PA) before injection. The injection volume was 5 μL. Quantitation was made by means of the relative response factor. The limit of detection for L-phenylalanine is 0.5 mg/L.

HPLC-DAD/ESI-MS. A Hewlett-Packard series 1100 MSD quadrupole mass spectrometer system equipped with an electrospray interphase (ESI) was used. Samples were introduced by direct injection. The column used was a 150 mm × 3.9 mm i.d., 4 μm, C18 Nova-Pack (Waters). The ESI parameters were as follows: drying gas, N_2; 10 L/min at 330 °C; nebulizer pressure, 40 psi; and spray capillary voltage, 4000 V. The ESI was operated in negative mode, scanning from m/z 100−800, using a variable fragmentator voltage gradient.

RESULTS AND DISCUSSION

Although biological decontamination of mycotoxins from foods by means of the use of microorganisms is one of the strategies used for the management of mycotoxins, little published information is available regarding biological methods for the removal of ochratoxin A. To address this important point and because it is known that soil bacteria are able to transform a wide range of aromatic compounds, we analyzed the presence of ochratoxin A in actinobacteria and *Pseudomonas* spp. grown in liquid BSM synthetic culture medium.

Initially, screenings of ochratoxin A degradation capacity were performed using *P. putida* and several actinobacteria from the genera *Rhodococcus* and *Brevibacterium*. Bacterial strains were grown in liquid synthetic (BSM) culture medium in the presence of ochratoxin A (about 11 μg/L). As displayed in Table 1, only 8−28% decrease in ochratoxin A concentration was recorded in the cell-free supernatants for *Rhodococcus* and *P. putida* strains, respectively, and no degradation products were observed on the HPLC chromatograms. Even though pellets were not analyzed for a confirmation, our data suggest that ochratoxin A is not degraded but adsorbed by the cells, as it has already been described for strains of lactic acid bacteria[23] and yeasts.[24] On the contrary, results in Table 1 also showed that ochratoxin A completely disappeared from the cell-free supernatants of *B. casei* RM101 strain, indicative of the presence of a mechanism of ochratoxin A degradation.

The genus *Brevibacterium* is a heterogeneous group of nine coryneform species that are capable of degrading insecticides (DTT, DDE, etc.) and produce self-processing extracellular proteases. They are found in diverse habitats, including soil, poultry, fish, human skin, and food. *Brevibacterium* species differ from other bacteria for their ability to metabolize compounds of heterocyclic and polycyclic ring structures, a trait that is also common in fungi. To analyze whether the above-mentioned observed ochratoxin A degradation is a specific characteristic of *Brevibacterium* strain, species or genus, a medium containing a high concentration of ochratoxin A (40 μg/L, rarely found in food products or beverages) was used with strains belonging to different species from the *Brevibacterium* genus. A complete disappearance of ochratoxin A was observed in all of the culture supernatants tested (Table 2); moreover, no traces of ochratoxin A were present in pellets or pellet's washing solutions after methanol extraction. Therefore, these results indicate that ochratoxin A degradation seems to be a characteristic of the *Brevibacterium* genus.

Next, to further study the mechanism of ochratoxin A degradation followed by *Brevibacterium* spp., *B. casei* RM101 and *B. linens* DSM 20425^T strains were grown at a 1000 times greater ochratoxin A concentration (40 mg/L), on different BSM medium compositions, with or without glycerol as a carbon source. Results indicated that both *Brevibacterium* strains were able to completely degrade higher ochratoxin A concentrations (40 mg/L) also in a medium devoid of a traditional carbon source such as glycerol, where growth is slight, as determined by measuring the turbidity of the media (results not shown).

Analyses of the chromatograms from the supernatant showed that while the ochratoxin A peak was absent, a new peak with a different retention time and spectrum was present in the elution profile (Figure 2). In fact, the UV/vis and fluorescence spectra of

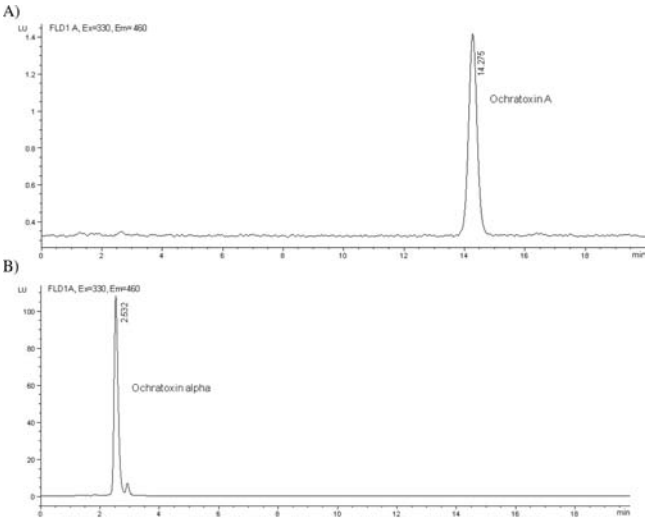

Figure 2. Chromatograms obtained from the supernatant of *B. casei* RM101 grown in BSM devoid of a carbon source but containing ochratoxin A (40 mg/L). (A) Supernatant at time 0 and (B) supernatant after 10 days of growth showing the disappearance of the ochratoxin A peak and the appearance of the ochratoxin α peak.

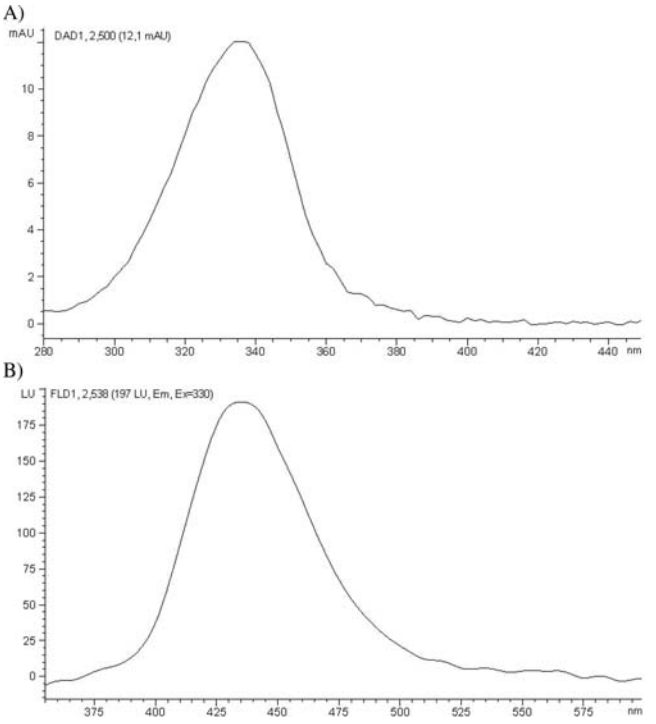

Figure 3. Ochratoxin α (A) UV/vis and (B) fluorescence spectra obtained from the supernatant of *B. casei* RM101 grown in BSM devoid of a carbon source but containing ochratoxin A (40 mg/L).

the produced compound found in the supernatants (Figure 3) were identical to the spectra corresponding to the ochratoxin α standard. Furthermore, HPLC-MS confirmed the identification of this compound, since the peak showed a molecular ion $[M - H]^-$ at m/z 255.1 in the MS (ochratoxin-α molecular weight = 256).

As quantitated by HPLC, similar amounts of ochratoxin α and L-β-phenylalanine were present in the supernatants of the two strains, *B. casei* RM101 and *B. linens* DSM 20425^T, irrespectively of the presence or not of glycerol, and these quantities corresponded to the theoretical concentration calculated from the complete hydrolysis of the ochratoxin A added to the medium. Our results indicate that L-β-phenylalanine is not further transformed by *Brevibacterium*. Furthermore, evaluation of the degradation of ochratoxin A over time showed that degradation already started 48 h after inoculation of the strain (Figure 4A). A decrease of the ochratoxin A concentration, production of ochratoxin α, and growth of *B. casei* RM101 in BSM media containing ochratoxin A, in the presence or absence of glycerol, was recorded (Figure 4). These results strongly suggest that *Brevibacterium* spp. strains possessed an enzyme, possibly a carboxypeptidase, which hydrolyzed the amide bond in the ochratoxin A molecule.

It has been reported that *B. linens* has highly active and multiple proteolytic enzymes that are mainly extracellular and intracellular proteases or peptidases.[25,26] Electrophoretic studies have shown that sonicated extracts of a *B. linens* strain have at least six different peptide hydrolases of varying dipeptide specificities.[27] In fact, zymograms obtained using 14 dipeptides of L-amino acids have shown that two peptides having a L-phenylalanine at the C terminus (Ala-Phe and Gly-Phe) were hydrolyzed by five extracts, whereas Ala-Trp and Ala-His dipeptides, containing the same N terminus, were hydrolyzed just by one of the cell extracts assayed.[27] Therefore, a possible carboxypeptidase activity acting on a peptide with L-phenylalanine as C terminus can be found in *B. linens* extracts. Moreover, two aminopeptidases purified from a culture filtrate of a *B. linens* strain[28] that hydrolyzed a variety of substrates have been shown to have specificity for N-terminal leucine. Additionally, one of the purified peptidases was also able to hydrolyze the tripeptide Phe-Phe-Phe with a relative activity of 49.1% as compared to its activity on Leu-Leu; however, when a Phe residue in the D-configuration was added at the C terminus, the activity decreased to 21.8%. One possible interpretation of this result is that the activity of the peptidase is higher when a L-Phe residue is located at the C terminus than when the residue is a D-Phe. Even though these data need to be further confirmed, it is worth noting that the hydrolytic reaction against ochratoxin A exerted by bacteria of this genus probably could be due to a carboxypeptidase activity, since ochratoxin A is an isocoumarin derivative linked, through the carboxyl group, to L-β-phenylalanine.

In this study, the presence of ochratoxin α was identified by UV/vis and fluorescence spectra and by HPLC-MS, as a product of the degradation of ochratoxin A by *Brevibacterium* strains. On the other hand, further HPLC analysis of supernatants in the conditions for the determination of amino acids showed the appearance of L-β-phenylalanine, in the presumed theoretical concentration. These results are in agreement with previous data indicating that the amide bond present in ochratoxin A could be enzymatically hydrolyzed by CPA, rendering L-β-phenylalanine and ochratoxin α, which is significantly less toxic than ochratoxin A. The production of only ochratoxin α and L-β-phenylalanine is interesting from the point of view of the possible use of *Brevibacterium* strains for detoxification of ochratoxin A in foodstuffs, as no other toxic degradation products were found.

In spite of many years of research and the introduction of good agricultural practices in food production and good manufacturing practices in the storage and distribution chain, mycotoxins continue to be a problem; their impact on animal and their potential impact on human health and welfare are wide-ranging. The usual methods to reduce the levels of ochratoxin A included physical—chemical washes, treatment with absorbent materials, solvent

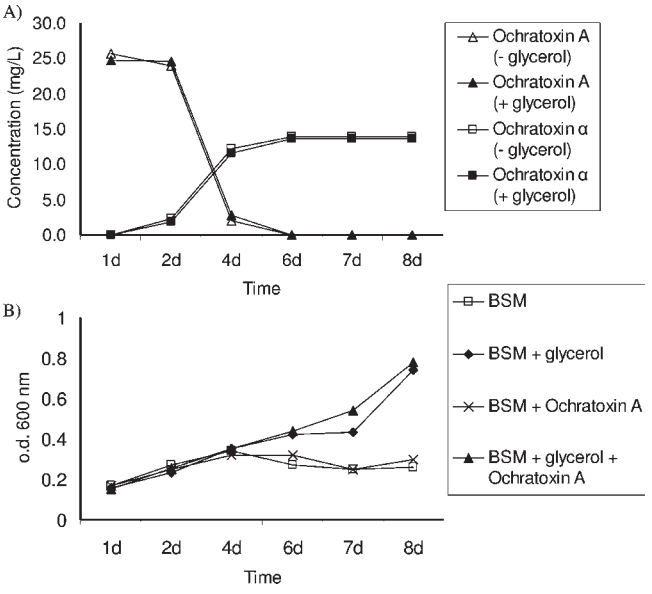

Figure 4. (A) Decrease of ochratoxin A concentration and production of ochratoxin α and (B) growth of *B. casei* RM101 in BSM containing ochratoxin A (25 mg/L) with or without glycerol (0.2%).

extraction, etc.; however, these methods are expensive and can remove nutrients or important compounds from an organoleptic point of view. In this regard, to our knowledge, currently no biological treatment is being used to reduce the content of ochratoxin A in foods, beverages, and feed, since the various microorganisms able to degrade it are not food-related. In contrast, the genus *Brevibacterium* is widely used in food technology, as *B. linens*, *B. casei*, and *B. iodinum* have been isolated from milk and cheese curd and contribute to the aroma, surface coloration, and the ripening of several types of cheese.[29,30] In this study was demonstrated that *Brevibacterium* spp. strains are able to totally degrade ochratoxin A, even at a concentration as high as 40 mg/L, a concentration 1000 times greater than the ochratoxin A concentration usually found in foodstuffs.

Because *Brevibacterium* spp. biomass can be produced, concentrated, freeze-dried, and possibly stabilized without losing its hydrolytic ability toward aromatic compounds (*B. linens* is commercially available as a starter for cheese ripening), the biological detoxification mechanism of ochratoxin A by *Brevibacterium* described here is therefore highly attractive and its use, for example, as a feed additive, for mycotoxin degradation seems practicable. Even more, future elucidation of the genetic basis of the detoxification reaction and cloning of the corresponding gene(s) may contribute to the development of new enzymatic detoxification systems or to engineer this detoxification pathway in other organisms.

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