1 # DEGRADATION OF OCHRATOXIN A BY *BREVIBACTERIUM spp*

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16 ## ABSTRACT

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18 The ability to degrade Ochratoxin A (OTA) was studied in different bacteria with a well-
19 known capacity to transform aromatic compounds. Strains belonging to *Rhodococcus,*
20 *Pseudomonas* and *Brevibacterium* genera were grown in liquid synthetic culture medium
21 containing OTA. *Brevibacterium spp* strains showed 100% degradation of OTA.
22 Ochratoxin α (OTα) was detected and identified by high-performance liquid
23 chromatography-mass spectrometry (HPLC-MS) as degradation product in the cell-free
24 supernatants. We demonstrated for the first time the degradation of OTA by bacteria
25 belonging to the food chain, which is of public concern for food and environmental safety.

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27

28 **Keywords**

29 ochratoxin A; ochratoxin α; *Brevibacterium*; degradation; mycotoxin

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31

32 **INTRODUCTION**

33

34 Ochratoxin A (OTA), an isocumarin derivative linked via the carboxyl group to L-β-
35 phenylalanine, is a mycotoxin produced by several species of fungi belonging principally
36 to the genera *Aspergillus* and *Penicillium.* These fungi are capable to grow under a wide
37 range of conditions, including moisture, pH, and temperature, and on a variety of foods,
38 such as cereals, coffee, fruits, cocoa, nuts, spices, meat, milk, wine and beer (1, 2).
39 Cereals and cereal derivatives remain the major contributors to OTA human and animal
40 exposure (3).

41 OTA is suspected to be nephrotoxic, teratogenic, hepatotoxic and carcinogenic (4) and
42 has been detected in human blood after the consumption of contaminated foods (5). To
43 protect consumer from the risk of exposure to this mycotoxin, reliable methods are needed
44 for reducing the levels of OTA in foodstuff. In this regard, it has been shown that
45 decaffeination with solvents significantly reduced OTA levels in coffee (6), and it has also
46 been suggested that contaminated grains can be detoxified of OTA by ozone treatment
47 (7). Likewise, several studies have focused on the reduction of OTA in musts and wines in
48 the winery, and different decontamination procedures based on either physical, chemical,
49 or biological removal have been proposed (8, 9, 10, 11).

50 With regard to the biological degradation of OTA, enzymes with carboxypeptidase A
51 activity (CPA), such as that obtained from bovine pancreas, have been described. These
52 enzymes can hydrolyze the amidic bond in the OTA molecule with the production of L-
53 phenylalanine (PHE) and ochratoxin α (OTα) (**Figure1**), which is 500 times less toxic
54 than OTA (12). Likewise, degradation of OTA by *Phenylobacterium immobile* (13), by
55 *Acinetobacter calcoaceticus* (14) and by *Aspergillus* species (15, 16) by a mechanism of
56 action similar to that of CPA, have been reported, although in the later case OTα was
57 produced. More recently, a protein produced by the fungus *Aspergillus niger* able to

58 hydrolyze OTA to PHE and OTα has been isolated (17). Even more, this mechanism has
59 been also implicated in the capability of *Trichosporon mycotoxinivorans* to degrade OTA
60 (18). Therefore, biological degradation of toxins is a very promising approach for
61 decontamination of foodstuffs, as use of chemical or physical tools in food may also
62 remove, along with the mycotoxin, other organoleptic important substances and nutrients.

63 On the other hand, soil bacteria, such as *Pseudomonas* spp. and actinobacteria, are able
64 to transform a wide range of aromatic compounds and, thus, they are considered to play a
65 crucial role in the biodegradation of toxic pollutants in soil (19, 20, 21). For instances,
66 among actinobacteria, *Rhodococcus* strains are able to degrade a large variety of organic
67 compounds. In fact, over 200 genes that encode for oxygenases and more than 30
68 metabolic pathways involved in the catabolism of aromatic compounds have been
69 identified in the complete genome sequence of *Rhodococcus jostii* RHA1 (22). Moreover,
70 it has recently been published that cell extracts of *Rhodococcus erythropolis* can degrade
71 aflatoxin B1, a mycotoxin similar to OTA that also possess an aromatic ring in its
72 structure (23). Furthermore, some species from another actinobacteria genus,
73 *Brevibacterium*, that are usually found in milk and cheese curd, such as *B. casei*, *B.*
74 *iodinum*, and *B. linens* (which usual habitat is the exterior of surface-ripened cheeses such
75 as Limburger, Romadour, Munster, Tilsiter, Appenzeller, Gruyère, Brick and others) have
76 also been isolated from soil. In addition to their versatility, another important
77 characteristic of the actinobacteria from the genus *Brevibacterium* is their extracellular
78 proteinase production. Intracellular and extracellular cell-wall associated proteinases have
79 been reported for *B. linens*, but most studies has focused on the later largely because of
80 their high activity and importance respect to cheese ripening (24, 25).

81 As mentioned above, given its potential health hazard, presence of OTA in foods is of
82 considerable public concern for the food industry and regulatory agencies and,
83 consequently, there is a growing demand from control authorities to reduce the allowable

84 limits of OTA in foods and beverages. Therefore, and based on the aforementioned
85 characteristics of some microorganisms, in this study we screened bacteria with the ability
86 to degrade aromatic compounds, such as *Pseudomonas putida* and several actinobacteria
87 from the genera *Rhodococcus* and *Brevibacterium*, for the ability to degrade OTA.
88
89 ## MATERIALS AND METHODS
90
91 **Microorganisms and growth conditions.** Cultures of the strains *Rhodococcus*
92 *erythropolis* CECT 3008, *Rhodococcus eryhtropolis* IGTS8, *Pseudomonas putida* DSMZ
93 291, *P. putida* KT2442 and seven strains of *Brevibacterium* species were screened for
94 their ability to degrade OTA.
95 *Rhodococcus erythropolis* CECT 3008 (DSMZ 43060) was purchased from the Spanish
96 Type Culture Collection (CECT). *Pseudomonas putida* DSM 291^T and the six
97 *Brevibacterium* strains included in the study (*Brevibacterium epidermidis* DSM 20660^T,
98 *Brevibacterium iodinum* DSM 20626^T, *Brevibacterium linens* DSM 20425^T,
99 *Brevibacterium casei* DSM 20657^T, *B. casei* DSM 9657, *B. casei* DSM 20658) were
100 purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). A
101 *B. casei* strain, RM101, molecularly identified by 16S rDNA sequencing, was isolated at
102 the Instituto de Fermentaciones Industriales IFI-CSIC. *Rhodococcus erythropolis* IGTS8
103 and *Pseudomonas putida* KT2442 strains were kindly provided by Dr. Eduardo Diaz,
104 from the Centro de Investigaciones Biológicas, CSIC, Spain.
105 All bacteria assayed were routinely grown in Luria-Bertani broth (LB) supplemented
106 with 0.5% glucose and incubated at 30 ºC under aerobic conditions. For the OTA
107 degradation assay, bacteria were grown in a basal salts medium (BSM) that contained
108 0.2% glycerol, 4 g of NaH_2PO_4-H_2O, 4 g of K_2HP0_4-$3H_2O$, 2 g of NH_4Cl, 0.2 g of $MgCl_2$-
109 $6H_2O$, 0.001 g of $CaCl_2$-$2H_2O$, and 0.001 g of $FeCl_3$-$6H_2O$ (26). Glycerol was omitted

110 from experiments designed to determine the potential use of OTA as carbon source by the
111 bacteria assayed.
112

113 **Standards.** OTA was purchased from Sigma (Sigma Chemical Co, Poole, Dorset, UK)
114 and suspended in 99% methanol under sterile conditions to make a stock solution of 500
115 mg/L. A standard solution of OTα (11.9 mg/L) was purchased from LGC Standards
116 (Germany) and diluted with acetonitrile (1:1, v/v) to make a working solution of 5.9
117 mg/L.

118 Phenylalanine was purchased from VWR (Milano, Italy) and suspended in HCl 0,1 M to
119 make a stock solution of 1 g/L. The standard solution was made in 75% methanol and
120 contained 15 mg/L of PHE. Norvaline was purchased from Sigma (Sigma Chemical Co,
121 Poole, Dorset, UK) and suspended in HCl 0,1 M to make a stock solution of 500 mg/L.
122

123 **OTA degradation assay.** Actinobacteria (*Rhodococcus erythropolis* CECT 3008,
124 *Rhodococcus erythropolis* IGTS8, and *Brevibacterium casei* RM101) and *Pseudomonas*
125 spp. (*P. putida* DSMZ 291^T and *P. putida* KT2442) strains were grown in 25 mL of BSM
126 containing OTA (about 10 μg/L) under aerobic conditions at 30°C until the late
127 exponential growth phase, time at which supernatants were collected for the analysis of
128 OTA by high-performance liquid chromatography (HPLC). To confirm OTA degradation
129 by *Brevibacterium* spp. strains, the bacterial strains were grown in BSM containing a 4-
130 fold increase on the OTA concentration (40 μg/L) under aerobical conditions on a rotary
131 shaker at 150 rpm for 10 days. To determine the ability of *Brevibacterium* spp. (*B. casei*
132 RM101 and *B. linens* DSM 20425^T strains) to degrade OTA at higher concentrations (40
133 mg/L, 1000 times greater than previously assayed) and to assess the use of OTA as carbon
134 source, bacteria were grown in BSM with or without glycerol (0.2%) and OTA (40 mg/L),
135 and OTA degradation was verified after 10 days of incubation at 30° C. In addition, to

136 determine the timecourse of OTA degradation and to quantify OTα production, samples
137 from *B. casei* RM101 strain grown in BSM containing OTA (25 mg/L), with or without
138 glycerol (0.2%), were collected twice daily and analyzed by HPLC. In all these
139 degradation assays, culture supernatants were separated by centrifugation at 3000 x g for
140 10 min at 4º C and further analyzed by HPLC. The *Brevibacterium* spp. cell pellets were
141 stored at -80 ºC for subsequent analysis. BSM controls with OTA and without bacteria
142 were always prepared.

143

144 **OTA, Otα and Phenylalanine quantification by HPLC.** Concentration of OTA in
145 supernatants, pellets and pellet's washing solutions was quantified as described (27) using
146 a Hewlett–Packard I model 1100 series HPLC instrument (Hewlett–Packard, Palo Alto,
147 CA), equipped with a degasser, quaternary pump, auto sampler, Uv/vis (DAD) and
148 fluorescence (FLD) detector and a 200 mm–4,6 mm Alltima C18 (5 μm) column. The
149 mobile phase was: solvent (A), acetonitrile; solvent (B), water (HPLC
150 grade)/acetonitrile/glacial acetic acid (89:10:1 by vol.); solvent A:B=37:63 (v/v), isocratic
151 method; flow, 1.3 mL/min; analysis temperature, 30° C; analysis time, 20 min; FLD
152 detector (λ_{ex}=330 nm, λ_{em}=460 nm); injection volume, 100 μL. The limit of detection for
153 OTA in the aforementioned conditions is 0.02 μg/L. OTA standard was injected at two
154 different concentrations: 20 and 50 μg/L; OTα standard was injected at a concentration of
155 5.9 mg/L. Samples were appropriated diluted with solvent A:B before the analysis HPLC.
156 The *Brevibacterium* spp. bacterial pellets were resuspended twice in 2 ml of absolute
157 methanol for 1 h to extract the OTA. After centrifugation at 3,000 x g for 15 min at 20º C,
158 the methanolic supernatants were separated, collected in 5 ml vials and evaporated to
159 dryness with a stream of dry nitrogen gas. The dry residues were reconstituted with the
160 mobile phase immediately before analysis for the determination of OTA concentration.

161 Concentration of PHE in supernatants was quantified as described (28) by using the
162 same instrument and column mentioned for OTA analysis. Briefly, samples were
163 subjected to automatic precolumn derivatization using o-phthaldialdehyde with 2-
164 mercaptoethanol (OPA Reagent, Agilent Technologies, Palo Alto, CA). Two eluents were
165 used as mobile phases: eluent A (1.224 g of sodium acetate trihydrate, 500 mL of water,
166 0.09 mL of triethylamine, and 1.5 mL of tetrahydrofuran) and eluent B (1.088 g of
167 sodiumacetate trihydrate, 100 mL of water, 200 mL of acetonitrile, and 200 mL of
168 methanol). A 65 min gradient program commenced with an initial concentration of 10%
169 eluent B at a flow rate of 0.450 mL/min and terminated with 100% eluent B at a flow rate
170 of 0.700 mL/min. The fluorescence wavelengths were 340 nm for excitation and 450 nm
171 for emission. Supernatant samples were added of Norvaline (15 mg/L) as internal
172 standard, diluted 1:1 with methanol and filtered through a 0.2 µm pore-size filter
173 (Millipore) before injection. Injection volume was 5 µL.
174 **HPLC-DAD/ESI-MS.** A Hewlett-Packard series 1100 MSD (Palo Alto,
175 CA) quadrupole mass spectrometer system equipped with an electrospray interphase (ESI)
176 was used. Samples were introduced by direct injection. The ESI parameters were: drying
177 gas N_2, 10 L/min at 330 ºC; nebulizer pressure, 40 psi; spray capillary voltage, 4000 V.
178 The ESI was operated in negative mode, scanning from *m/z* 100 to *m/z* 800, using a
179 variable fragmentator voltage gradient.
180
181 **RESULTS AND DISCUSSION**
182 Mycotoxins are well-known natural contaminants in foods. Among the more than 300
183 mycotoxins isolated and described until now, OTA is one of the most important because
184 of its teratogenic, embryotoxic, genotoxic, neurotoxic, immunosuppressive, carcinogenic
185 (IARC group 2B), and nephrotoxic effects (Joint FAO/WHO Expert Committee on Food
186 Additives, JECFA) (29). Aside from the health and toxicological perspective, OTA

187 exposure also has an impact on livestock economics, where the associated decrease in
188 productivity (milk, eggs, weight loss) and the increase of mortality rate are of concern (3).
189 In fact, levels of OTA found in food products or beverages have been strictly fixed
190 (ranging from 2-10 µg/kg) by the European Union (UE) in cereals, dried vine fruits,
191 roasted coffee beans, ground coffee, soluble coffee, wine and grape juice (30), and the
192 World Health Organization (WHO) has proposed a maximum limit of 5 µg/kg of OTA in
193 cereals (31). In spite of it, and although biological decontamination of mycotoxins from
194 foods by means of the use of microorganisms is one of the strategies used for the
195 management of mycotoxins, little published information is available regarding biological
196 methods for the removal of OTA.

197 To address this important point, and since it is known that soil bacteria are able to
198 transform a wide range of aromatic compounds, we analyzed the presence of OTA in
199 actinobacteria and *Pseudomonas* spp. grown in liquid BSM synthetic culture medium.

200 Initially screenings of OTA degradation capacity were performed using *Pseudomonas*
201 *putida* and several actinobacteria from the genera *Rhodococcus* and *Brevibacterium*.
202 Bacterial strains were grown in liquid synthetic (BSM) culture medium in the presence of
203 OTA (10 µg/L). As displayed in **Table 1**, only 8-28% decrease in OTA concentration was
204 recorded in the cell-free supernatants for *Rhodococcus* and *Pseudomonas putida* strains,
205 respectively, and no degradation products were observed on the HPLC chromatograms,
206 suggesting that OTA is not degraded but adsorbed by the cells, as it has already been
207 described for strains of lactic acid bacteria (27) and yeasts (32).

208 On the contrary, results in **Table 1** also showed that OTA completely disappeared from
209 the cell-free supernatants of *Brevibacterium casei* RM101 strain, indicative of the
210 presence of a mechanism of OTA degradation.

211 *Brevibacterium* spp. is a heterogeneous group of nine coryneform species that are
212 capable of degrading insecticides (DTT, DDE, etc.), and produce self-processing

213 extracellular proteases. They are found in diverse habitats, including soil, poultry, fish,
214 human skin, and food. *Brevibacterium* species differ from other bacteria for their ability to
215 metabolize compounds of heterocyclic and polycyclic ring structure, a trait that is also
216 common in fungi. Then, to analyze whether the above mentioned observed OTA
217 degradation is a specific characteristic of *Brevibacterium* strain, species or genus, a media
218 containing high concentration of OTA (40 μg/L, rarely found in food products or
219 beverages) was used with strains belonging to different species from the *Brevibacterium*
220 genus. A complete disappearance of OTA was observed in all the culture supernatants
221 tested (**Table 2**), moreover, no traces of OTA were presented in pellets or pellet`s
222 washing solutions after methanol extraction. Therefore, these results indicate that OTA
223 degradation is a characteristic of the *Brevibacterium* genus.

224 Next, to further study the mechanism of OTA degradation followed by *Brevibacterium*
225 spp., *B. casei* RM101 and *B. linens* DSM 20425^T strains were grown at a 1000 times
226 greater OTA concentration (40 mg/L), on different BSM medium compositions, including
227 or not glycerol as carbon source. Results indicated that both *Brevibacterium* strains were
228 able to completely degrade higher OTA concentrations (40 mg/L) also in a medium
229 devoid of a traditional carbon source such as glycerol, where growth is slight, as
230 determined by measuring the turbidity of the media (results not shown).

231 Analyses of the chromatograms from the supernatant showed that whilst the OTA peak
232 was absent, a new peak with a different retention time and spectrum was present in the
233 elution profile (**Figure 2**). In fact, the UV/Vis and fluorescence spectra of the produced
234 compound found in the supernatants (**Figure 3**) were identical to the spectra
235 corresponding to the OTα standard. Even more, HPLC-MS confirmed the identification of
236 this compound, since the peak showed a molecular ion [M-H]− at *m/z* 255.1 in MS (PM
237 OTα = 256).

238 As quantified by HPLC, similar amounts of OTα and PHE were presented in the
239 supernatants of the two strains, *B. casei* RM101 and *B. linens* DSM 20425^T, irrespectively
240 of the presence or not of glycerol, and these quantities corresponded to the theoretical
241 concentration calculated from the complete hydrolysis of the OTA added to the medium.

242 Furthermore, evaluation of the degradation of OTA over time showed that degradation
243 already started 48 hours after inoculation of the strain (**Figure 4A**). Decrease of OTA
244 concentration, production of OTα and growth of *Brevibacterium casei* RM101 in BSM
245 media containing OTA, in the presence or absence of glycerol, was recorded (**Figure 4**).

246 These results strongly suggest that *Brevibacterium* spp. strains possessed an enzyme,
247 possibly a carboxypeptidase, which hydrolyzed the amidic bond in the OTA molecule.

248 It has been reported that *B. linens* has highly active and multiple proteolytic enzymes
249 that are mainly extracellular and intracellular proteases or peptidases (33, 34, 35).
250 Electrophoretic studies have shown that sonicated extracts of a *B. linens* strain have at
251 least six different peptide hydrolases of varying dipeptide specificities (36). In fact,
252 zymograms obtained using 14 dipeptides of L-amino acids have shown that two peptides
253 having a L-phenylalanine at the C-terminus (Ala-Phe, and Gly-Phe) were hydrolyzed by
254 five extracts, whereas Ala-Trp and Ala-His dipeptides, containing the same N-terminus,
255 were hydrolyzed just by one of the cell extracts assayed (36). Therefore, a possible
256 carboxipeptidase activity acting on a peptide with L-phenylalanine as C-terminus can be
257 found in *B. linens* extracts. Moreover, two aminopeptidases purified from a culture filtrate
258 of a *B. linens* strain (37) that hydrolyzed a variety of substrates, have been shown to have
259 specificity for N-terminal leucine. Additionally, one of the purified peptidases was also
260 able to hydrolyze the tripeptide Phe-Phe-Phe with a relative activity of 49.1% compared
261 to its activity on Leu-Leu; however, when a Phe residue in the D-configuration was added
262 at the C-terminus, the activity decreases to 21.8%. One possible interpretation of this
263 result is that the activity of the peptidase is higher when a L-Phe residue is located at the

264 C-terminus than when the residue is a D-Phe. Even thought these data need to be further
265 confirmed, it is worthy to note that the hydrolytic reaction against OTA exerted by
266 bacteria of this genus could be probably due to a carboxipeptidase activity, since OTA is
267 an isocumarin derivative linked, through the carboxyl group, to L-β-phenylalanine.

268 In the present study, our analysis showed a compound, OTα, identified by UV/Vis and
269 fluorescence spectra and by HPLC-MS, as a product of the degradation of OTA by
270 *Brevibacterium* strains. On the other hand, further HPLC analysis of supernatants in the
271 conditions for the determination of amino acids showed the appearance of PHE in the
272 theoretical concentration expected. These results are in agreement with previous data (12)
273 indicating that the amidic bond present in OTA could be enzymatically hydrolyzed by
274 CPA, rendering PHE and OTα, which is 500 times less toxic than OTA. The production of
275 only OTα and PHE is interesting from the point of view of the possible use of
276 *Brevibacterium* strains for detoxification of OTA in foodstuffs, as no other toxic
277 degradation products were found.

278 In spite of many years of research and the introduction of good agricultural practices in
279 food production and good manufacturing practices in the storage and distribution chain,
280 mycotoxins continue to be a problem, and their impact in both human and animal health
281 and welfare is wide-ranging. The usual methods to reduce the levels of OTA included
282 physical-chemical washes, treatment with absorbent materials, solvent extraction, etc;
283 however, these methods are expensive and can remove nutrients or important compounds
284 from an organoleptic point of view. In this regard, to our knowledge, currently no
285 biological treatment is being used to reduce the content of OTA in foods, beverages and
286 feed, since the various microorganisms able to degrade it (see introduction) are not food-
287 related microorganisms. In contrast, the genus *Brevibacterium* is widely used in food
288 technology, as *B. linens*, *B. casei* and *B. iodinum* have been isolated from milk and cheese

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289 curd and contribute to the aroma, surface coloration and the ripening of several types of
290 cheese (38, 39).

291 In this study we have demonstrated that *Brevibacterium spp* strains are able to totally
292 degrade OTA, even at a concentration as high as 40 mg/L, a concentration 1000 times
293 greater than the OTA concentration usually found in foodstuffs.

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

303

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421

422 **Table 1.** Reduction in concentration of OTA in BSM by Actinobacteria and

423 *Pseudomonas* spp.

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Strain	OTA [μg/L] *	OTA reduction (%)
BSM + OTA (Control)	11.01 (0.07)	0
Rhodococcus erythropolis CECT 3008	7.88 (0.70)	28.47
Rhodococcus erythropolis IGTS8	8.81 (0.98)	19.98
Brevibacterium casei RM101	n.d.	100
Pseudomonas putida DSM 291^T	10.07 (0.09)	8.54
Pseudomonas putida KT2442	8.18 (0.65)	25.70

426 n.d., not detected

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428 *Results are expressed as mean values of two experimental replications for each strain;

429 standard deviations are shown in parentheses

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434 **Table 2.** Reduction in concentration of OTA in BSM by *Brevibacterium* spp.

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Strain	OTA (μg/L)[a]	OTA reduction (%)
BSM + OTA (Control)	39.81	0
Brevibacterium casei DSM 20657[T]	n.d.	100
Brevibacterium casei DSM 9657	n.d.	100
Brevibacterium casei DSM 20658	n.d.	100
Brevibacterium casei RM101	n.d.	100
Brevibacterium linens DSM 20425[T]	n.d.	100
Brevibacterium iodinum SM20626[T]	n.d.	100
Brevibacterium epidermidis DSM 20660[T]	n.d.	100

436 [a] Determined from supernatants of three independent cultures

437 n.d., not detected

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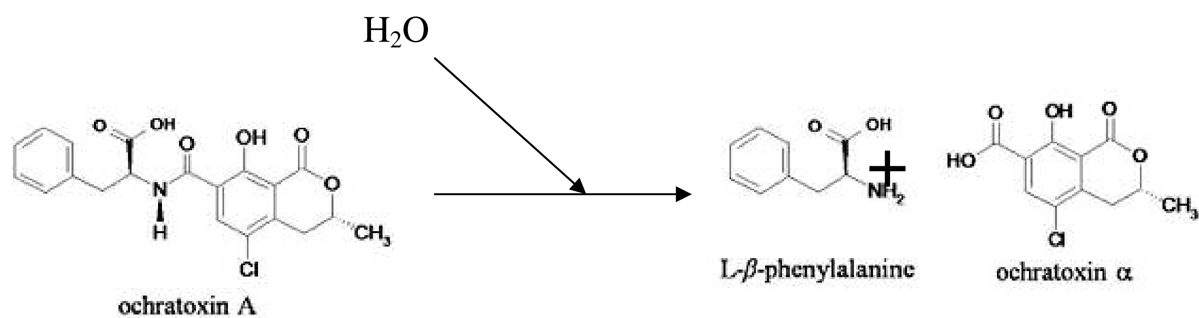

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447 **Figure 1.** Conversion of OTA in L-β-phenylalanine and OTα

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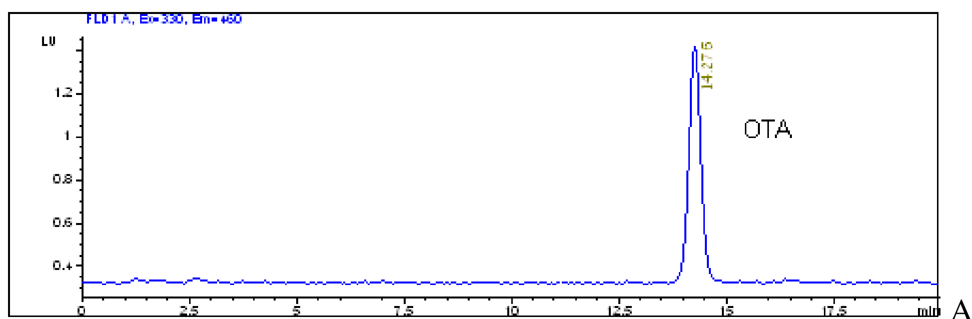
A)



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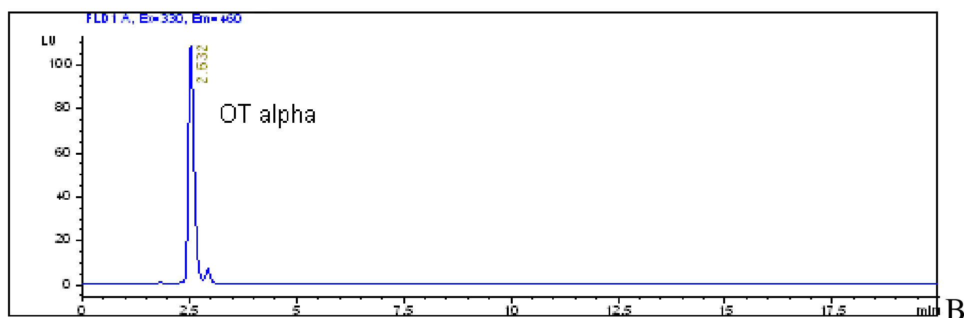
B)



B

Figure 2: Chromatogram obtained from the supernatant of *B. casei* RM101 grown in BSM devoid of a carbon source but containing OTA (40 mg/L).

A) Supernatant at time 0, B) Supernatant after 10 days of growth showing the disappearance of the OTA peak and the appearance of the OTα peak.

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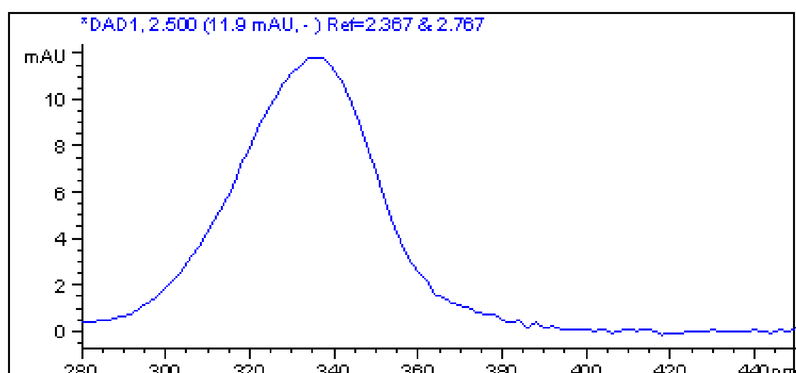

A)

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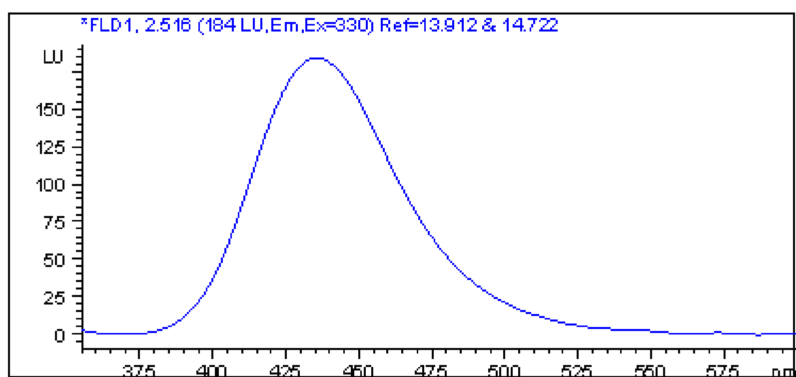

B)

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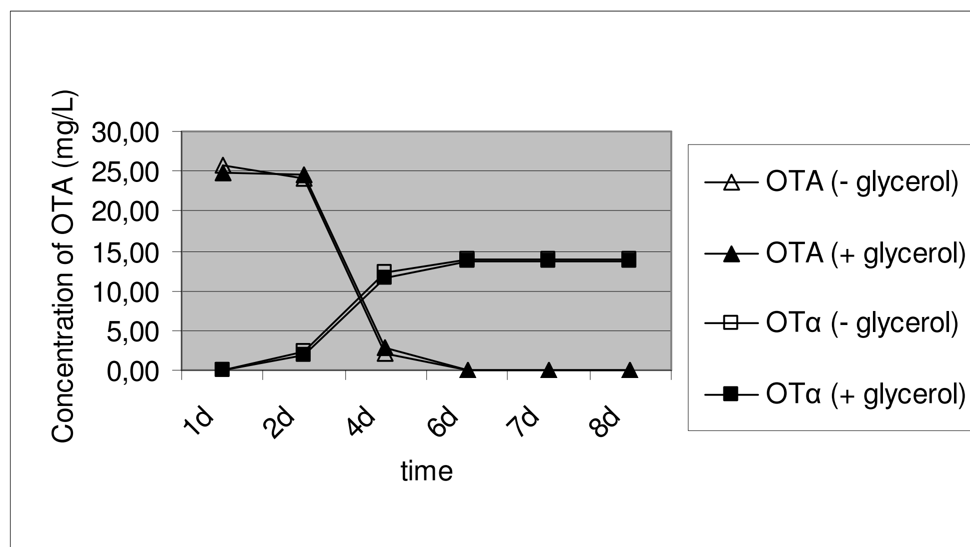
494 **Figure 3:** OTα (A) UV/Vis and (B) fluorescence spectra obtained from the supernatant

495 of *B. casei* RM101 grown in BSM devoid of a carbon source but containing OTA (40

496 mg/L).

497 **A)**



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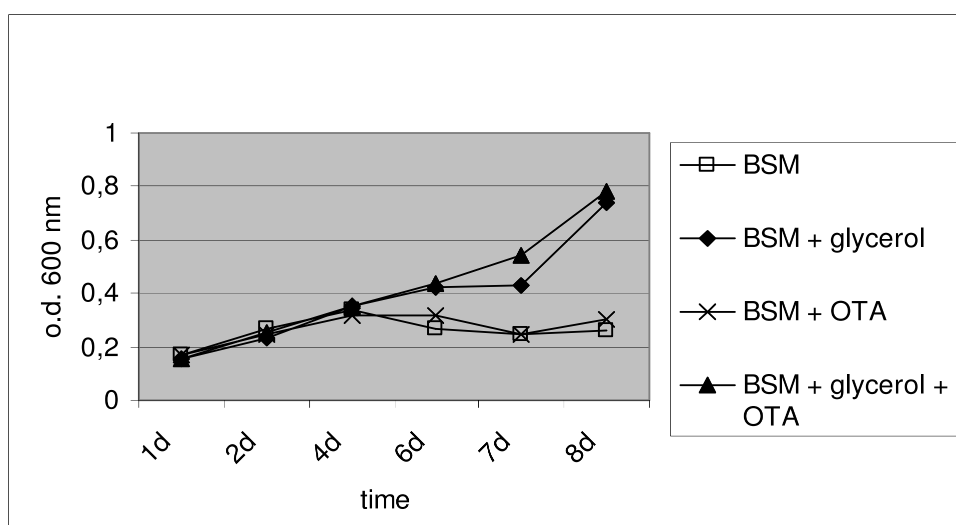
501 B)



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505 **Figure 4.** (A) Decrease of OTA concentration and production of OTα and (B) growth of

506 *B. casei* RM101 in BSM containing OTA (25 mg/L) with or without glycerol (0.2%).

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