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Degradation of the Bioactive Compounds Enniatins A, A_1, B, B_1 Employing Different Strains of *Bacillus Subtilis*

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Abstract

The biological reduction of the *Fusarium* mycotoxins ENs by six *Bacillus subtilis* strains and the formation of the new ENs degradation products produced by microbial fermentations were studied.

The fermentations were carried out in the liquid medium of Triptic Soy Broth during 48 h at 37°C, under aerobic conditions. The extraction of ENs and of the degradation products was performed using ethyl acetate, and the detection and quantification of these bioactive compounds, was carried out using liquid chromatography coupled to the Mass Spectrometry Detector (LC-MS). All the bacteria analyzed in this study showed a significant ENs reduction during the fermentation processes, in particular the mean reduction evidenced ranged from 64 to the 99%. Also two degradation products related to the ENs B and B_1 were identified.

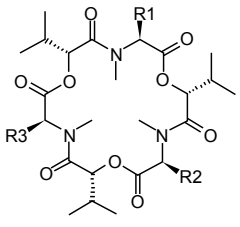
Keywords: Enniatins; *Bacillus subtilis*; Mycotoxin degradation; LC-MS/MS

Introduction

The enniatins (ENs) (Figure 1) are fungal metabolites produced by several *Fusarium* species [1]. They are cyclic depsipeptides, which are commonly composed of three D-a-hydroxyisovaleric acid (HyLv) residues linked alternatively to three L-configured N-methyl amino acid residues to give on 18-membered cyclic skeleton. ENs are phytotoxins, with antibiotic, herbicidal and insecticidal activities. They also have enzymatic inhibiting and oxidative phosphorylation properties [2], and some of them could have important clinical applications in combination with chemotherapeutic drugs [3-6].

The presence of ENs, BEA and FUS in food commodities has been recently reported during the last decade in some European countries (Finland, Norway, Spain, Slovakia, Croatia, Switzerland and Italy), in USA, in South Africa and in Australia. Recently our research group has reported the contamination of cereals (maize, wheat and barley) and cereal products (breakfast cereals) available in Morocco and Spain [7-10].

In the scientific literature only a US patent is available on the biological reduction of the mycotoxin BEA; in particular the authors reduced the contamination of BEA present in corn employing a strain of *Norocardia glubera* [11]. The degradation of other fusarotoxins has been studied by other authors.



ENs	MW	R1	R2	R3
A	682.92	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$
A_1	668.89	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)_2$
B	640.84	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$
B_1	654.87	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$

Figure 1: ENs general chemical structures.

Poppenberger et al. [12] reported the isolation of a UDP-glycosyltransferase from *A. thaliana*, able to detoxify the *Fusarium* mycotoxin deoxinivalenol (DON). The enzyme catalyzes the transfer of glucose from UDP-glucose to the hydroxyl group at carbon 3 of deoxynivalenol (DON). This deoxynivalenol-glucosyltransferase (DOGT1) was also found to detoxify the acetylated derivative 15-Ac-DON.

The degradation of 12 trichothecenes by microorganisms isolated by chicken gut was studied by Young et al. [13]. The two principally degradation pathways evidenced were: deacetylacion and deepoxydation. The authors reported also that the deepoxydation was the prevalent reaction in HT-2 toxin and T-2 triol, whereas T-2 toxin showed only deacetylation. The percentage of reduction detected ranged from 40 to 95%.

Guan et al. [14], studied the degradation of *Fusarium* mycotoxin DON, using microorganisms isolated from fish digesta. The authors evidenced that the microbial pathway related to the fish *Ameiurus nebulosus*, transformed DON to deepoxy DON (dE-DON) in full medium after 96 h incubation.

The degradation of the fumonisisn B_1 (FB_1) by microbial enzymes was studied by Heinl et al. [15]. The authors using chromatographic techniques isolated enzymes by liquid culture of the bacterium *Sphingopyxis* sp. MTA144, responsible of the detoxification of the FB_1.

Awad et al. [16], studied the detoxification properties of an *Eubacterium* strain (BBSH 797) isolated from bovine rumen fluid, to

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transform completely the mycotoxin DON into dE-DON [17], using different soil samples isolated microorganisms that have the capacity to transform DON to dE-DON. The authors evidenced that a microbial culture originated by a soil sample showed the 100% DON to dE-DON biotransformation in Mineral Salt Broth (MSB), after 144 h incubation at 25°C. The analysis of some genetic sequences indicated that the bacterial population employed was related to the genera *Serratia*, *Clostridium*, *Citrobacter*, *Enterococcus*, *Stenotrophomonas* and *Streptomyces*.

Considering the lack of data related to the biological degradation of the minor *Fusarium* mycotoxins ENs, the aims of this study were: a) to evaluate the reduction *in vitro* of the ENs employing different *Bacillus subtilis* strains and b) to study the degradation products of ENs employing the technique of the liquid chromatography coupled to the mass spectrometry linear ion trap (LC-MS-LIT).

Material and methods

Chemicals

A stock standard solution of ENs A, A_1, B, B_1 (98% of purity) (Sigma-Aldrich, St. Luis, USA) were prepared by dissolving 1 mg of standard in 1 mL of pure methanol. This stock solution was then diluted with methanol in order to obtain the appropriated work solutions with concentrations of 1, 10 and 100 mg/L. All ENs solutions were stored in darkness at 4°C until the LC-MS/MS analysis. Acetonitrile, methanol, water, ethyl acetate, sodium chloride (all of LC grade) and acetic acid were purchased from Merck (Whitehouse Station, NJ, USA).

Strains and methodology

The study was carried out using six strains of *Bacillus subtilis* obtained by the Spanish Type Culture Collection (CECT Valencia, Spain), in sterile 18% glycerol.

For longer survival and higher quantitative retrieval of the cultures, the strains were stored at -80°C. When needed, recovery of strains was carried out by two consecutive subcultures in appropriate media before to use.

The microbes were cultured in 15 mL sterile plastic centrifuge tubes utilizing as growth medium 10 mL of Tryptic Soy Broth (TSB, Oxoid Madrid, Spain) and incubated at 37°C in aerobic atmosphere during 48 h. After that each bacterial suspensions at concentrations of 10^8 CFU/ml were added to a fresh 10 mL of TSB contaminated with 5 mg/L of the mycotoxins ENs A, A_1, B, and B_1, incubated at 37°C in an anaerobic atmosphere with 95% CO_2 and 5% H_2 during 48 h.

The mediums were analyzed in order to determinate the residual concentration of ENs present in the growth medium and also to identify the possible degradation products.

ENs and degradation products extraction from fermented mediums

The fermentation tubes were centrifuged at 4000 rpm (Centrifuge 5810R, Eppendorf, Germany) during 5 min at 4°C in order to separate the fermented medium from the cells. ENs contained in fermented medium were extracted as follows [18]: 5 mL of fermented TSB were introduced in a 20 mL plastic tube, and extracted three times with 5 mL of ethyl acetate using a vortex VWR international (Barcelona, Spain) during 1 min. After that the mixtures were centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 4000 rpm for 10 min at 4°C. The organic phases were completely evaporated by a rotary evaporator (Buchi, Switzerland) operating at 30°C and 30 mbar pressure, resuspended in 1 mL of methanol, filtered with 0.22 μM filters (Pheomenex, Madrid, Spain) and analyzed by LC-MS/MS [8] or by LC-MS-LIT.

LC-MS/MS analysis of ENs

The optimization of MS/MS conditions was performed by direct injection of individual standards at 100 μg/mL in "full SCAN". The most abundant mass-to-charge ratio (m/z) was selected for each compound of interest. The mycotoxins exhibited precursor ions and product ions with reasonably high signal intensities in positive ESI mode (ESI^+), being found protonated molecules $[M+H]^+$, sodium adduct ions $[M+Na]^+$ and potassium adduct ions $[M+K]^+$. A Quattro LC triple quadrupole mass spectrometer from Micromass (Manchester, UK), equipped with an LC Alliance 2695 system (Waters, Milford, MA) consisting of an autosampler, a quaternary pump, and a pneumatically assisted electrospray probe, a Z-spray interface and Mass Lynx NT software Version 4.1, were used for the MS/MS analyses. The separation was achieved by a Gemini-NX C_{18} (150 mmx4.6 mm I.D., 5 μm particle size) analytical column supplied by Phenomenex (Barcelona, Spain), preceded by a guard column C_{18} (4 mmx2 mm I.D.). The analytical separation for LC-MS/MS was performed using gradient elution with 90% of methanol (with 5 mM of formate ammonium) as mobile phase A, and 10% of acetonitrile as mobile phase B, increasing linearly to 50% B for 10 min; then, decreasing linearly to 40% B for 3 min, and then gradually up to 10% B for 5 min. Finally, initial conditions were maintained for 3 min. Flow rate was maintained at 0.2 ml/min. The ESI source values were as follows: capillary voltage, 3.50 kV; source temperature, 100°C; desolvation temperature, 300°C; cone gas 50 l/h; desolvation gas (nitrogen 99.95% purity) flow, 800 l/h.

The analyzer settings were as follows: resolution 12.0 (unit resolution) for the first and third quadrupoles; ion energy, 0.5; entrance and exit energies, -3 and 1; multiplier, 650; collision gas (argon 99.99% purity) pressure, $3.83x10^{-3}$ mbar; interchanel delay, 0.02 s; total scan time, 1.0 s; dwell time 0.1 ms. The mass spectrometer was programmed in Multiple Reaction Monitoring (MRM) mode. All time measurements were carried out in triplicate. The MRM optimized parameters cone voltages and collision energies were: 35 Ev and 40 V, respectively. The precursor and product-ions selected were 681.9 $[M+H]^+$, 228.2 and 210.0 for EN A; 667.9 $[M+H]^+$, 228.2 and 210.0 for EN A_1; 639.8 $[M+H]^+$, 214.2 and 196.2 for EN B; 654.9 $[M+H]^+$, 214.2 and 196.2 for EN B_1.

Method performance

Method validation was carried out according to the guidelines established by the European Commission [19,20]. The method validation included the determination of linearity, Limits of Detection (LODs), Limits of Quantification (LOQs), recoveries; repeatability (intra-day precision) and reproducibility (inter day precision).

In order to determine the linearity, calibration curves for each studied mycotoxin were constructed from the standards prepared in methanol and from the standards prepared in extract of blank sample. All mycotoxins exhibited good linearity over the working range, and the regression coefficient of calibration curves was higher than 0.992.

The LODs and LOQs were estimated from an extract of a blank sample, fortified with decreasing concentrations of the analytes. For 6 days additions were performed from three different blank samples (n=18), to the estimated concentrations for each mycotoxin. The LODs and LOQs were calculated using the criterion of $S/N \geq 3$ and $S/N \geq 10$ for LOD and LOQ, respectively. LODs for ENA, ENA_1, ENB and ENB_1

were 0.15, 0.08, 0.15 and 0.15 µg/kg, respectively. LOQs for ENA, ENA_1, ENB and ENB_1 were 0.5, 0.25, 0.5 and 0.5 µg/kg respectively.

The recoveries, intra-day precision and inter-day precision were evaluated by spiking different levels of standard analyte to samples at two spiked levels (LOQ and 100 times LOQ). Recovery ranges for the low spiked level (LOQ) and the high spiked level (100xLOQ) were 85-110% and 86-112%, respectively. RSD values ranged between 4 and 11% for the intra-day precision, and between 5 and 15% for the inter-day precision. These values agree with EU criteria [19].

Determination of the ENs degradation products with LC-MS-LIT

The separation of ENs was achieved by LC Agilent 1100 (Agilent Technologies, Santa Clara, California) coupled to a mass spectrometer, Applied Biosystems/MDS SCIEX Q TRAP TM linear ion trap mass spectrometer (Concord, Ontario, Canada). A Gemini (150x2.0 mm, 5 µm) Phenomenex (Torrance, California) column was used. LC conditions were set up using a constant flow at 0.3 ml/min and acetonitrile/water (80:30, v/v with 0.1% of HCOOH) as mobile phases in isocratic condition were used. The instrument was configured in the positive ion electrospray mode using the following parameters: cone voltage 40 V, capillary voltage 3.80 kV, source temperature 350°C, desolvation temperature 270°C and collision gas energy 5 eV. The analyses of the ENs degradation products employing the technique of the liquid chromatography coupled to the ion trap was carried out using the following procedure:

1. Characterization of the compound isolated with the modality of ER scan, using the m/z range from 200 to 900 Da to obtain the general spectra of the degradation compound;

	% of Reduction			
Strains	**A**	**A_1**	**B**	**B_1**
Bacillus subtilis CECT 35	77.7 ± 1.2	67.1 ± 3.1	64.7 ± 3.6	79.7 ± 3.2
Bacillus subtilis CECT 39	87.7 ± 1.5	83.0 ± 2.6	85.3 ± 3.8	90.0 ± 2.0
Bacillus subtilis CECT 371	96.2 ± 2.2	94.2 ± 2.9	96.7 ± 4.1	96.7 ± 1.9
Bacillus subtilis CECT 497	95.1 ± 2.4	95.4 ± 3.0	98.0 ± 2.6	99.0 ± 3.6
Bacillus subtilis CECT 498	90.6 ± 2.6	86.4 ± 2.9	86.2 ± 2.9	92.8 ± 3.3
Bacillus subtilis CECT 4522	81.7 ± 2.4	72.9 ± 3.5	76.5 ± 3.3	84.0 ± 2.7

Table 1: Degradation of the ENs A, A_1, B, B_1 in TSB medium through fermentation in submerged culture by different strain of *Bacillus subtilis*.

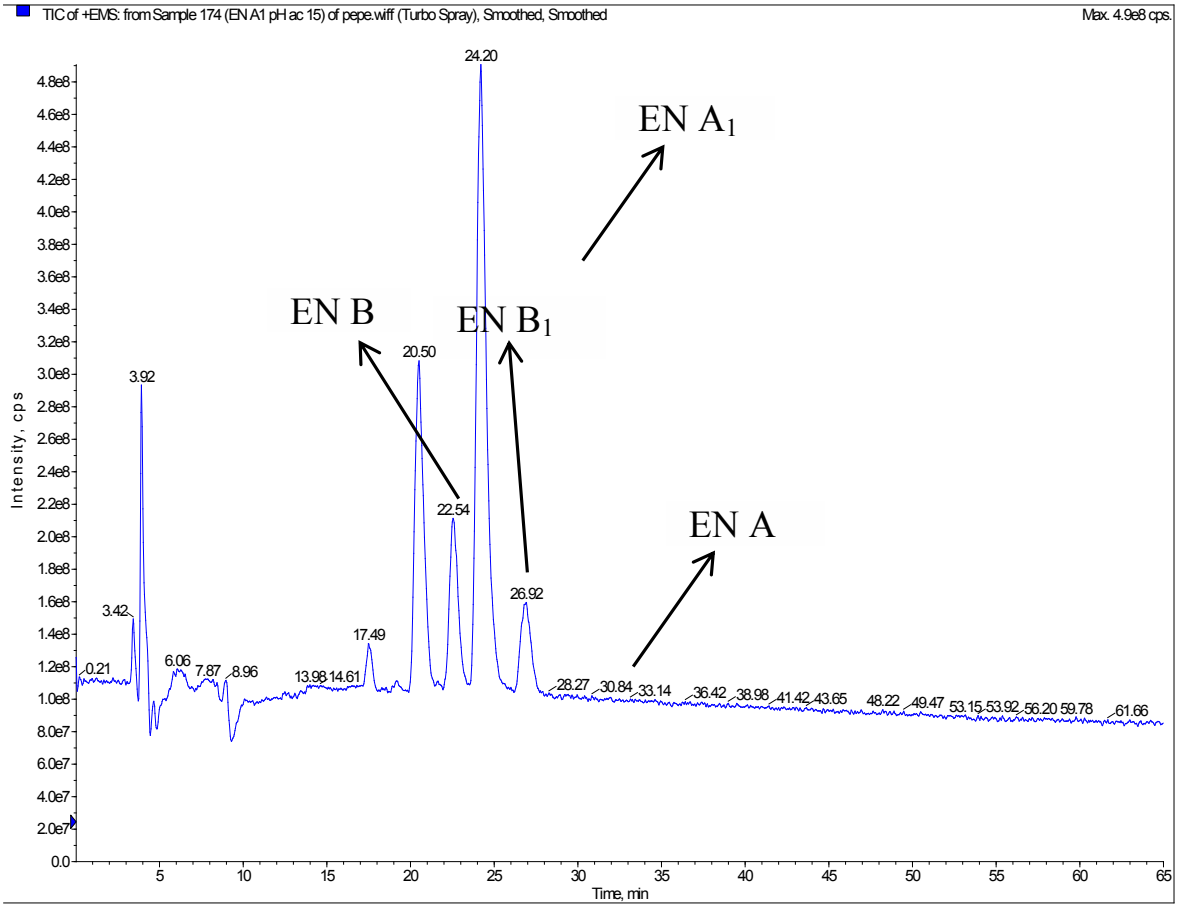

Figure 2: LC-MS/MS chromatogram of the ENs A, A_1, B, B_1, present in TSB medium before fermentation.

2. Characterization of the fragments obtained in the ER scan with the modality EPI scan to obtain a MS^2 scan of a fragment of the degradation product.

The utilization of the mass spectrometry associated at the detection with the linear ion trap, utilized in these two modalities permitted us to obtain a total characterization of the compound isolated Meca et al. [21].

Results and discussions

ENs degradation by *Bacillus subtilis* strains in TSB medium

All the *Bacillus subtilis* strains tested showed a degradation activity on the minor *Fusarium* mycotoxins ENs A, A_1, B and B_1 in TSB medium as is possible to observe in the data (each EN degradation experiment was repeated five times) shown in (Table 1).

In figure 2 is evidenced the LC-MS/MS chromatogram of the ENs present in growth mediums after the fermentation with *Bacillus subtilis* CECT 4522.

As is possible to evidence in table 1 the mean percentage of EN A degradation was 88.1%. The highest degradation data was evidenced by the strain of *Bacillus subtilis* CECT 371 with 96.2% whereas the lowest was detected in the sample fermented by *Bacillus subtilis* CECT 35 with 77.7%. The mean percentage of reduction of the EN A_1 was 83.1%. The highest reduction was performed by the strain of *Bacillus subtilis* CECT 497 with 95.4%, whereas the lowest reduction was obtained by *Bacillus subtilis* CECT 35 with 67.1%. The ENs of the B group presented mean degradations data similar to the values evidenced for the ENs A and A_1. In particular the mean degradation evidenced for the EN B was of 84.5%; the highest reduction was evidenced in the medium fermented by the strain of *Bacillus subtilis* CECT 497 with 98.0, whereas the lowest degradation was observed by the strain of *Bacillus subtilis* CECT 35 with 64.7%, that can be considered the lowest degradation data evidenced in this study. The EN B_1 evidenced a mean reduction of 92.0%, and the highest and lowest degradation activities were performed by the strains *Bacillus subtilis* CECT 497 and *Bacillus subtilis* CECT 35 with 99.0 and 79.7 respectively.

In the scientific literature only one reference is available on the microbial degradation of the minor *Fusarium* mycotoxins ENs. In particular Roig et al. [22] evaluated the degradation of the *Fusarium* mycotoxins ENs by 9 bacterial strains characteristic of the gastrointestinal tract like *Bb. longum, Bb. bifidum, Bb. breve, Bb. adolescentes, Lb. rhamnosus, Lb. casei–casei, S. termofilus, Lb. ruminis, Lb. casei* and twenty two strains of *Saccharomyces cerevisiae*. The degradation of the bioactive compounds ENs was also studied in a food system composed

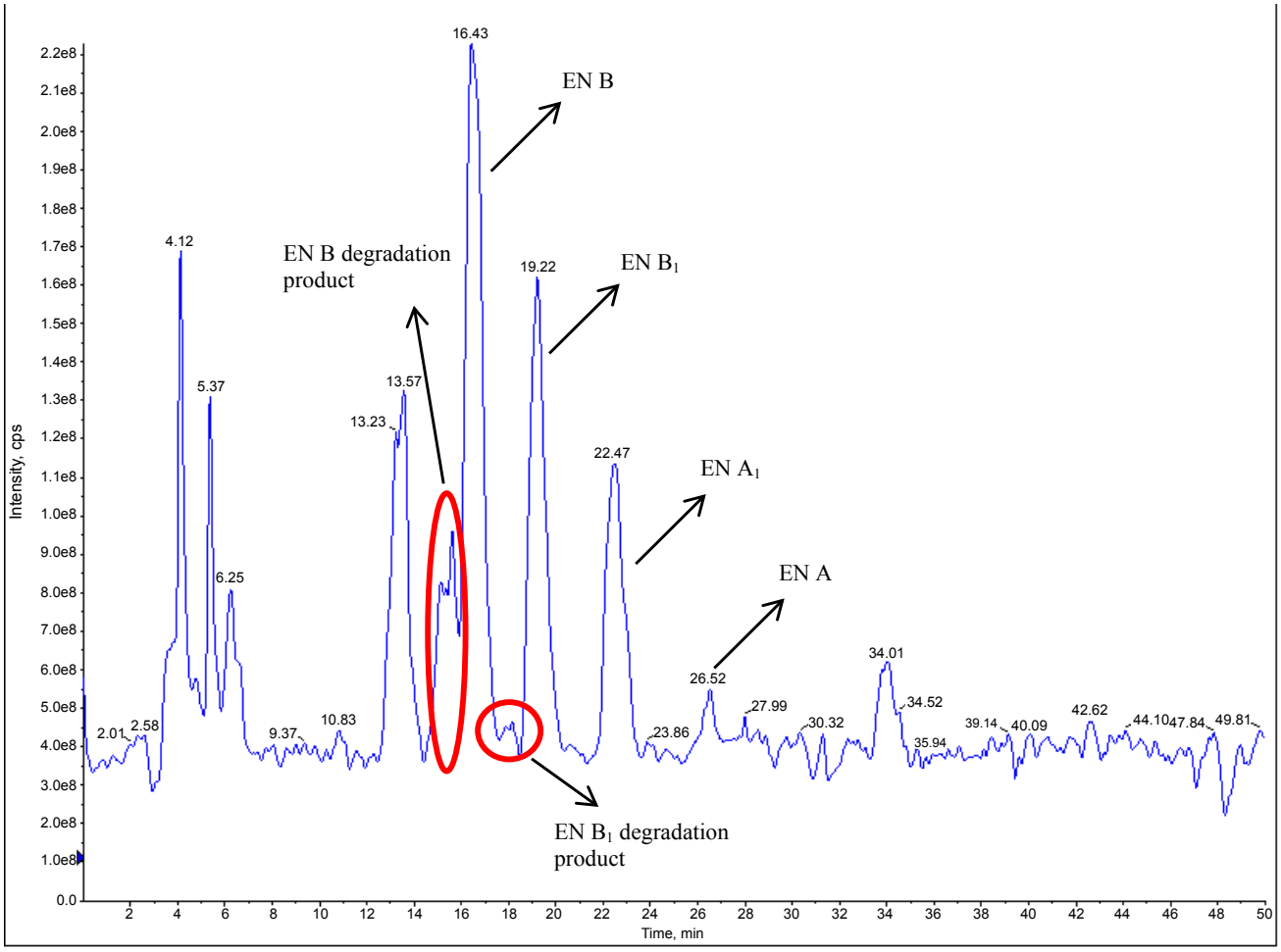

Figure 3: LC-MS-LIT chromatogram in ER mode of the ENs and its degradation products present in TSB medium after the fermentation by the strain of *Bacillus subtilis* CECT 4522.

by wheat flour naturally contaminated by ENs through fermentation by a strain of *Fusarium tricinctum*. The determination of the ENs in the fermentation mediums was performed using the technique of the liquid chromatography coupled to the mass spectrometry detector in tandem (LC–MS/MS). All the bacteria analyzed showed a significant ENs reduction in vitro during the fermentation processes, with degradation data ranging from 5 to the 99%. In the food system, the ENs degradation data evidenced ranged from 1.3 to 49.2%.

Meca et al. [21] evaluated the interaction between the minor *Fusarium* mycotoxin BEA a minor *Fusarium* mycotoxin structurally similar to the ENs, by 13 bacterial strains characteristic of the gastrointestinal tract as *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium adolescentes, Lactobacillus rhamnosus, Lactobacillus casei-casei, Lactobacillus plantarum, Eubacterium crispatus, Salmonella fecalis, Salmonella termofilus, Lactobacillus ruminis, Lactobacillus casei* and *Lactobacillus animalis*. The fermentations were carried out in the liquid medium of MRS during 4, 12, 16, 24 and 48 h at 37°C, under anaerobic conditions. All the bacteria studied showed a significant BEA reduction during the fermentation process, in particular the mean diminution ranged from 66 to 83%.

Considered that the mycotoxins BEA and ENs are characterized by similar chemical structures (cyclic compounds composed by three aminoacids and three HyLv units), this study confirms that these compounds can be degraded by bacteria of different species.

LC-MS-LIT characterization of the ENs degradation products

The samples positive to the ENs degradation, by microbial fermentation, were also injected in the LC-MS-LIT in the modality ER scan (m/z=200-900). In (Figure 3) is shown the chromatogram of the ENs presents in the fermentation extract produced by the strain of *Bacilllus subtilis* CECT 35, where is possible to evidence the presence of the ENs A, A_1, B and B_1 with the retention time (RT) of 16.4, 19.2, 22.4 and 26.5 min. In the chromatogram are also present some other peaks corresponding to the ENs degradation products. In particular close to the peak of the EN B and with the retention time of 14.9 minutes is evidenced the degradation product of this compound, whereas the EN B_1 degradation product is presents at the RT of 18.0 min. The formation of the EN B degradation product is confirmed by the fragments related to the structure of this new compound presents in the ER mass spectra showed in the figure 4a. In particular figure 4 it is shown that,the mass spectrum related to the EN B degradation product identified as the potassium adduct of the EN B, with the loss of a ENs structural component as the HyLv. The formation of this degradation product is confirmed by the fragment with a pseudomolecular weight of 561.2, and also by other important diagnostic signals as the fragments with a m/z of 547.1 and 503.2, that represent the potassium adduct of the EN B with the loss of the amino acid valine (Val) associated also with the loss of a molecule of water and of a carboxylic group, respectively. The fragment with a m/z of 459.2 confirms the presence in the structure of the EN B degradation product of the HyLv with the loss of two units. To obtain more information and also to confirm the structure of the EN B degradation product formed, the sample was also injected in the modality EPI scan to obtain the MS^2 scan of the degradation product isolated using as fragmenting signal, the ion with a m/z of 561.2. In (Figure 5a), is possible to observe the LC-MS-LIT chromatogram of the degradation product identified, and in (Figure 5b) the EPI-LIT spectrum with the presence of two important fragments that confirm the structure of the reduction product isolated that are the signals with m/z of 563.5 and 350.3. These signals confirmed the presence in the degradation product structure of the two components of the ENs structure as the Val and HyLv.

In (Figure 4b) is shown the ER-LIT spectrum related to the degradation product of the EN B_1, and identified as the potassium adduct of the EN B_1 with the loss of a structural component as the HyLv. In the spectra there are some important diagnostic signals that confirm the formation of this degradation product. The fragment with a m/z of 573.1 represents the MW of the EN B_1 degradation product, whereas the fragments with m/z of 517 and 473.2 represent the EN B with the loss of other important structural components as the amino acid Val for the signals with m/z of 517.2 and of the contemporary loss of the amino acids Ile and Val for the signal with m/z of 473.2. To confirm the formation of these new degradation product the ion corresponding to its MW was fragmented in the modality EPI (MS^2) scan with a mass range variable from 200 to 540 Da. In (Figure 6a) is evidenced the EPI-LIT chromatogram of the EN B_1 degradation product and in (Figure 6b) the MS^2 spectrum that confirm, with the presence of two diagnostic signals, the structure of the degradation product detected. In particular the fragment with a m/z of 415.4 was identified as the EN B_1 degradation products with the loss of another HyLv unit, whereas the signal with a m/z of 309.2 represents the degradation compound formed with the loss of the structural amino acids that compose the EN B_1 as the Val and Ile. The utilization of the LC-MS-LIT in the ER and EPI modalities permitted to characterize completely the two degradation products formed.

In the scientific literature only one reference is available on the identification of the degradation products of the minor *Fusarium* mycotoxins ENs through microbial fermentations mediated by probiotic bacteria, whereas the microbial degradation of other *Fusarium* mycotoxins and also the identification of the degradation products was studied by many authors.

In particular the degradation of the *Fusarium* mycotoxins ENs by nine bacterial strains characteristic of the gastrointestinal tract like *Bb. longum, Bb. bifidum, Bb. breve, Bb. adolescentes, Lb. rhamnosus, Lb. casei-casei, S. termofilus, Lb. ruminis*, *Lb. casei* and twenty two strains of *Saccharomyces cerevisiae* was studied was studied by Roig, Meca, Ferrer, Mañes [20]. The authors identifies three degradation products produced by the microbial fermentation of the EN A_1, B and B_1, that were characterized using the technique of the LC-MS-LIT and corresponding to the ENs with the loss of several structural components and in particular of the ENB-HyLv, EN B_1-Val and of the EN A-Ile. The degradation products identified by the authors are comparable with the degradation products identified in our article.

Meca et al. [23] evaluated the influence of the technological process and of the formation of degradation products of the minor *Fusarium* mycotoxin BEA during the beer and bread making studying the interaction between the mycotoxin and 13 bacterial strains characteristic of the gastrointestinal tract. Employing the technique of the LC-MS-LIT was identified a BEA degradation product composed by the mycotoxin with the loss one structural component of the BEA composed by the phenylalanine (Phe). The result obtained by the authors is comparable with the results obtained in our study considering that the principal's compounds lost by the mycotoxins during the fermentation are the structural amino acids that compose those bioactive compounds.

Guan et al. [14], evaluated the degradation of the Fusarium mycotoxin DON, employing microorganisms of fish digesta. The authors evidenced that the microbial pathway related to the fish

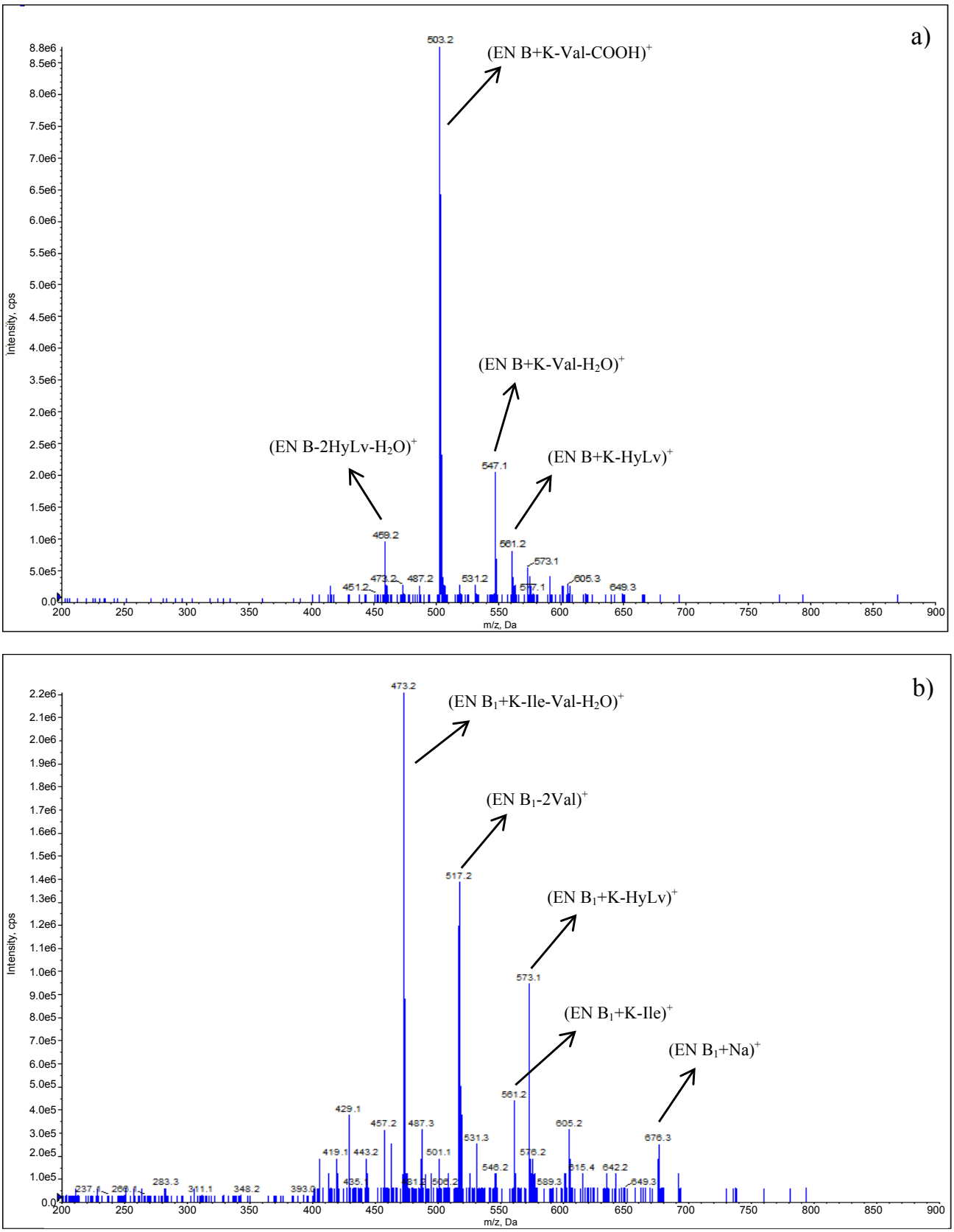

Figure 4: LC-MS-LIT mass spectra obtained in ER mode (MS^1) of the degradation products of the EN B a) and of the EN B_1 b).

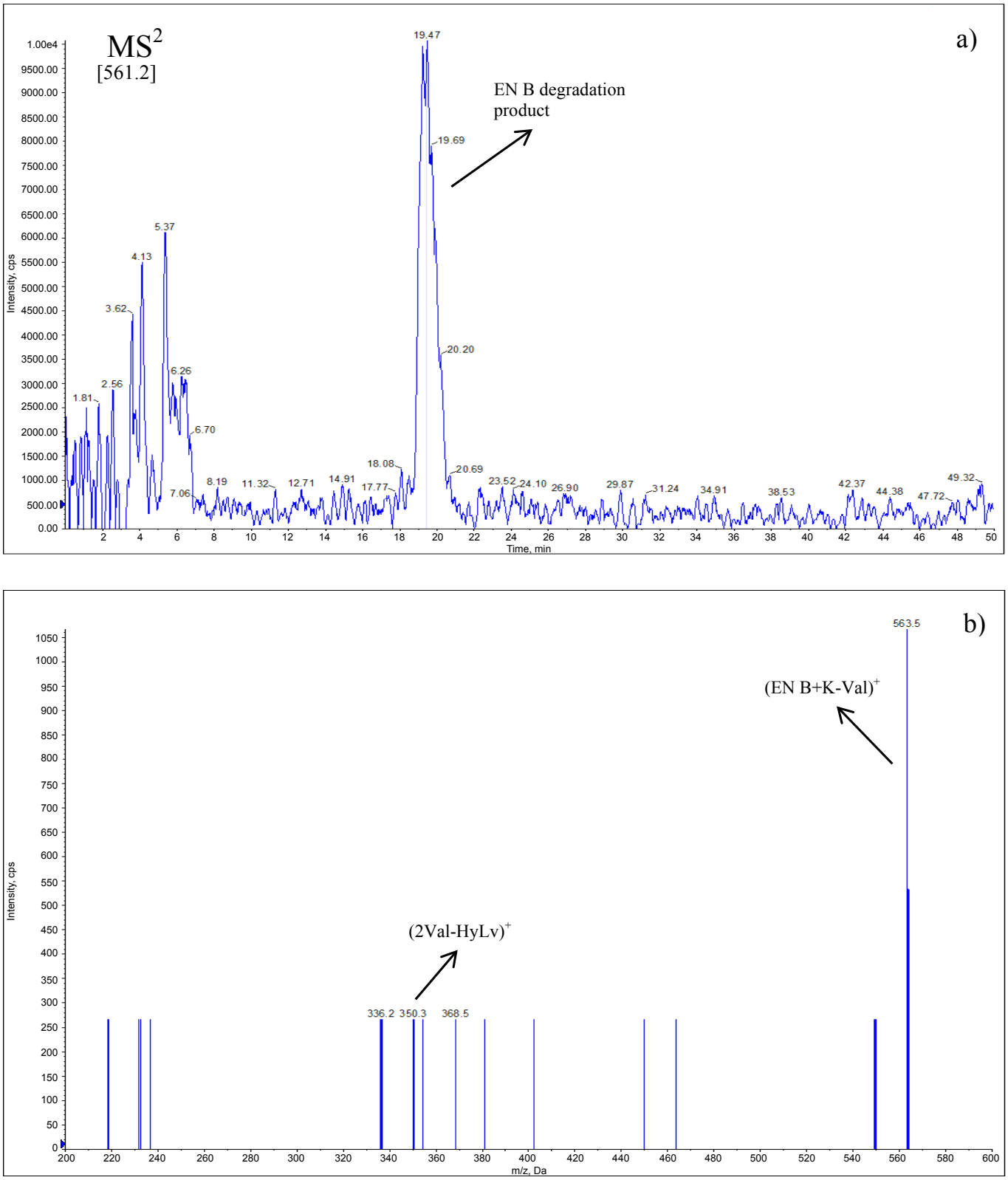

Figure 5: Chromatogram obtained in EPI mode (MS^2) using as fragmenting ion the signal with a *m/z* of 561.2 of the EN B degradation product a) and EPI mass spectrum b).

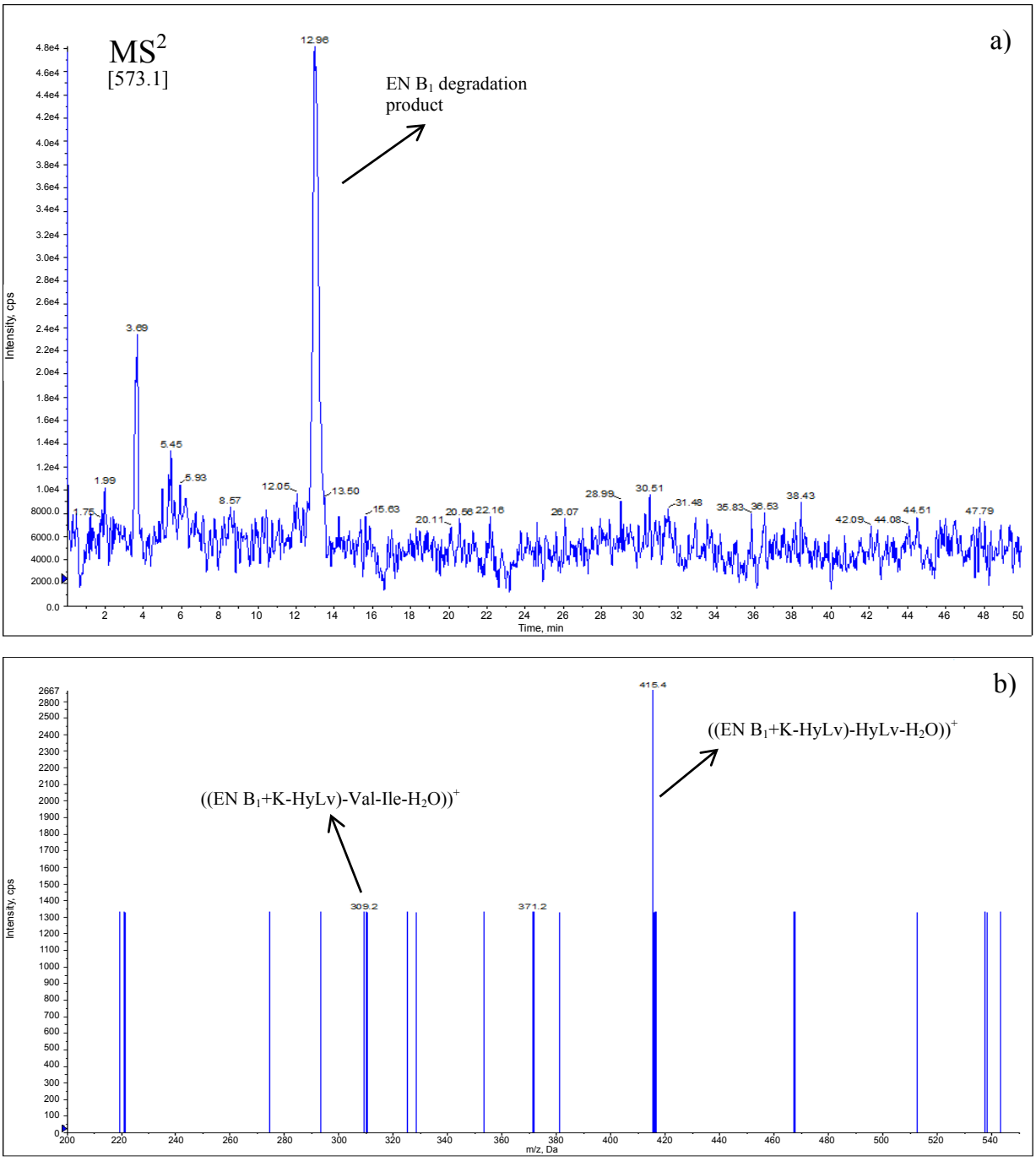

Figure 6: Chromatogram obtained in EPI mode (MS^2) using as fragmenting ion the signal with an m/z of 573.10 of the EN B_1 degradation product a) and EPI mass spectrum b).

Ameiurus nebulosus, transformed DON to deepoxy DON (dE-DON) at 15°C in full medium after 96 h incubation.

Awad et al. [16], explained the detoxification properties of an Eubacterium strain (BBSH 797), to transform completely the mycotoxin DON into 3a, 7a, l5-trihydroxytrichothec-9, 12-dien-8-one (deepoxy DON), which has been named DOM-1.

Islam et al. [17], employed different soil samples to carried out a screening of the micrograms that have the capacity to transform DON to dE-DON. The authors evidenced that a microbial culture originated by a soil sample showed the 100% DON to dE-DON biotransformation in Mineral Salt Broth (MSB), after 144 h incubation at the temperature of 25°C.

Conclusions

The present study evidences the capacity of several *Bacillus subtilis* strains to reduce the presence of the mycotoxins ENs in growth medium

forming two degradation products characterized with the technique of the LC-MS-LIT.

Further investigation will be focused on the application of the *Bacillus subtilis* strains to reduce the contamination by mycotoxins of food and feed employing fermentation techniques and also to study the toxicity of the ENs degradation products. The characterization of the toxicological parameters of these newly discovered substances is importance to evaluate the possible utilization of microorganisms to reduce the ENs level in food and feed matrices.

Acknowledgement

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References

1. Ivanova L, Skjerve E, Eriksen GS, Uhlig S (2006) Cytotoxicity of enniatins A, A1, B, B1, B2 and B3 from Fusarium avenaceum. Toxicon 47: 868-876.
2. Tomoda H, Huang XH, Cao J, Nishida H, Nagao R, et al. (1992) Inhibition of acyl-CoA: cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. J Antibiot 45: 1626-1632.
3. Lee HS, Song HH, Jeong JH, Shin CG, Choi SU, et al. (2008) Cytotoxicities of enniatins H, I, and MK1688 from fusarium oxysporum KFCC 11363P. Toxicon 51: 1178-1185.
4. Behm C, Degen GH, Follmann W (2009) The Fusarium toxin enniatin B exerts no genotoxic activity, but pronounced cytotoxicity in vitro. Molecular Nutrition and Food Research 53: 423–430.
5. Hyun U, Lee DH, Lee C, Shin CG (2009) Apoptosis induced by enniatins H and MK1688 isolated from Fusarium oxysporum FB1501. Toxicon 53: 723-728.
6. Tonshin AA, Teplova VV, Andersson MA, Salkinoja-Salonen MS (2010) The Fusarium mycotoxins enniatins and beauvericin cause mitochondrial dysfunction by affecting the mitochondrial volume regulation, oxidative phosphorylation and ion homeostasis. Toxicology 276: 49-57.
7. Mahnine N, Meca G, Elabidi A, Fekhaoui M, Saoiabi A, et al. (2011) Further data on the levels of emerging fusarium mycotoxins enniatins (A, A1, B, B1), beauvericin and fusaproliferin in breakfast and infants cereals from Morocco. Food Chem 124: 481-485.
8. Meca G, Zinedine A, Blesa J, Font G, Mañes J (2010) Further data on the presence of fusarium emerging mycotoxins enniatins, fusaproliferin and beauvericin in cereals available on the Spanish markets. Food Chem Toxicol 48: 1412-1416.
9. Zinedine A, Meca G, Mañes J, Font G (2011) Further data on the occurrence of Fusarium emerging mycotoxins enniatins (A, A1, B, B1), fusaproliferin and beauvericin in raw cereals commercialized in Morocco. Food Control 22: 1-5.
10. Oueslati S, Meca G, Mliki A, Ghorbel A, Mañes J (2011) Determination of fusarium mycotoxins enniatins, beauvericin and fusaproliferin in cereals and derived products from Tunisia. Food Control 22: 1373-1377.
11. Duvick J, Rod TA (1998) Beauvericin detoxification composition and methods. United States Patents, USA.
12. Poppenberger B, Berthiller F, Lucyshyn D, Sieberer T, Schuhmacher R, et al. (2003) Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from Arabidopsis thaliana. The Journal of Biological Chemistry 278: 47905-47914.
13. Young JC, Zhou T, Yu H, Zhu H, Gong J (2007) Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food and Chemical Toxicology 45: 136-143.
14. Guan S, He J, Young JC, Zhu H, Li XZ, et al. (2009) Transformation of trichothecene mycotoxins by microorganisms from fish digesta. Aquaculture 29: 290-295.
15. Heinl S, Hartinger D, Thamhesl M, Vekiru E, Krska R, Schatzmayr G, Moll WD, Grabherr R (2010) Degradation of fumonisin B1 by the consecutive action of two bacterial enzymes. Journal of Biotechnology 145: 120-129.
16. Awad WA, Ghareeb K, Bohm J, Zentek J (2010) Decontamination and detoxification strategies for the Fusarium mycotoxin deoxynivalenol in animal feed and the effectiveness of microbial biodegradation. Food Additive and Contaminants 27: 510-520.
17. Islam R, Zhou T, Young JC, Goodwin PH, Pauls KP (2012) Aerobic and anaerobic de-epoxydation of mycotoxin deoxynivalenol by bacteria originating from agricultural soil. World Journal of Microbiology and Biotechnology 28: 7-13.
18. Jestoi M (2008) Emerging fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: a review. Critical Review in Food Science and Nutrition 48: 21-49.
19. European Commission (EC) (2002) Regulation (EC) No 2002/657 of 12 August implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities L221.
20. European Commission (EC) (2006) Regulation (EC) No 401/2006 of 23 February laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of European Communities L70.
21. Meca G, Ritieni A, Mañes J (2012) Reduction in vitro of the minor fusarium mycotoxin beauvericin employing different strains of probiotic bacteria. Food Control 28: 435-440.
22. Roig M, Meca G, Ferrer E, Mañes J (2013) Reduction of the enniatins A, A1, B, B1 by an in vitro degradation employing different strains of probiotic bacteria: Identification of degradation products by LC–MS–LIT. Toxicon 70: 44-53
23. Meca G, Zhou T, Li XZ, Mañes J (2013) Beauvericin degradation during bread and beer making. Food Control 34: 1-8.