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Original Research

In vitro study of L-asparaginase enzyme activity by two yeast strains on food matrixes and the relative effect on fungal pathogens growth

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Abstract

Asparagine is one of the precursors of acrylamide and toxic fungal secondary metabolites, both carcinogenic compounds. In the present study, the optimal conditions to deplete asparagine by *Aureobasidium pullulans* (L1 and L8) from potato and wheat flour matrices were investigated. Through a colorimetric plate-assay with phenol red as indicator dye, both strains demonstrated to be able to produce L-asparaginase from 20 °C to 30 °C for L1 and only at 20 °C for L8 strain starting from 48 h of incubation. The ability of both yeasts to reduce asparagine content in potato and wheat flour was studied by *in vitro* spectrophotometric assay. Both strains showed a great ability to totally reduce asparagine at 20 °C after 15 min of incubation in potato homogenate, conversely in wheat flour, the highest reduction was detected after a longer exposition time (60 min). As known, L1 and L8 diamine asparaginase to aspartic acid. For this reason, both amino acids were tested to verify the antifungal effect against *Rhizoctonia solani* (Rs1) and *Fusarium graminearum* (F3) mycelial growth. Asparagine (120 mg/L) increased Rs1 and F3 mycelial growth respectively by 4.4% and 18.9%; conversely, aspartic acid significantly inhibited both respectively by 8.2% and 12.0%.

Keywords: Amino acids; *Aureobasidium*; Potato; Wheat flour; Temperature; Fungi

1. Introduction

L-asparaginase is an amid-hydrolase that catalyzes L-asparagine to L-aspartate and L-ammonia [1]. Recently, its important applications in the pharmaceutical industry as cancers treatment determined a great interest from different international industrial sectors [2]. L-asparaginase enzyme is also used in the food industry for acrylamide prevention formation in foods processed with high temperatures [3], especially for baked and fried foods. It is known that plants, animals, and microorganisms can produce asparaginase enzyme [4,5]. Among yeasts, L-asparaginase or L-glutaminase occurred frequently in *Hansenula* spp., *Cryptococcus* spp., and *Rhodotorula* spp. [6]. More recently, *Saccharomyces cerevisiae* stands up between filamentous fungi with a high asparaginase activity [7]. Much effort has been invested in the study of asparagine, related to acrylamide levels mitigation in food to reduce its exposure to the consumers. Acrylamide is a carcinogenic molecule to humans, resulting from the Maillard reaction that involves asparagine, reducing sugars, and high temperatures [8]. The yeast *Aureobasidium pullulans* can assimilate asparagine and determine a reduction of acrylamide content in fried potatoes [9–11]. Application of L-asparaginase in food process represent an alternative method to reduce acrylamide content in fried, baked, and toasted products. At the same time, the activity of this enzyme can improve the antagonistic capability of a biocontrol agent (BCA) against the development of some fungal pathogens [12]. It is known that a specific nutritional composition can enhance BCAs activity but inhibit the growth of the pathogens [13]. The amino acids involved in the studied food systems such as potato and wheat flour are mainly based on asparagine and aspartic acid. Both amino acids were studied because the first one can be utilized readily by the antagonists and poorly by the pathogens, and the second one showed an inhibiting effect on the growth of some molds [9]. About potatoes, *Rhizoctonia solani* represents an economically important disease [14] causing both quantitative and qualitative damages. In the case of wheat, *Fusarium graminearum* can cause severe economic losses for cereal farmers due to significant reductions in grain yield and quality, especially for mycotoxins production [15].

In this regard, the goals of the research were to (i) verify *A. pullulans* strains extracellular L-asparaginase enzyme activity at different growth conditions (temperatures and times) through *in vitro* assays and (ii) to confirm their ability to reduce asparagine content in presence of different vegetable matrices, such as potato and wheat flour, at different co-culturing conditions. (iii) The last aim was to verify how amino acids content influence *R. solani* and *F. graminearum* mycelial growth.

2. Materials and methods

2.1 Microorganisms

Aureobasidium pullulans L1 and L8 strains [16] were grown on nutrient yeast dextrose agar (Oxoid, UK) in 1 L of distilled water, and stored at 4 °C until use. Before the experiments, the strains were cultured in nutrient yeast dex-

trose broth (50 mL) in conical flasks (250 mL) on a rotary shaker at 200 rpm for two days at 25 °C. Cells were washed with sterile distilled water (SDW) by centrifugation of the liquid culture samples. Cells were resuspended in SDW and adjusted to a concentration of 10^8 cells/mL. *Rhizoctonia solani* (Rs1) [17] and *F. graminearum* (F3) [18] were grown on Potato Dextrose Agar (39 g in 1 L of distilled water, Oxoid, UK) at 20 °C for 7 d.

2.2 Food matrices: potato and wheat flour

Potatoes ("Primura") from experimental fields (Bologna, Italy) were stored at 8 °C at high relative humidity (90%). Tubers were washed, peeled, mixed with an electric mixer (Imetec, Italy). Grain samples *cv* "Achille" (~100 g) were previously cleaned and then milled on an analytical mill (model IKA A11 BS32) to obtain wheat flour.

2.3 L1 and L8 l-asparaginase activity

Asparaginase enzymatic activity was tested by a rapid plate assay made by suspending: glucose 2 g, L-asparagine (Sigma Aldrich, Saint Louis, MO, USA) 10 g, KH_2PO_4 1.52 g, KCl 0.52 g, $CuNO_3.3H_2O$ (trace), $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, agar technical 15 g in 1000 mL of distilled water. Agar was supplemented with 3 mL of a phenol red stock solution (2.5%) dissolved in ethanol (pH 6.2). Agar plates were inoculated with yeasts plug (5 mm Ø) derived from two days colonies, suddenly incubated at 20 °C, 25 °C, 30 °C. The control consisted of agar inoculated with NYDA plugs. After 2, 4, and 7 days of incubation, the colony diameters were measured and the agar shading to pink color was detected. At 7 days of incubation the pH values were evaluated (model pH 25+, Crison, Spain). At least 15 plates for each strain and growth condition of treated and control samples were analyzed. The experiment was conducted twice.

2.4 L-asparagine quantitative assay

Samples of homogenized potato (1 g) and wheat flour (1 g) were amended with L1 and L8 suspension concentrated 10^8 cells/ml or with distilled water (ratio 1:3, respectively) for the control. The effect of carbon and nitrogen sources on L-asparagine samples content was assayed by L-asparagine kit assay (Megazyme, Ireland, EU) after 15, 30, 60 min of incubation at 20 °C, 25 °C, 30 °C on a rotary shaker (250 rpm). The sample cultures were centrifuged at 12,000 g (for 8 min) and the supernatant thus obtained was used as a crude extract for L-asparagine quantification. Absorbance was measured at 340 nm with a spectrophotometer (Tecan Infinite F50, Switzerland). Three replicates for each treatment were set up and the experiment was conducted three times.

2.5 In vitro assay: amino acids effect on fungal growth

Asparagine and aspartic acid effect on fungal growth were assayed on PDA plates. Agar medium was amended with different concentrations of the above-reported amino acids (0 mg/L, 15 mg/L, 40 mg/L, 80 mg/L, and 120 mg/L) (Sigma Aldrich, MO, USA). Subsequently, substrates were inoculated with a mycelial pathogen agar plug (6 mm, Ø) and incubated at 20 °C. PDA without amendments represented the control. Fungal colonies diameter was measured after 5 days. Five plates (replicates) represented the sample unit for each concentration and microorganism. The experiment was conducted twice.

2.6 Statistical analysis

Statistical analysis was conducted by using Statgraphics software (version centurion 15.0, The Plains, VA, USA). One-way ANOVA was used to analyze data and the least significant differences test (LSD) to separate differences among the means ($p < 0.05$).

3. Results

3.1 L-asparaginase activity by L1 and L8 strains

To screen the asparaginase activity of the two yeasts a plate assay was set up. Phenol red was added to the agar to reveal the pH alterations induced by the release of ammonia ions by the L-asparaginase activity. The pH indicator is normally yellow at acid conditions and turns pink at alkaline pH. L-asparaginase activity by the yeasts was detected through a pink halo around the colony. By culturing L1 and L8 on the screening plate amended with asparagine, a more intensive pink halo was detected around the colony, starting from the two days of incubation at 20 °C by both strains, reaching the largest halo diameter at 7th day (39 mm and 18 mm respectively) (Table 1). No halos were displayed by the control plates at each temperature and exposition time. However, despite the dye did not inhibit the yeast growth, at 25 °C and 30 °C the L8 strain did not produce halos and showed higher values of colony diameter at each incubation temperature concerning L1 strain. By the plate assay, *A. pullulans* strains, especially L1, were found to be good producers of L-asparaginase (**Supplementary Fig. 1**). A direct correlation was found between the halo diameters and pH values: at higher diameter halo values corresponded higher pH values (Table 1). Instead, an inverse correlation was shown by L8 between colony diameter and halo diameter, where at more developed yeast colonies (mm) corresponded lowest or even absent halos.

3.2 L-asparagine quantitative assay

Yeast strains resulted both good consumers of asparagine contained in the target food matrices. At each tested temperature the asparagine amount increased over time in control samples. Especially at 60 min of incubation at each temperature, the increase of the asparagine content in potato homogenate and wheat flour was respectively of 83%, 84%, 66% and 66%, 48%, 96%, for the recorded values at 15 min.

Table 1. *Aureobasidium pullulans* asparaginase enzyme activity.

Incubation time							
20 °C	2 d		4 d		7 d		
	Colony diameter (mm)	Halo diameter (mm)	Colony diameter (mm)	Halo diameter (mm)	Colony diameter (mm)	Halo diameter (mm)	pH
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	6.2 ± 0.0a
L1	9.7 ± 0.9b	21.5 ± 1.7c	13.7 ± 0.5b	32.2 ± 2.8c	16 ± 1.1b	39 ± 2.0c	7.2 ± 0.2c
L8	11.7 ± 1.2c	18.0 ± 1.8b	17.5 ± 1.0c	18 ± 1.8b	23.5 ± 1.3c	18 ± 1.8b	6.9 ± 0.1b
Incubation time							
25 °C	2 d		4 d		7 d		
	Colony diameter (mm)	Halo diameter (mm)	Colony diameter (mm)	Halo diameter (mm)	Colony diameter (mm)	Halo diameter (mm)	pH
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	6.2 ± 0.0a
L1	11.7 ± 0.5b	21.5 ± 1.3b	17.7 ± 1.2b	37.7 ± 2.9b	20.5 ± 3.0b	56.2 ± 4.3b	7.2 ± 0.1c
L8	15.7 ± 2.0c	0.0 ± 0.0a	22.7 ± 2.5c	0.0 ± 0.0a	29 ± 2.4c	0.0 ± 0.0a	6.4 ± 0.2b
Incubation time							
30 °C	2 d		4 d		7 d		
	Colony diameter (mm)	Halo diameter (mm)	Colony diameter (mm)	Halo diameter (mm)	Colony diameter (mm)	Halo diameter (mm)	pH
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	6.2 ± 0.0a
L1	9.5 ± 1.0b	17 ± 0.8b	10.2 ± 0.9b	29.7 ± 1.7b	12.5 ± 0.5b	29.7 ± 1.7b	6.8 ± 0.2b
L8	11.2 ± 0.9c	0.0 ± 0.0a	16 ± 1.1c	0.0 ± 0.0a	20.2 ± 0.9c	0.0 ± 0.0a	6.2 ± 0.3a

The halos were recorder after 2, 4, 7 days of incubation at 20 °C, 25 °C, and 30 °C. pH values were observed after 7 days of incubation. Different letters within the same temperature, incubation time, and pH value indicate significant differences, LSD test ($p < 0.05$).

In the potato matrix, L1 and L8 strains showed a great ability to completely reduce asparagine content especially at 20 °C immediately after 15 min of incubation, instead of at 30 °C the strains' efficacy decreases (Fig. 1), mainly for L8.

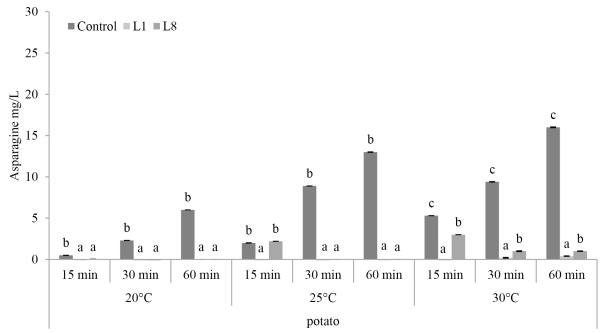

Fig. 1. Asparagine amount in potato homogenate after 15, 30 and 60 min of incubation with *Aureobasidium pullulans* L1 and L8 strains at 20 °C, 25 °C, and 30 °C. Different letters within the same temperature and incubation time indicate significant differences, LSD test ($p < 0.05$).

In wheat flour, the two strains showed different behavior. L1 showed faster asparagine consumption, with the best performances at 60 min of incubation at each tested temperature, almost completely reducing the target amino acid (Fig. 2).

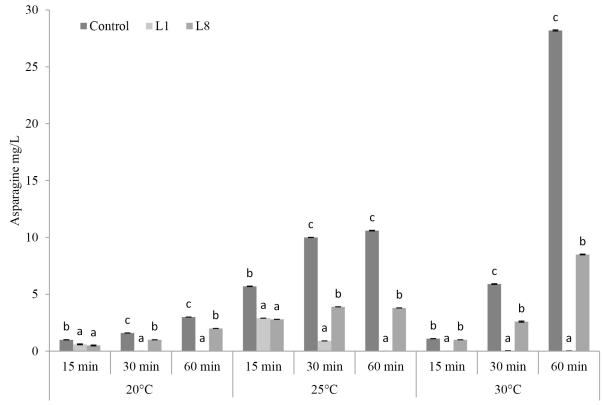

Fig. 2. Asparagine amount in wheat after 15, 30 and 60 min of incubation with *Aureobasidium pullulans* L1 and L8 strains at 20 °C, 25 °C, and 30 °C. Different letters within the same temperature and incubation time indicate significant differences, LSD test ($p < 0.05$).

Also *A. pullulans* L8 strain reduced asparagine content in wheat flour but as described above for potato homogenate, its efficacy (68.6%) concerning L1 (100%) was reduced by the highest temperature (30 °C), that conversely increased the asparagine levels.

3.3 In vitro assay: amino acids effect on fungal growth

Different concentrations of asparagine and aspartic acid (0, 15, 40, 80, 120 mg/L) were infused on MEA plates to evaluate their effect on Rs1 and F3 mycelial growth. The presence of the highest concentration of aspartic acid determined a significant reduction of both pathogens' mycelial diameter with respect to the control, respectively by 8.2% and 12.0%. About the asparagine, the amino acid concentration of 120 mg/L resulted the most effective with respect to the control by significantly stimulating *R. solani* and *F. graminearum* growth by 4.4% and 18.9% respectively (Fig. 3a,b).

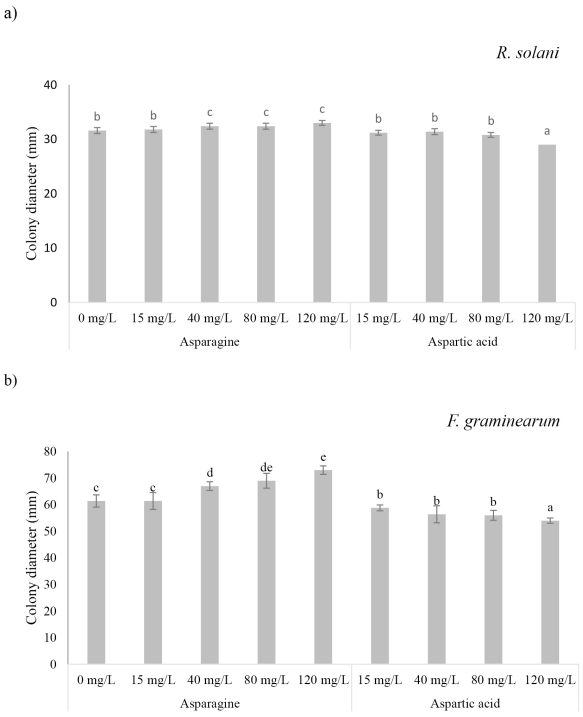

Fig. 3. Asparagine and aspartic acid concentrations effect on *Rhizoctonia solani* (a) and *Fusarium graminearum* (b) mycelial growth (colony diameter, mm) after 7 days of incubation at 20 °C. Malt extract plates were amended with different amino acids concentrations (0, 15, 40, 80, 120 mg/L) and inoculated with a 6 mm (Ø) mycelial plug. Different letters indicate significant differences, LSD test ($p < 0.05$).

4. Discussion

In a previous study, L1 and L8 displayed their capability to drastically alter the amino acids pattern of peach juice with a strong consumption of asparagine [9]; this activity was confirmed also in fried potato chips by 80% of reduction of acrylamide content [10]. The present study reports the asparaginase activity exerted by both yeast

strains through the direct method of the plate assay, where both strains showed a different capability to produce L-asparaginase enzyme. This was more expressed by L1 with respect to L8 strain. However, the enzymatic activity exerted by microorganisms was strictly influenced by environmental conditions such as temperature and pH [19].

L1 strain displayed more intense halos at each temperature notably increasing pH, with the halos diameters directly connected to the pH values. Conversely, L8 showed asparaginase halos only at 20 °C of smaller size and with lower pH values with respect to L1. The plate assay results were confirmed by the spectrophotometer experiments where the asparagine content was reduced by L1 at each tested temperature of incubation in potato homogenate and by L8 only at 20 °C. The temperature and the exposition time increased the asparagine levels in food matrices, especially in wheat flour exposed to the highest temperature and incubation time. Nevertheless, L1 confirmed is pronounced asparaginase activity with respect to L8.

However, this activity is strictly connected to L1 and L8 strains mechanisms of action: competition for nutrients [9]. In fact, through the capability of the BCAs to compete for asparagine, pathogens' aggressiveness was reduced, as showed also against *Botrytis cinerea* and *Monilinia laxa* [9, 12].

Results showed how each fungal growth was stimulated by the presence of asparagine. As reported by Giese *et al*. [20], asparagine was found to be a preferential nitrogen source for *F. graminearum.* About *R. solani,* its virulence was increased by organic sources of nitrogen such as asparagine [21].

Yeasts use asparagine as their nitrogen source through its deamination to aspartic acid [22]; this was the reason why the study provided the same assay for the evaluation of the aspartic acid effect on pathogens' growth.

Amino acids are fundamental compounds, sometimes toxic if at high intracellular levels [23]. For example, bacterial cells growth can be inhibited by valine and phenylalanine, meanwhile causing the enzymes repression involved in isoleucine and tyrosine synthesis [24,25].

However, amino acids such as aspartic acid, glutamic acid, glycine, serine, alanine, and leucine can reduce *B. cinerea* growth *in vitro* tests [12]. Nevertheless, amino acids were often used as antiseptic agents due to their low toxicity level [26–29].

The application of yeasts or their enzymes in various technological processes may have also an inhibitory effect on certain toxins producers' fungi such as *F. graminearum.* These microorganisms can accumulate mycotoxins from agricultural products decontaminating them [30].

Mycotoxigenic fungi are capable to grow on a broad range of substrates. However, not all of these substrates are equally good for mycotoxin production.

Bullerman *et al*. [31] asserted that the formation of aflatoxin is stimulated by the presence of certain amino acids, fatty acids, and zinc. Asparagine has been reported to be a good nitrogen source for aflatoxin production in stationary cultures [32].

Based on the obtained results, other experiments should be conducted for more details on the influence of the two yeasts on the asparagine content in food matrices and if they could represent an innovative, environment friendly, and effective method to mitigate toxic fungal secondary metabolites and acrylamide formation in cooking.

5. Conclusions

The present study reports the versatility use of BCAs, especially yeasts, such as mitigating agents of toxic substances in food and fungal pathogens antagonists. It is in fact known how some BCAs have the capacity to detoxify foods from toxic compounds and control fungal pathogens development ensuring food security and quality. The presented results could be added to the others with the aim to streamline procedures for the application and regulation of BCAs in agriculture and food technologies. However, all the food industry starting from the agricultural practices and storage management should be considered and improved to reduce pathogens' development and toxic compounds formation. By the way, novel enzymes' production by *A. pullulans* with other potential effects is still to be investigated and studied in-depth.

Author contributions

ADF conducted all the experiments, analysis and writing of the manuscript.

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Ethics approval and consent to participate

Not applicable.

Acknowledgment

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Conflict of interest

The author declares no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBE/14/1/10.31083/j.fbe1401006.

References

[1] Doriya K, Kumar DS. Isolation and screening of L-asparaginase free of glutaminase and urease from fungal sp. *3 Biotech* **2016**; 6: 239.

[2] Appel IM, van Kessel-Bakvis C, Stigter R, Pieters R. Influence of two different regimens of concomitant treatment with asparaginase and dexamethasone on hemostasis in childhood acute lymphoblastic leukemia. *Leukemia* **2007**; 21: 2377–2380.

[3] Mohan Kumar NS, Shimray CA, Indrani D, Manonmani HK. Reduction of Acrylamide Formation in Sweet Bread with l-Asparaginase Treatment. *Food Bioproc. Technol.* **2013**; 7: 741–748.

[4] Sahu MK, Poorani E, Sivakumar K, Thangaradjou T, Kannan L. Studies on L-asparaginase enzyme of actinomycetes isolated from estuarine fishes. *J. Env. Biol.* **2007**; 28: 645–650.

[5] Eisele N, Linke D, Bitzer K, Na'amnieh S, Nimtz M, Berger RG. The first characterized asparaginase from a basidiomycete, Flammulina velutipes. *Bioresour. Technol.* **2011**; 102: 3316–3321.

[6] Imada A, Igarasi S, Nakahama K, Isono M. Asparaginase and Glutaminase Activities of Micro-organisms. *J. Gen. Microbiol.* **1973**; 76: 85–99.

[7] Akıllıoglu HG, Gökmen V. Mitigation of acrylamide and hydroxymethyl furfural in instant coffee by yeast fermentation. *Food Res. Int.* **2014**; 61: 252–256.

[8] Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. *Nature* **2002**; 419: 448–449.

[9] Di Francesco A, Ugolini L, D'Aquino S, Pagnotta E, Mari M. Biocontrol of Monilinia laxa by Aureobasidium pullulans strains: Insights on competition for nutrients and space. *Int. J. Food Microbiol.* **2017**; 248: 32–38.

[10] Di Francesco A, Mari M, Ugolini L, *et al*. Reduction of acrylamide formation in fried potato chips by Aureobasidum pullulans L1 strain. *Int. J. Food Microbiol.* **2019**; 289: 168–173.

[11] Schouten MA, Genovese J, Tappi S, *et al*. Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips. *Innov. Food Sci. Emerg. Technol.* **2020**; 64: 102397.

[12] Di Francesco A, Mari M, Ugolini L, Baraldi E. Effect of Aureobasidium pullulans strains against Botrytis cinerea on kiwifruit during storage and on fruit nutritional composition. *Food Microbiol.* **2018**; 72: 67–72.

[13] Gramisci BR, Lutz MC, Lopes CA, Sangorrín MP. Enhancing the efficacy of yeast biocontrol agents against postharvest pathogens through nutrient profiling and the use of other additives. *Biol. Control* **2018**; 121: 151–158.

[14] Tsror L. Biology, Epidemiology and Management of Rhizoctonia solani on Potato. *J. Phytopathol.* **2010**; 158: 649–658.

[15] Parry DW, Jenkinson P, Mcleod L. Fusarium ear blight (scab) in small grain cereals–a review. *Plant Pathol.* **1995**; 44: 207–238.

[16] Di Francesco A, Calassanzio M, Ratti C, Mari M, Folchi A, Baraldi E. Molecular characterization of the two postharvest biological control agents Aureobasidium pullulans L1 and L8. *Biological Control* **2018**; 123: 53–59.

[17] Di Francesco A, Di Foggia M, Corbetta M, Baldo D, Ratti C, Baraldi E. Biocontrol Activity and Plant Growth Promotion Exerted by Aureobasidium pullulans Strains. *J. Plant Growth Regul.* **2021**; 40: 1233–1244.

[18] Senatore MT, Ward TJ, Cappelletti E, *et al*. Species diversity and mycotoxin production by members of the Fusarium tricinctum species complex associated with Fusarium head blight of wheat and barley in Italy. *Int. J. Food Microbiol.* **2021**; 358: 109298.

[19] Li X, Zhang X, Xu S, *et al*. Simultaneous cell disruption and semi-quantitative activity assays for high-throughput screening of thermostable L-asparaginases. *Sci. Rep.* **2018**; 8: 7915.

[20] Giese H, Sondergaard TE, Sørensen JL. The AreA transcription factor in Fusarium graminearum regulates the use of some non-preferred nitrogen sources and secondary metabolite production. *Fungal Biology* **2013**; 117: 814–821.

[21] Weinhold AR, Bowman T, Dodman RL. Virulence of *Rhizoctonia solani* as affected by nutrition of the pathogen. *Phytopathology.* **1969**; 59: 1601–1605.

[22] Drainas C, Kinghorn JR, Pateman JA. Aspartic Hydroxamate Resistance and Asparaginase Regulation in the Fungus Aspergillus nidulans. *J. Gen. Microbiol.* **1977**; 98: 493–501.

[23] Ruiz SJ, van 't Klooster JS, Bianchi F, Poolman B. Growth Inhibition by Amino Acids in Saccharomyces cerevisiae. *Microorganisms.* **2021**; 9: 7.

[24] LEAVITT RI, UMBARGER HE. Isoleucine and valine metabolism in Escherichia coli. XI. Valine inhibition of the growth of Escherichia coli strain K-12. *J. Bacteriol.* **1962**; 83: 624–630.

[25] Bhatnagar RK, Berry A, Hendry AT, Jensen RA. The biochemical basis for growth inhibition by L-phenylalanine in Neisseria gonorrhoeae. *Mol. Microbiol.* **1989**; 3: 429–435.

[26] Minami M, Ando T, Hashikawa S, *et al*. Effect of glycine on Helicobacter pylori in vitro. *Antimicrob. Agents Chemother.* **2004**; 48: 3782–3788.

[27] Baisa G, Stabo NJ, Welch RA. Characterization of Escherichia coli D-cycloserine transport and resistant mutants. *J. Bacteriol.* **2013**; 195: 1389–1399.

[28] Barreteau H, Kovac A, Boniface A, Sova M, Gobec S, Blanot D. Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* **2008**; 32: 168–207.

[29] Fox SW, Fling M, Bollenback GN. Inhibition of Bacterial Growth by d-Leucine. *J. Biol. Chem.* **1944**; 155: 465–468.

[30] Pfliegler WP, Pusztahelyi T, Pócsi I. Mycotoxins - prevention and decontamination by yeasts. *J. Basic Microbiol.* **2016**; 55: 805–818.

[31] Bullerman LB. Effects of Potassium Sorbate on Growth and Aflatoxin Production by *Aspergillus parasiticus* and *Aspergillus flavus*. *J. Food Prot.* **1983**; 46: 940–942.

[32] Reddy TV, Viswanathan L, Venkitasubramanian TA. Factors Affecting Aflatoxin Production by Aspergillus parasiticus in a Chemically Defined Medium. *J. Gen. Microbiol.* **1979**; 114: 409–413.