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Production of plant growth regulatory metabolites of *Rhizopus arrhizus* KB-2

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Abstract

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The genus *Rhizopus* is a well recognized filamentous fungus, inhabiting soil, rotting fruits, vegetables, food, etc. *Rhizopus arrhizus* is widely used in biotechnology and is technologically important producer of organic acids and hydrolytic enzymes.

The aim of this study was to estimate the capability of *Rh. arrhizus* KB-2 strain to produce metabolites with plant growth regulatory activity. Morphological characteristics, biosynthesis of hydrolytic enzymes and production of regulatory metabolites were defined during the batch cultivation. The presence of some plant regulatory metabolites in the free-cell cultural liquid, such as gibberellic acid, trans-zeatin, indol–3 acetic acid and cis, trans-abscisic acid were determined by liquid chromatography-tandem mass spectrometry analysis.

A molecular phylogenetic identification of *Rh. arrhizus* was done for the first time in the present study. The identity of our strain *Rhizopus arrhizus* KB-2 was conformed to the species *Rhizopus arrhizus*. The strain produced various metabolites with different concentrations in different fermentation media and time and the cell free supernatant from 216 h and 264 h increased 32% of the root length of *Lactuca sativa*. In cell free supernatant of *Rh. arrhizus* KB-2 was determined the presence of the phytohormones (auxins and gibberellins), which influenced the seed germination and root formation.

Keywords: Rhizopus arrhizus; regulatory metabolites; hydrolytic enzymes; seed germination

Introduction

Soils have a large number of microorganisms that exist in the rhizosphere or on the root surface of the plants. Most of them play important roles in the degradation of organic matter. Some suppress harmful effect and inhibit a plant growth (Agrios, 2004). They affect the structure of the plant community and may also have effects on the plant performance. Pathogenic fungi are the major infectious agents in the plants, which cause changes during the stages of development, including postharvest. Fruits and vegetables have a wide variety of fungal genes causing quality problems.

Some of the microorganisms associated with plants known as Plant Growth-Promoting Rhizobacteria (PGPR) or Plant Growth-Promoting Fungi (PGPF) can stimulate the plant growth (Zehnder et al., 2001). Most of the studies were focused on the interactions between PGPF, PGPR and phytopathogens, while molecular mechanisms of resistance of the plants are not well known. The beneficial effects of some fungi of the rhizosphere in regard to the stimulation of the plant growth and biological control have been reported by many authors (Waqas et al., 2012; Tsavkelova et al., 2006). Colonization of fungi with PGPF may also result in systemic resistance in the distant parts of the plant. PGPF also produce several

other substances to promote growth, including indole-acetic acid (IAA), gibberellic acid GA3 (GA3), trans-zeatin (TZ) and indol-3-butyric acid (IBA) (Perrig et al., 2007; Naz et al., 2009; Nath et al., 2015). Soil fertility is mainly important for the microbiological production of plant growth regulators. Regulatory metabolites as IAA and GA3 can be produced, as secondary metabolites, during the cultivation process (Tsavkelova et al., 2006; Perrig et al., 2007; Nath et al., 2015; Bilkay et al., 2010). GA and IAA have been synthesized from *Aspergillus* and *Rhizopus* strains (Waqas et al., 2012; Bilkay et al., 2010; Ahmad et al., 2008). Fungi from *Rhizopus* sp. are important producers of different enzymes, organic acid and metabolites suitable for esterification of fats and oils in food and pharmaceutical industry (Yordanova et al., 2014).

Rhizopus arrhizus is a producer of organic acids (L-lactic acid, fumaric acid) and various lytic enzymes (amylases, pectinases, cellulases, lipases, proteases and phytases). At present, many investigations have been focused on the ability of the *Rh. arrhizus* to produce metabolites with regulatory activity on the plant growth (Tsavkelova et al., 2006; Perrig et al., 2007; Nath et al., 2015; Bilkay et al., 2010; Yordanova et al., 2014; Huang et al., 2003; Chatterjee et al., 2008; Sumantha et al., 2006; Suhair Ahmed Abdelwahab, 2015; Peeran et al., 2018).

The aim of this study was to estimate the capability of *Rh. arrhizus* KB-2 strain to produce metabolites with plant growth regulatory activity.

Materials and Methods

Fungal culture and batch fermentation conditions

In this study, the strain *Rh. arrhizus* KB-2 was obtained from the microbial collection of Department of Biotechnology, Faculty of Biology, Sofia University "St. Kliment Ohridski" and was investigated for biosynthesis of different enzymes and secondary metabolites, which have effect on the plant growth. Potato dextrose agar (Fluka) was used for cultivation and submerge nutrient medium for biosynthesis of enzymes with hydrolase activities, as previously described by Li et al. (2006) and modify by Yordanova et al. (2014). The modified media was consisted of carbon source glucose (Sigma–Aldrich) and soybean flour. The samples were prepared in 500 cm^3 Erlenmeyer flasks with 100 cm^3 modification nutrient media and agitated on rotary shaker at 150 rpm at 30°C, 250 rpm. Samples from the 264 h of the batch fermentation process were collected and used for further analysis.

Extraction and amplification of Fungal genomic DNA

The mycelium of *Rh. arrhizus,* previously treated with liquid nitrogen and a HiPurA™ Fungal DNA Purification Kit (Himedia, India) were used for DNA extraction. The amplification of fungal ITS region was done using ITS1/ITS4 primers in Macrogen Ltd, South Korea (Dolatabadi et al., 2014). The obtained sequences were compared by BLAST algorithm and phylogenetic tree was done by MEGA 6.0 software.

API ZYM assay

API ZYM test (Biomerieux, France) was used for detection of the 19 enzyme activity of the cell culture and cell-free culture supernatant of *Rh. arrhizus* KB-2 obtained by batch cultivation, according to the manufacturer's instructions. The results of the API-ZYM system are color reactions of the presence of the enzymes.

Lytic enzyme assay

The α-amylase activity of free cell supernatant of *Rh. arrhizus* KB-2 was determined by the SKB method (Sandstedt, 1939). One amylase unit (SKB unit) was defined as the amount of enzyme that will dextrinize 1g of soluble starch for 1h at 30°C.

The endoxylanase activity of free cell supernatant of *Rh. arrhizus* KB-2 was determined by a colorimetric assay, based on the Somogyi-Nelson method by measured the hydrolysis of birchwood xylan (Sigma) (Nelson, 1944; Somogyi, 1952). One unit of endoxylanase activity was defined as the amount of enzyme required to liberate 1 μmol of reducing sugars (xylose) per minute at 40°C per milliliter.

Endo-1,4-β-glucanase activity was detected on sodium carboxy-methyl cellulose (Na-CMC) as substrate according to Wood and Bhat (1978). One unit of cellulose activity was defined as the amount of enzyme that released 1 mmol glucose per minute at 50°C, pH 4,8.

α-galactosidase activity was assayed according to the method of Dey & Pridham (1972). One unit of enzyme activity was defined as the amount of enzyme, which produced 1 μmol of paranitrophenol for minute under assay conditions.

Lipase activity was estimated with olive oil emulsion using the Fungi Lipase-International F.I.P. Standard (Enzyme Technical Association, 2002). One unit of enzyme activity (FIP Unit) is defined as that quantity of lipase that liberates the equivalent of 1μmole of fatty acid from olive oil per minute at 35°C and pH 7.0.

Seed germination assay

To study the effects of regulatory metabolites, different dilutions (10; 100; 500 and 1000) of cell free supernatant were used for germination of *Lactuca sativa* seeds. For seed germination, 25 seeds were transferred to petri dish with 5 ml of different dilutions, containing moist filter paper. Seeds

were incubated for 48 h at 28°C in dark. As a control was used petri dish with water containing moist filter paper (Turhan et al., 2011). Different samples at the 120 h, 168 h, 216 h and 264 h of batch cultivation of cell free culture suspensions and their different dilutions were investigated. The length of the seed germination was given as mm and was compared to the control probe. All experimental results were statistical analyzed. The experiments were performed in triplicate.

Analysis on regulatory metabolites of cell free culture supernatant

Cell free supernatant at 264 h of batch fermentation was investigated for regulatory metabolites. The crude metabolites of *Rh. arrhizus* were analyzed through HPLC system with XTerra® C18 column (Waters Co,USA) and IonMax® electrospray ionization module (ThetmoScientific Co, USA). The obtained results were comparable with the standard chemical components in HPLC/MS data library. Data analysis and mass spectrometric evaluation were done using XCalibur® software and kindly provided by Medical University, Sofia, Bulgaria.

Results

Molecular phylogenetic identification of Rhizopus arrhizus

A molecular phylogenetic identification of *Rh. arrhizus* was done for the first time in the present study. The PCR product of ITS1 and ITS4 regions were obtained by Macrogen Ltd, South Korea. The sequences of the standard nucleotide were BLAST analysis and compared with NCBI database for construction of the phylogenetic tree (Figure 1). Our strain *Rh. arrhizus* KB-2 was a member of the genus *Rhizopus* and identified as *Rh. arrhizus.* It had 99% DNA sequences similarity with the strains from species *Rh. oryzae* and similar to other *Rh. delemar* strains.

Enzymes production in culture supernatant by Rhizopus arrhizus KB-2

The strain *Rh. arrhizus* KB-2 was investigated for the exstracellulare and intracellulare enzymes production in cell culture and cell free culture supernatant by API ZYM test after batch cultivation (Figure 2). Strong expressive activity of alkaline phosphatase, leucine amylamidase, and acid phosphatase was detected in the cell culture. Less activity were demonstrated α-chymotrypsin, naphthol-AS-BI- phosphohydrolase, β-glucosidase, valine amylamidase, esterase (C-4), esterase lipase (C-8), lipase (C-14), cysteine arylamidase, α-galactosidase. In comparison, in the cell free supernatant were detected low activity of extracellular enzymes only for esterase (C-4), esterase lipase(C-8), lipase (C-14) and β-glucosidase.

The hydrolytic enzyme production was determined quantitatively into batch fermentation up to 313 hour at modified cultural media containing. The production of α-amylase was 0.52 [U/cm^3] at the 216 h, endoxylanase activity was 2.26 [U/cm^3] at the 168 h, endocellulase activity was 1.52 [U/cm^3] at the 120 h and lipase activity was 11.21 FIP/cm^3 to 120 h (Figure 3).

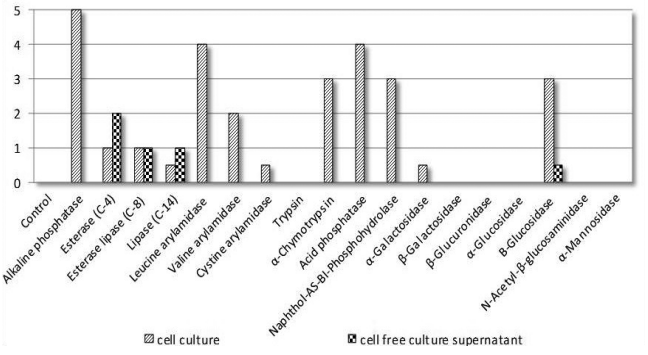

Fig. 2. Semi-quantitative analysis of enzyme activities of *Rh. arrhizus* KB-2 in the cell culture and cell free culture supernatant

Figure 1

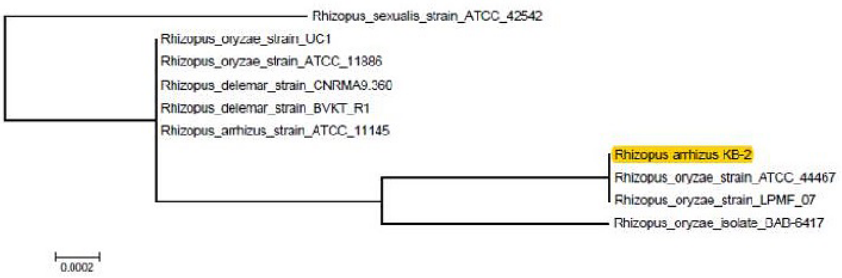

Fig. 1. Phylogenetic tree of *Rh. arrhizus* KB-2

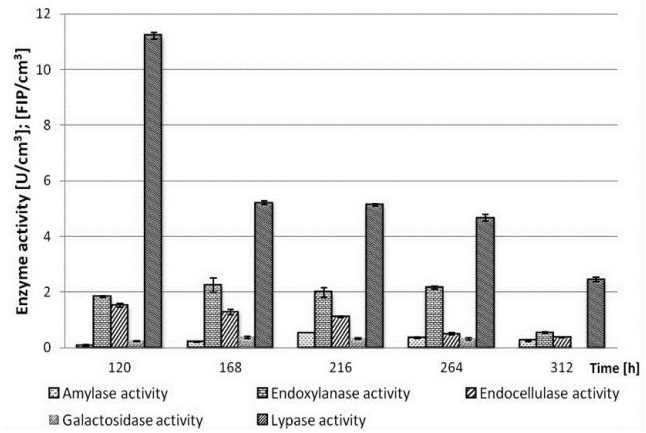

Fig. 3. Hydrolytic enzyme production of the *Rh. arrhizus* KB-2 in submerge cultivation with carbon source glucose and soybean flour

Effect of regulatory metabolites on the germination of Lactuca sativa seeds

The biological effects of the cell free supernatants from batch cultivation of the *Rh. arrhizus* KB-2 were observed on the germination of *Lactuca sativa* seeds. Four probes with different dilutions (10, 100, 500 and 1000) were used (Figure 4).

The results demonstrated that the strain produced various metabolites with different concentrations in different fermentation media and time and the cell free supernatant from 216 h and 264 h increased 32% of the root length of *Lactuca sativa.* The effect of biosynthesis secondary metabolites was observed early in the fermentation process and at 10x dilution the seed germination was inhibited.

Produce phytohormones and regulators metabolites by Rhizopus arrhizus KB-2

The complex of regulatory metabolites and similar substances were determined in cell free supernatant at 216 h of cultivation process (Table 1). The values for the GA3 were 27.88 μg/cm³, IAA – 30.30 μg/cm³ and TZ – 49.04 μg/cm³ and probably they have effects on the plant growth.

Discussion

Identification of Rhizopus arrhizus strain

Rh. arrhizus KB-2 strain had been used from the microbial collection of the Department of Biotechnology, Faculty of Biology, Sofia University "St. Kliment Ohridski" (Bulgaria) since 1989. In our laboratory the strain was determined as *Rh. arrhizus* KB-2 strain and has been used for production of lipase enzyme. Primary this strain was morphologically and physiologically studied as lipase producer (Yordanova

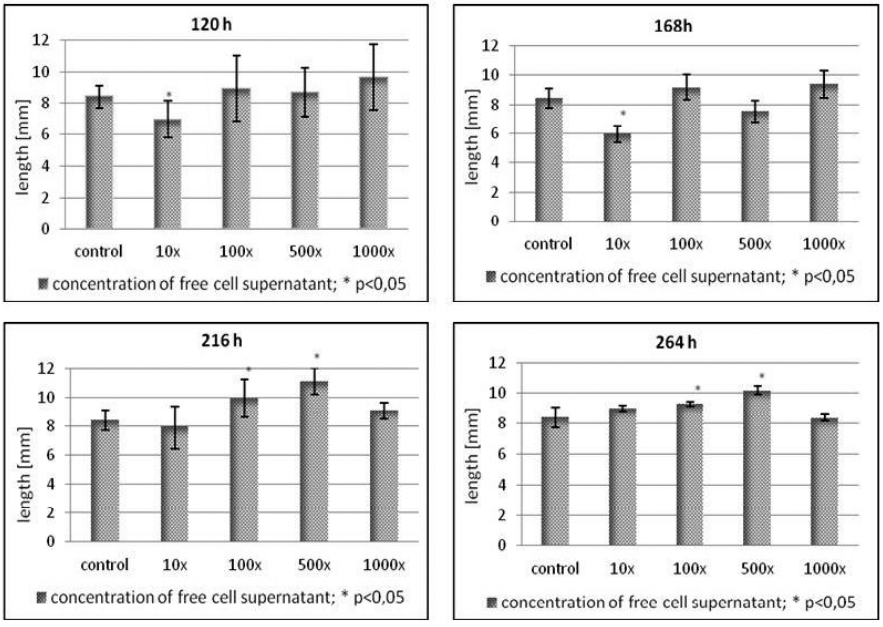

Fig. 4. Biological effects of different dilutions on cell free supernatants of *Rh. arrhizus* KB-2 on the seed germination

Table 1. Regulatory metabolites in cell free supernatant of *Rh. arrhizus* KB-2

Compounds	*Rhizopus arrhizus* KB-2
	μg/cm^3
GIBBERELLIC ACID GA3	27.88
INDOLE-3-ACETIC ACID	30.30
PACLOBUTRAZOL	0.00031
UNICONAZOLE	0.00014
INDOLE-3-BUTYRIC ACID	0.00033
FLURPRIMIDOL	n.d
GIBBERELLIN A4	0.00083
N(2-CHLORO-4-PYRYDYL)-N-PHENYLUREA	0.00055
GIBBERELLIN A7	n.d
TRANS-ZEATIN	49.04
(±)-CIS,TRANS-ABSCISIC ACID	0.0182
2,4-DICHLOROPHENOXYACETIC ACID	n.d
ANCYMIDOL	0.00013
THIDIAZURON	0.00353

n.d. – no detected

et al., 2014). Our strain *Rh. arrhizus* KB-2 had 99% DNA sequences similarity with the strains from species *Rh. oryzae* and similar to other *Rh. delemar* strains. These results were observed by other authors, who considered that *Rh. arrhizus* and *Rh. oryzae* were the synonym and have the same morphological and cultivation characteristics (Dolatabadi et al., 2014; Zhang et al., 2007). According to these results, identity of the strain KB-2 was conformed to the species *Rhizopus arrhizus*.

Enzymes production of Rhizopus arrhizus KB-2

The strain *Rh. arrhizus* KB-2 was investigated for production of hydrolytic enzymes. The cell culture and cell free culture supernatant were analyzed and was determined that the cell free supernatant was consisted only esterase, lipase and glucosidase enzymes. The absence of proteinase and peptidase activities was due to penetration of the cell wall of the mycelium of Rh. arrhizus KB-2. In cell cultural supernatant was established different hydrolytic enzymes which have probably effected on plant. The similar results have been observed by other groups, which were investigated fungal biomass of genus *Rhizopus* and show the different enzyme activities (Suhair, 2015; Guimarães et al., 2006; Kilcawley et al., 2002; Varzakas, 1998).

Rh. arrhizus KB-2 was produced higher lipase activity due to the submerge cultivation in the complex media for biosynthesis of this enzyme. The absence of the substrates inductors in the cultivation media and higher pH during the process probably are the reasons for the low amylase, endoxylanase and endocellulase activities. Many authors has been shown analogues results and found that the lipase activity was increased gradually and reached the highest activity after 168 h of cultivation of *Rhizopus oryzae* strain. Other hydrolytic enzymes as endoxylanase, endocellulase and amylase were produced in very low levels (Sumantha et al., 2006; Suhair, 2015; Adejuwon et al., 2015; Wang et al., 2013). Therefore, the *Rh. arrhizus* KB-2 is considered to be a potential of the lipase production.

Effect of regulatory metabolites on Lactuca sativa seeds and production of phytohormones

The results demonstrated that the strain produced various metabolites with different concentrations in different fermentation media and time. The production of IAA and GA3 were biosynthesized near the end of the growth phase or during the stationary phase. Therefore, it was expected that the production times of these plant regulators were long (Bilkay et al., 2010). Nath (2015) reported that some species of *Rhizopus*, *Aspergillus and Penecillium* have IAA and GA3 activities at different time of cultivation and were used as plant growth promoting activities *in vitro*. The influence of GA3 and Ca ions was regarded as defense mechanisms of plant to salinization (Hasan, 2002).

The ability of fungi to produce phytohormones and regulators metabolites was also frequently used in the crop production (Tsavkelova et al., 2006). It is known that the genus *Rhizopus* produce secondary metabolites as IAA, GA, trans-zeatin and etc. Phytohormones of the group of auxins (IAA), stimulate seed germination and increase the rate of xylem and root formation, whereas the gibberellins (GA and TZ) were affected the division and elongation of the cells of the plant (Nath et al., 2015; Bilkay et al., 2010). The presence of the regulatory metabolites in the cell free supernatant of *Rh. arrhizus KB-2* were demonstrated that the effect on the seed germination. Hassan (2002), reported similar results and found that the production of GA3 was in concentration 10 mg/50 ml in filtrate from *Rh. stolonifer.* IAA production of the fungi of genus *Aspergillus* and *Penicillium* revealed that their activity increased gradually after day 6 up to 33.07 μg/cm^3 and GA3 from 6.98 μg/cm^3 up to 12.46 μg/cm^3. The same data for the biosynthesis of the regulatory metabolites of *Penicillium, Aspergillus* and *Rhizopus* were reported by other studies (Waqas et al., 2012; Tsavkelova et al., 2006; Barroso et al., 1986).

Conclusion

In present study we investigated the production of plant growth regulatory metabolites of *Rhizopus arrhizus* KB-2. It was found that the cell supernatant of studied strain produces various esterase, lipase, galactosidase, peptidase and pectinase activities while the cell free supernatant was characterized only by extracellular enzymes as esterase and β-glucosidase activities. In submerge fermentation process we determined that in the cell free supernatant has high lipase activity due to different cultivation media for biosynthesis. A molecular phylogenetic identification of *Rh. arrhizus* was done for the first time in the present study. The identity of our strain *Rhizopus arrhizus* KB-2 was conformed to the species *Rhizopus arrhizus*. The strain produced various metabolites with different concentrations in different fermentation media and time and the cell free supernatant from 216 h and 264 h increased 32% of the root length of *Lactuca sativa*. In cell free supernatant of *Rh. arrhizus* KB-2 was determined the presence of the phytohormones (auxins and gibberellins), which influenced the seed germination and root formation.

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References

Adejuwon, A. O., Ajayi, A. A. & Ladokun, O. A. (2015). Alpha-Amylase from *Rhizopus arrhizus* Fisher. *Journal of Plant Sciences, 10(3):* 99.

Agrios, G. N. (2004). Losses caused by plant diseases. Plant pathology. Elsevier, Academic Press, Oxford, UK, 29-45.

Ahmad, F., Ahmad, I. & Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research, 163 (2*): 173-181.

Barroso, J., Neves, H. C. & Pais, M. S. (1986). Production of indole-3-ethanol and indole-3-acetic acid by the mycorrhizal fungus of *Ophrys lutea* (*Orchid aceae*). *New Phytologist, 103 (4):* 745-749.

Bilkay, I. S., Karakoç, Ş. & Aksöz, N. (2010). Indole-3-acetic acid and gibberellic acid production in *Aspergillus niger*. *Turkish Journal of Biology, 34(3):* 313-318.

Chatterjee, S., Chatterjee, S., Chatterjee, B. P. & Guha, A. K. (2008). Enhancement of growth and chitosan production by *Rhizopus oryzae* in whey medium by plant growth hormones. *International Journal of Biological Macromolecules, 42 (2):* 120-126.

Dey, P. M. & Pridham, J. B. (1972). Biochemistry of α-Galactosidases. *Advances in Enzymology and Related Areas of Molecular Biology*, 36: 91-130.

Dolatabadi, S., de Hoog, G. S., Meis, J. F. and Walther, G. (2014). Species boundaries and nomenclature of *Rhizopus arrhizus* (syn. *R. oryzae*). *Mycoses*, *57*: 108–127.

Enzyme Technical Association (2002). GRAS notification – exemption claim for lipase enzyme preparation produced by *Aspergllus niger* that is the subject of a GRAS affirmation petition, GRASP 3G00162002.

Guimarães, L. H. S., Peixoto-Nogueira, S. C., Michelin, M., Rizzatti, A. C. S., Sandrim, V. C., Zanoelo, F. F. & Polizeli, M. D. L. (2006). Screening of filamentous fungi for production of enzymes of biotechnological interest. *Brazilian Journal of Microbiology, 37 (4),* 474-480.

Hasan, H. A. H. (2002). Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction. *Rostlinna Vyroba, 48 (3),* 101-106.

Huang, L. P., Jin, B., Lant, P. & Zhou, J. (2003). Biotechnological production of lactic acid integrated with potato wastewater treatment by *Rhizopus arrhizus*. *Journal of Chemical Technology and Biotechnology, 78 (8),* 899-906.

Kilcawley, K. N., Wilkinson, M. G. & Fox, P. F. (2002). Determination of key enzyme activities in commercial peptidase and lipase preparations from microbial or animal sources. *Enzyme and Microbial technology, 31(3),* 310-320.

Li, L., Du, W., Liu, D., Wang, L. & Li, Z. (2006). Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium. *Journal of Molecular Catalysis B: Enzymatic, 43 (1-4),* 58-62.

Nath, R., Sharma, G. D. & Barooah, M. (2015). Plant growth promoting endophytic fungi isolated from tea (*Camellia sinensis*) shrubs of Assam, India. *Appl. Ecol. Environ. Res., 13,* 877-891.

Naz, I., Bano, A. & Ul-Hassan, T. (2009). Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. *African Journal of Biotechnology, 8(21),* 5762-5766.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem., 153 (2),* 375-380.

Peeran, M. F., Prasad, L. and Kamil, D. (2018). Characterization of secondary metabolites from *Rhizopus oryzae* and its effect on plant pathogens. *Int. J. Curr. Microbiol. App. Sci., 7(3),* 705-710.

Perrig, D., Boiero, M. L., Masciarelli, O. A., Penna, C., Ruiz, O. A., Cassán, F. D. & Luna, M. V. (2007). Plant-growth-promoting compounds produced by two agronomical important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied Microbiology and Biotechnology, 75(5),* 1143-1150.

Sandstedt, R. M. (1939). Starch in relation to some baking properties of flour. *Cereal Chem., 16,* 780-792.

Somogyi, M. (1952). Notes on sugar determination. *J. Biol. Chem., 195,* 19-23.

Suhair Ahmed Abdelwahab (2015). Production of glucoamylase enzyme by a *Rhizopus oryzae* strain. *Asian Journal of Biotechnology, 7 (1),* 32-38

Sumantha, A., Deepa, P., Sandhya, C., Szakacs, G., Soccol, C. R. & Pandey, A. (2006). Rice bran as a substrate for proteolytic

enzyme production. *Brazilian Archives of Biology and Technology, 49(5),* 843-851.

Tsavkelova, E. A., Klimova, S. Y., Cherdyntseva, T. A. & Netrusov, A. I. (2006). Microbial producers of plant growth stimulators and their practical use: a review. *Applied Biochemistry and Microbiology, 42(2),* 117-126.

Turhan, A., Kuşçu, H. & Şeniz, V. (2011). Effects of different salt concentrations (NaCl) on germination of some spinach cultivars. *Journal of Agricultural Faculty of Uludag University, 25(1),* 65-77.

Varzakas, T. (1998). *Rhizopus oligosporus* mycelial penetration and enzyme diffusion in soya bean temple. *Process Biochemistry, 33(7),* 741-747.

Wang, J. R., Li, Y. Y., Xu, S. D., Li, P., Liu, J. S. & Liu, D. N. (2013). High-level expression of pro-form lipase from *Rhizopus oryzae* in *Pichia pastoris* and its purification and characterization. *International Journal of Molecular Sciences, 15(1),* 203-217.

Waqas, M., Khan, A. L., Kamran, M., Hamayun, M., Kang, S. M., Kim, Y. H. & Lee, I. J. (2012). Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules, 17 (9),* 10754-10773.

Wood, T. M. & Bhat, K. M. (1988). Methods for measuring cellulase activities. In: *Methods in enzymology*, *160,* 87-112.

Yordanova, M., Ilieva, S., Goranov, B., Evstatieva, Y., Milanova, K., Bozhanchev, K. & Nikolova, D. (2014). Optimization of nutrient medium composition by mathematical modeling for production of *Rhizopus arrhizus* KB-2 lipase. *Bulgarian Journal of Agricultural Science, 20 (1),* 87-92.

Zehnder, G. W., Murphy, J. F., Sikora, E. J. & Kloepper, J. W. (2001). Application of rhizobacteria for induced resistance. *European Journal of Plant Pathology, 107 (1),* 39-50.

Zhang, Z. Y., Jin, B. & Kelly, J. M. (2007). Production of lactic acid and byproducts from waste potato starch by *Rhizopus arrhizus*: role of nitrogen sources. *World Journal of Microbiology and Biotechnology, 23 (2),* 229-236.

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