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β-AMYLASE PRODUCTION BY A NOVEL STRAIN *Paenibacillus chitinolyticus* CKS1 USING COMMERCIAL AND WASTE SUBSTRATES

PROIZVODNJA β-AMILAZA POMOĆU NOVOG BAKTERIJSKOG SOJA Paenibacillus chitinolyticus CKS1 NA KOMERCIJALNIM I OTPADNIM SUPSTRATIMA

Neda RADOVANOVIĆ[*], Slađana DAVIDOVIĆ[*], Miona MILJKOVIĆ[*], Marija PAVLOVIĆ[*], Aneta BUNTIĆ[*], Vesna LAZIĆ[**], Katarina MIHAJLOVSKI[*]
**University of Belgrade, Faculty of Technology and Metallurgy, Department for Biochemical Engineering and Biotechnology, Karnegijeva 4, Belgrade, Serbia*
*** University of Belgrade, Vinča Institute of Nuclear Science,Mike Petrovića Alasa 12-14, Serbia*
e-mail: kmihajlovski@tmf.bg.ac.rs

ABSTRACT

Amylases are industrially important enzymes which could convert starch to glucose, maltose and oligosaccharides. A bacterial strain designated as Paenibacillus chitinolyticus CKS1which was isolated from the soil of the coniferous forest, produced β-amylases using different commercial and waste substrates. Maximum β-amylases activity of 0.820 U/mL was obtained using a sugar alcohol-isomaltidex (0.5% w/v), as a substrate for microorganism growth and enzyme production. After 48 h of fermentation in a medium that contained starch (0.5%, w/v) and 0.05% v/v of ethanol, CKS1 produced β-amylase with the activity of 0.518 U/mL. The latest trends in enzyme production include utilisation of various waste products, mainly of agroindustrial origin, as a substrate for microorganisms growth. The strain CKS1 was also able to grow and produce β-amylases by using plant waste material. The plant waste substrate (PWS) contained plant biomass that is left after the ethanol extraction of various medicinal herbs (marigold and chamomile flowers, artichoke leaf, lemon balm leaf, nettle leaf, thyme leaf, yarrow shoot, yellow gentian shoot, primrose shoot, valerian shoot and chestnut and hawthorn seeds).This mixture of dried plant biomass is dissposed as such as a waste. In a medium with 0.1% (w/v) of PWS, CKS1 produced β-amylases with a maximum activity of 0.569 U/mL. The results show the potential of utilising waste plant biomass, left after ethanol extraction of medicinal herbs, in production of amylases. The application of microorganisms in β-amylase production using waste substrate is economically and environmentally accepted.

Key words:*Paenibacillus chitinolyticus CKS1, fermentation, β-amylase production, sugar alcohol, plant waste substrate (PWS)*

REZIME

Amilaze predstavljaju grupu industrijski veoma važnih enzima koji hidrolizuju skrob do glukoze, maltoze i različitih oligosaharida. Bakterijski izolat Paenibacillus chitinolyticus CKS1, izolovan iz zemljišta četinarske šume, pokazao je sposobnost proizvodnje β-amilaza tokom svog rasta na različitim komercijalnim ali i na otpadnim supstratima. Maksimalna aktivnosti β-amilaza koja je iznosila 0,820 U/mL postignuta je korišćenjem izomaltideksa, šećernog alkohola, u koncentraciji od 0,5% (w/v), kao supstrata za rast mikroorganizma i proizvodnju enzima. U podlozi sa skrobom (0,5% w/v) i sa dodatkom 0,05% (v/v) etanola, nakon 48h fermentacije, maksimum aktivnosti β-amilaza iznosila je 0,518 U/mL. Najnoviji trendovi u proizvodnji enzima odnose se na korišćenje različitih otpadnih sirovina agroindustrijskog porekla kao supstrata za rast mikroorganizma. Soj CKS1 je pokazao mogućnost korišćenja otpadnog biljnog materijala kao supstrata za rast i proizvodnju enzima amilaza. Otpadni biljni supstrat (PWS), činila je biljna masa zaostala nakon etanolne ekstrakcije različitog lekovitog bilja (cveta nevena i kamilice, lista matičnjaka, artičoke, koprive, timijana, nadzemnog dela hajdučke trave, lincure, jagorčevine i valerijane i semena divljeg kestena i gloga). Ova biljna biomasa, iz koje su ekstrahovane bioaktivne materije, meša se i odlaže kao takva u vidu otpada. Nakon rasta u podlozi sa 1,0 % (w/v) PWS, soj CKS1 proizveo je β-amilaze sa aktivnošću od 0,569 U/mL.

Ovi reziltati ukazuju na mogućnost iskorišćenja otpadne biljne biomase, zaostale nakon alkoholne ekstrakcije lekovitog bilja, u procesima ekonomičnije proizvodnje amilaza. Korišćenje otpadne sirovine u mikrobnim procesima proizvodnje enzima je i ekološki mnogo prihvatljivije usled uticaja na smanjene skladištenja otpada a samim tim i sveukupnog zagađenje životne sredine.

Ključne reči: *Paenibacillus chitinolyticus CKS1, fermentacija, aktivnost β-amilaza, šećerni alkohol, otpadni billjni supstrat(PWS)*

INTRODUCTION

Starch, after cellulose, is one of the most available carbohydrate on earth *(Van der Maarel et al., 2000);* it represents a major storage product of many crops like potato, corn, rice, wheat, barley etc.*(Agrawal et al. 2005)* and certainly is the most abundant raw material with a low cost *(Šuput et al., 2014).* Amylases are enzymes that hydrolyze starch molecules to glucose, maltose, oligosaccharides and dextrins *(Asgher et al., 2007).*The main components of starch are amylose, a linear molecule composed of glucose units linked by α-1,4 bonds, and amylopectin which is a highly branched molecule composed of glucose units linked by α-1,4 and α-1,6 bonds *(Šuput et al., 2013;Šuput et al., 2015).*

β-amylases belong to a group of exoamylases which act on the external glucose residues of the starch molecule and produce only maltose *(Pandey et al., 2000;Gupta et al., 2003).* Amylases have many industrial applications including food industry (bread and baking industry), textile and paper industry *(Pandey et al., 2000).* However, β-amylases are mostly used in food industry for the production of maltose syrups *(Gaouar et al., 1998;Teotia et al., 2001).*

Various microorganisms are able to produce amylases and among them the representatives of the genus *Bacillus* are the

most studied *(Ashraf et al., 2003;Gangadharan et al., 2008;Rajagopalan and Krishnan 2008). Paenibacillus* is a new genus of endospore-forming bacteria emerged from the genus *Bacillus (De Vos et al., 2009).* Recently, our previous invesigation showed that *Paenibacillus chitinolyticus* CKS1 produce amylases *(Mihajlovski et al., 2015b;Mihajlovski et al., 2016)* although it has been described in literature as a non-amylolitic species *(De Vos et al., 2009).*Various comercial substrates are utilized for amylases production, however nowadays, preference is given to waste substrates due to their abundancy and low price. Also, recent trends in enzyme production include valorization of different agricultural wastes.The use of waste biomass, as a renewable sources, for processes transformation and for obtaining biological compounds is highly recommended *(Modelska et al., 2015)*. In our previous work *(Mihajlovski et al., 2015a)*, plant waste biomass was used as a substrate for cellulases production. In this study we showed, for the first time, that plant biomass remaining after ethanol extraction of various medicinal herbs could be used as a substrate for amylase production. In addition we showed that commercial substrates based on alcohol sugars as well as waste plant biomass are suitable inducers for β-amylases activity.Therefore, the aim of this study was to investigate influence of different substrates, including commercial and waste, on β-amylase production by *Paenibacillus chitinolyticus* CKS1.

MATHERIALS AND METHODS

Microorganism and inoculum preparation

P. chitinolyticus CKS1 was isolated from the soil of a coniferous forest. In our previous study the strain CKS1 was characterized and identified by using standard morpho-physiological and molecular methods. Its squence is deposited to GenBank database under the accesion number: KP715850 *(Mihajlovski et al., 2015a)*. The inoculum was prepared by transfering one colony from International Streptomyces Project 1 (ISP1) agar plate medium (containing 3 g/L yeast extract; 5 g/L casein hydrolysate; 12 g/L agar agar) to 300 mL Erlenmeyer flask with 30 mL of ISP1 broth (same composition as ISP1 agar without agar) and the incubation was carried out at 30 °C for 24 hours in a rotary shaker at 150 rpm. This inoculum was used for the fermentation process.

Plant waste material for β-amylase production

Plant waste substrate (PWS), used in this study, presents dried plant biomass left after ethanol extraction of various medicinal herbs: *Calendulae flos, Chamomillae flos, Cynarae folium, Melissae folium, Urticae folium, Thymi folium, Millefolii herba, Hippocastani semen, Gentianae radix, Primulae radix, Valerianae radix and Crataegi summitates.* This mixture of dried plant biomass is an aliquot of the total of 150 kg of plant material with the following composition: 8.3 % ash, pH of 6.72, 0.27% total P, 1.07% total K, 1.47% total N, 45.84% total C, C/N ratio of 31.19% and particle size < 200 μm. This plant material is generated annually in the Institute of Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia and is disposed as waste.

Enzyme test for β-amylase

The activity of the β-amylase was measured by modified Bernfeld method *(Bernfeld 1955)*.

The reaction mixture consisted of 0.500 mL of 1% (w/v) soluble starch solution made in 0.016M sodium acetate buffer (pH 4.8) and 0.500 mL enzyme solution (crude bacterial supernatant). The mixture was incubated at 50 °C for 15min, and then the reaction was stopped by adding 1mL of 3,5 dinitrosalicylic acid (DNS). The reaction mixture was boiled for 5 min in a water bath. After cooling at room temperature, 5 mL of distilled water was added to each tube and absorbance of the solution was measured at 540 nm on a UV-VIS spectrophotometer (Ultrospec 3300 *pro*Amersham Bioscience). One unit of the enzyme was defined as the amount of enzyme producing reducing sugars corresponding to 1 μmol of maltose from the soluble starch per minute under the assay conditions.

β-amylase production

β-amylases were produced by growing *P. chitinolyiticus* CKS1 on media containing commercial substrates and PWS. In a medium containing ISP1 broth, different carbon sources including strarch, isomaltidex, xylitol and tapioca dextrin, were added in a concentration of 0.2% (w/v). After sterilization at 121°C for 20 min, the fresh medium with the coresponding carbon source, was inoculated with 24h old bacterial culture (inoculum size 10%, v/v) and placed at 30 °C in a rotary shaker (150 rpm) for 48h. After the incubation period, the culture medium was centrifuged at 6000×g for 15 min and the cell-free supernatant was analysed for β-amylase activity.

Therafter, comercial substrate-isomaltidex, that provided the highest β-amylase production by CKS1 was added to the ISP1 broth in different concentrations (0.05, 0.5 and 1.0%, w/v) and the activity of the β-amylase was evaluated as previously described (Bernfeld 1955).

In order to evaluate if the similar amounts of β-amylase can be obtained by replacing sugar alcohol with a cheaper substrate, different concentration of starch (0.05, 0.5 and 1.0%, w/v) were added to the ISP1 broth and β-amylase production by CKS1 was examined. Thereafter, the ISP1 broth containing the concentration of starch that resulted in the highest β-amylase activity was amended with ethanol. Ethanol was filter sterilized through a 0.2 μm pore size filter and added to the sterile starch medium to a final concentrations of: 0.05, 0.5 and 1.0% (v/v). In order to evaluate if ethanol can induce the production of β-amylase,it was also added to the ISP1 broth containing 0.5% (v/v) of isomaltidex to a final concentration of (0.05, 0.5 and 1.0%, v/v).

For β-amylase production on the waste material, ISP1 medium was further enriched with PWS. An amount of 0.5g and 1.0g of PWS were added separately into 10 mL of previously prepared agar medium (0.5% w/v of agar-agar in distiled water, and sterilized in an autoclave at 121°C for 20 min). After sterilization 10 mL of this medium was poured into sterile Petri dishes (ϕ 4.5 cm). After solidification the agar medium with plant residues was added in 100 mL of previously sterilized ISP1 broth, making a final concentration of 0.5 and 1.0% (w/v) of PWS in the medium. This medium was inoculated with a 24 h old bacterial culture with inoculum size of 10 % (v/v). The incubation was carried out at 30 °C for 48h in a rotary shaker at 150 rpm. The cell-free supernatant was obtained as previously described and was further analysed for β-amylase activity.

Statistical analysis

All experiments were done in triplicates. All values are expressed as mean ± standard deviation. Mean values of single experiments were compared by the analysis of variance (one-way ANOVA) followed by Tukey test for mean differences testing. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The influence of different carbon sources on β-amylase production by *Paenibacillus chitinolyticus* CKS1 was tested in this study. The results showed that the strain CKS1 produced the

largest ammount of β-amylase (0.766 U/mL) using isomaltidex as a growth substrate. CKS1 also produced β-amylase using other tested substrates: xylitol (0.548 U/mL), tapioca dextrin (0.502 U/mL) and starch (0.331 U/mL), but with lower activities in comparison to isomaltidex (Figure 1).

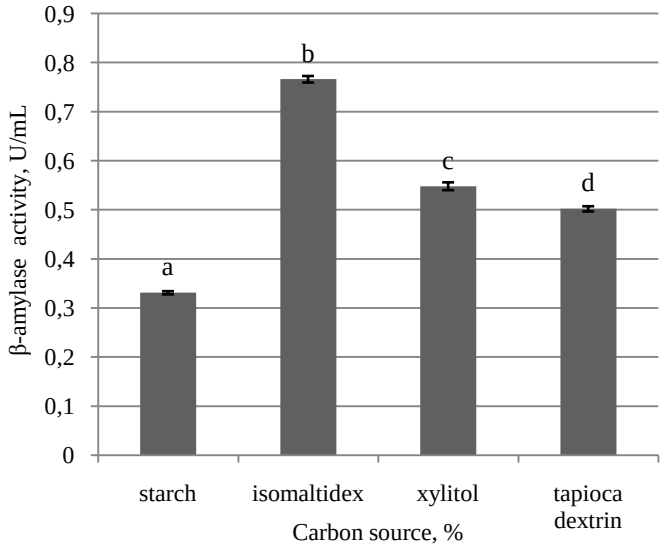

Fig.1. The influence of different carbon sources on β-amylase production by P. chitinolyticus CKS1. Values with different superscripts (a, b, c, d) within each column were significantly different (p < 0.05)

Previous studies have shown that the production of bacterial amylases is inducible and highly affected by the nature of the carbon source used as a substrate in the fermentations process *(Hensley et al., 1980;Olufunke and Azeez 2012)*. Isomaltidex or isomalt and xylitol are types of sugar alcohols. Literature data show that starch is predominantly used as an inducer for amylase production *(Ajadi and Fagade, 2003).* A combinations of starch and sugar alcohol, such as glycerol, is also reported to induce the production of amylase *(Cotârleţ 2013)*. Ohno and co-workers reported that the addition of glycerol, as a carbon source in the fermentation medium, induced amylase production in fungus *Fusidium* sp. BX -1 more successfully than starch *(Ohno et al., 1992)*. However, there is no literature data about amylase production using isomaltidex or xylitol, or any sugar alcohol as inducers of amylase production by *Paenibacillus* sp. Our results show that the addition of a carbohydrate in the fermentation medium, in the form of sugar alcohol, have stimulative effect on β-amylase production by *Paenibacillus chitinolyticus* CKS1(Fig.1). Since the highest β-amylase activity in this study was obtained using isomaltidex as a carbon source, different amount of this substrate was added into the ISP1 broth in order to evaluate the influence of its concentration on β-amylase production by CKS1. The concentration of isomaltidex that resulted in the highest β-amylase production was 0.5 % (w/v), providing a 0.820 U/mL of enzyme activity (Fig. 2). *P. chitinolyticus* CKS1 is the first reported *Paenibacillus* for which a sugar alcohol is shown to be better amylase inducer than starch. The decrease in amylase yield at higher substrate concentartion (1.0 % w/v) might be due to the change of conditions in the fermentation medium, production of inhibitory by-products and denaturation or decomposition of amylase, as similar has been reported for other bacterial strains *(Gangadharan et al., 2008)*. Although isomaltidex gave the hihghest β-amylase activities its utilization in the enzyme production processes is not cost effective since it is an expensive material. Nowadays, preference is given to low cost substrates or waste materials, due to their abundancy and low prices. In order to achieve more economically accepted process of production of amylase, starch representing the cheapest of the four tested substrates, amended with ethanol, was selected as a substrate for β-amylase production by CKS1. Firstly the concentration of starch in the fermentation medium which resulted in the highest β-amylase activity was evaluated and it was 0.5 % (w/v) providing 0.463 U/mL of enzyme activity (Fig.2) . Therafter, different concentrations of ethanol was added to evaluate whether the combination of ethanol and cheap substrate such as starch could be a suitable replacement for expensive isomaltidex for the production of β-amylase by CKS1. The results showed that the addition of 0.05 % (v/v) of ethanol in a medium with starch displayed a stimulative effect on β-amylase production by CKS1.β-amylase activity reached 0.518 U/mL, which is higher than 0.463 U/mL obtained when the bacterium was grown in a medium complemented only with starch. Further increase of the ethanol concentration led to a decrease in β-amylase activity (Fig. 3).

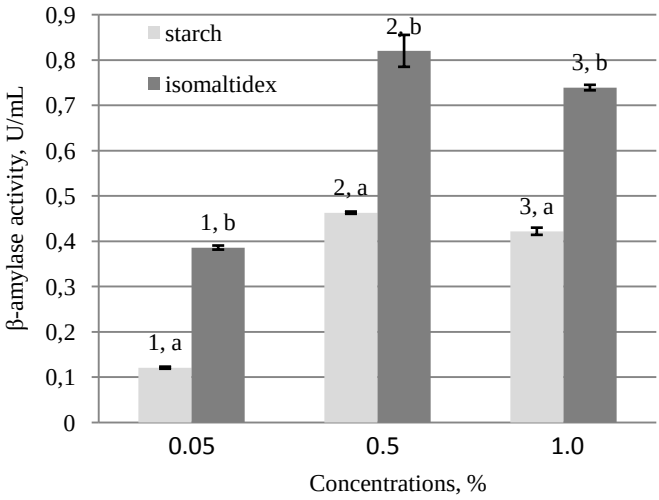

Fig. 2.The influence of different concentrations of starch and isomaltidex on β-amylase production by P. chitinolyticus CKS1.Values with different superscripts (a, b) within each concentration were significantly different (p<*0.05)*

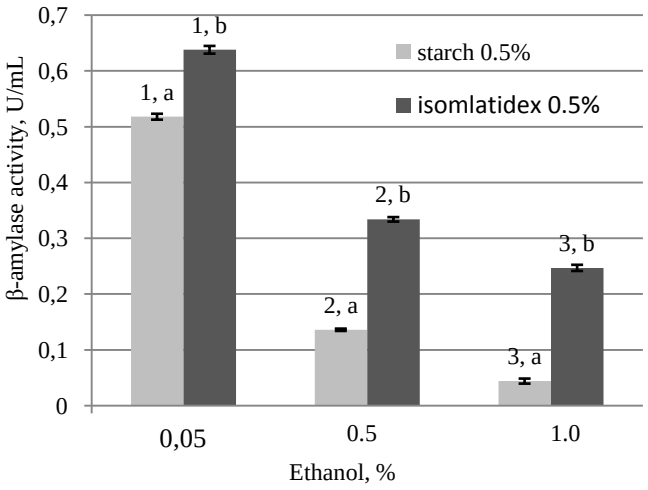

Fig.3. The influence of different ethanol concentrations on β-amylase production by P. chitinolyticus CKS1
. Values with different superscripts (a, b) within each concentration were significantly different (p < 0.05)

To test if the additional concentrations of ethanol have a positive influence on enzymatic activity, different ethanol concentrations were added into a medium with isomaltidex (0.5 %). In contrast to previously obtained results with starch, the addition of a small amount of ethanol 0.05 % (v/v) to a medium with isomaltidex showed an inhibitory effect on β-amylase production. β-amylase activity decreased to 0.638 U/mL compared to 0.820 U/mL when CKS1 was grown in a medium containing only the optimal concentration of isomaltidex (Figure 3).The possible explanation is that the presence of this sugar alcohol in a medium for amylase production, for the strain CKS1, is sufficient for the bacterial growth and enzyme production, thus any increase in alcohol concentration establishes unfavorable or toxic conditions which eventually led to a reduced growth and a desrease in amylase production.

P.chitinolyticus CKS1 could produce β-amylase in a medium with plant waste material as a susbtrate (PWS). PWS contains mixed plant biomass left after ethanol extraction of various medicinal herbs, and thus it is enriched with a certain amount of alcohol. As previously stated, alcohol enriched substrates could serve as inducers for amylase production (Ohno et al., 1992;Cotârleţ 2013). Therefore, PWS was chosen as a low cost substrate to evaluate its potential use as an inducer for amylase production by CKS1. After 48 h of incubation, maximum β-amylase activity of 0.569 U/mL was reached in a sample with 1.0% (w/v) of PWS (Fig.4.).

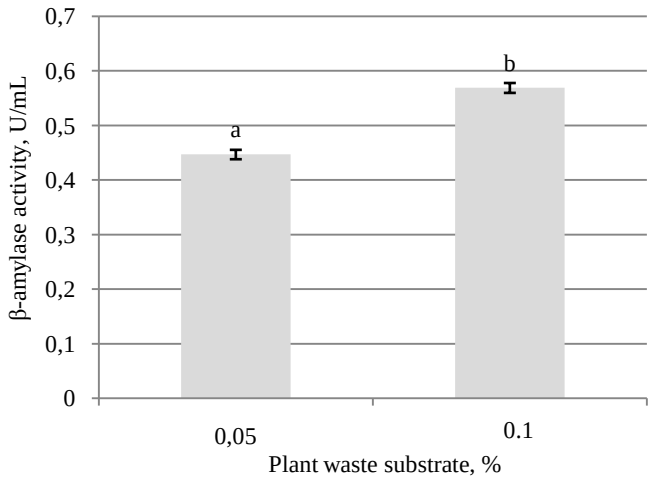

Fig.4. Effect of two concentrations of plant waste substrate on β-amylase production by P. chitinolyticus CKS1.*Values with different superscripts (a, b) within each concentration were significantly different (p< 0.05)*

Our previous investigations showed that strain CKS1 could use also PWS entraped in agar medium for cellulases production (Mihajlovski et al., 2015a). Addition of this plant material in its original form in the fermentation medium was not suitable for the growth of strain *Paenibacillus chitinolyticus* CKS1 or the production of the enzyme β-amylase (unpublished data). The used PWS has particle size that is smaller than 200 μm and the strain CKS1 could not use it as a substrate for growth and enzyme production. Entrapment of the plant material in the agar and its addition to the fermentation medium provided more suitable environment for the bacteria enabling the growth and the production of β-amylase by CKS1.When CKS1 was grown in a medium that contained only agar particles without plant material β-amylase activity was not recorded. In addition to this, plant waste material which did not undergo ethanol exstraction was not suitable for the production of β-amylases (unpublished data). According to this, the presence of ethanol in PWS could have an inducible effect on the production of β-amylases by the strain CKS1. Although the concentration of ethanol in the tested sample was not determined, it was considered to be very low due to its inducible effect on the growth of CKS1 strain and the production of enzyme amylase.

There is no little literature data about amylase production using waste generated after the ethanol extraction of medicinal herbs as substrate, especially by *Paenibacillus* sp. Many agricultural-industrial wastes (wheat bran, rice husk, rice bran, maize bran, etc.) were used as substrates for amylases induction in *Paenibacillus amylolyticus (Ikram-Ul-Haq et al., 2012)* and in *Bacillus* sp. *(Anto et al., 2006;Saxena and Singh 2011)*. The type of carbon source in a medium for microorganism fermentation is of great importance for amylases production. For example, maximum β-amylase activity obtained in this study (0.569 U/mL), for the strain CKS1, using 1.0% (w/v) PWS, is higher than 0.322 U/mL obtained using wastewater as carbon source in our previous study *(Mihajlovski et al., 2015b)* but is also lower than 2.187 U/mL obtained using molasses and sugar beet pulp (Mihajlovski et al., 2016).

CONCLUSION

Results obtained in this study indicate that *Paenibacillus chitinolyticus* CKS1 could use different commercial susbstrates in the form of sugar alcohols for β-amylase production.The strain CKS1 is the frist reported *Paenibacillus* species with the possibility of using isomaltidex as a substrate for growth and amylase production. The strain CKS1 is also able to produce β-amylases using starch with the addition of ethanol as a substrate. For the first time we showed that the strain CKS1 could use plant waste material containing biomass of medicinal herbs left after ethanol extraction, for β-amylases production. Maximum β-amylase activity is obtained using commercial substrate isomaltidex, folowed by plant waste substrate in a fermentation medium. Microbial processes of enzyme production using low cost and waste materials are preferable from both economical and environmental aspects.

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REFERENCES

Ajayi, A.O., Fagade, O.E. (2003). Utilization of corn starch as substrate for ß-amylase by *Bacillus* spp. African Journal of Biomedical Research, 6 (1), 37-42.

Anto, H., Trivedi, U.,Patel, K. (2006). α-Amylase production by *Bacillus cereus* MTCC 1305 using solid-state fermentation. Food Technology and Biotechnology, 44 (2), 241-245.

Asgher, M., Asad, M. J., Rahman, S.,Legge, R. (2007). A thermostable α-amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing. Journal of Food Engineering, 79 (3), 950-955 .

Ashraf, H., Iqbal, J.,Qadeer, M. (2003). Production of alpha amylase by< i>*Bacillus licheniformis*</i> using an economical medium. Bioresource Technology, 87 (1), 57-61.

Bernfeld, P. (1955). Amylases, α and β. Methods in enzymology, 1, 149-158

Cotârleţ, M. (2013). Medium optimization for the production of cold-active beta amylase by psychrotrophic *Streptomyces*

MIUG 4 alga using response surface methodology. Microbiology, 82 (2), 147-154.

De Vos, P., Garrity, G., Jones, D., Krieg, N., Ludwig, W., Rainey, F., Schleifer, K.-H.,Whitman, W. 2009. Bergey's manual of systematic bacteriology (Volume three: The firmicutes). Editor in Chief: Michael Goodfellow. Segundaedición. London New York: Springer Dordrecht Heidelberg.

Gangadharan, D., Sivaramakrishnan, S., Nampoothiri, K. M., Sukumaran, R. K.,Pandey, A. (2008). Response surface methodology for the optimization of alpha amylase production by< i>*Bacillus amyloliquefaciens*</i>. Bioresource Technology, 99 (11), 4597-4602.

Gaouar, O., Zakhia, N., Aymard, C.,Rios, G. (1998). Production of maltose syrup by bioconversion of cassava starch in an ultrafiltration reactor. Industrial Crops and Products, 7 (2), 159-167.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K.,Chauhan, B. (2003). Microbial α-amylases: a biotechnological perspective. Process Biochemistry, 38 (11), 1599-1616.

Hensley, D. E., Smiley, K. L., Boundy, J. A.,Lagoda, A. A. (1980). Beta-Amylase production by *Bacillus polymyxa* on a corn steep-starch-salts medium. Applied and Environmental Microbiology, 39 (3), 678-680.

Ikram-Ul-Haq, H. U., Zeeshan, M.,Mohsin, J. M. (2012). Solid state fermentation for the production of α-amylase by *Paenibacillus amylolyticus*. Pakistan Journal of Botany, 44, 341-346.

Mihajlovski, K. R., Carević, M. B., Dević, M. L., Šiler-Marinković, S., Rajilić-Stojanović, M. D.,Dimitrijević-Branković, S. (2015a). Lignocellulosic waste material as substrate for Avicelase production by a new strain of *Paenibacillus chitinolyticus* CKS1. International Biodeterioration & Biodegradation, 104, 426-434.

Mihajlovski, K. R., Radovanovic, N. R., Miljkovic, M. G., Siler-Marinkovic, S., Rajilic-Stojanovic, M. D.,Dimitrijevic-Brankovic, S. I. (2015b). [small beta]-Amylase production from packaging-industry wastewater using a novel strain *Paenibacillus chitinolyticus* CKS 1. RSC Advances, 5 (110), 90895-90903.

Mihajlovski, K. R., Radovanović, N. R., Veljović, Đ. N., Šiler-Marinković, S. S.,Dimitrijević-Branković, S. I. (2016). Improved β-amylase production on molasses and sugar beet pulp by a novel strain *Paenibacillus chitinolyticus* CKS1. Industrial Crops and Products, 80 115-122.

Modelska, M., Binczarski, M., Dziugan, P., Karski, S.,Witońska, I. (2015). Waste biomass as a raw material for furfural production. Journal on Processing and Energy in Agriculture, 19 (3), 120-122.

Ohno, N., Ijuin, T., Song, S., Uchiyama, S., Shinoyama, H., Ando, A.,Fujii, T. (1992). Purification and properties of amylases extracellularly produced by an imperfect fungus, Fusidium sp. BX-1 in a glycerol medium. Bioscience, Biotechnology, and Biochemistry, 56 (3), 465-471.

Olufunke, F.-O. T.,Azeez, I. I. (2012). Purification and characterization of beta-amylase of *Bacillus subtilis* isolated from Kolanut Weevil. Journal of Biology and Life Science, 4 (1), 68-78.

Pandey, A., Nigam, P., Soccol, C., Soccol, V., Singh, D.,Mohan, R. (2000). Advances in microbial amylases. Biotechnology and Applied Biochemistry, 31, 135-152.

Rajagopalan, G.,Krishnan, C. (2008). α-Amylase production from catabolite derepressed< i>*Bacillus subtilis*</i> KCC103 utilizing sugarcane bagasse hydrolysate. Bioresource Technology, 99 (8), 3044-3050.

Saxena, R.,Singh, R. (2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus* sp. Brazilian Journal of Microbiology, 42 (4), 1334-1342.

Šuput, D., Lazić, V., Hromiš, N., Popović, S., Pezo, L., Lončar, B.,Nićetin, M. (2014). Effect of black cumin oil on mechanical and structural characteristics of starch based edible films. Journal on Processing and Energy in Agriculture, 18 (4), 154-157.

Šuput, D., Lazić, V., Pezo, L., Radulović, A., Popović, S., Hromiš, N.,Bulut, S. (2015). Structural changes in starch during starch based edible films synthesis. Journal on Processing and Energy in Agriculture, 19 (3), 139-142.

Šuput, D. Z., Lazić, V. L., Jelić, A., Lević, L. B., Pezo, L. L., Hromiš, N. M.,Popović, S. (2013). The effect of sorbitol content on the chracteristics of starch based edible films. Journal on Processing and Energy in Agriculture, 17 (3), 106-109.

Teotia, S., Khare, S.,Gupta, M. (2001). An efficient purification process for sweet potato beta-amylase by affinity precipitation with alginate. Enzyme and microbial technology, 28 (9), 792-795

Van der Maarel, M., Veen, A.,Wijbenga, D. (2000). *Paenibacillus granivorans* sp. nov., a new *Paenibacillus* species which degrades native potato starch granules. Systematic and applied microbiology, 23 (3), 344-348.

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