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Characterization of Cellulase Enzyme Produced by Two Selected Strains of *Streptomyces Macrosporeus* Isolated from Soil in Indonesia

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Abstract

This study was conducted to characterize the cellulase enzymes produced by *Streptomyces macrosporeus* BB 32 and *S. microspores* KRC 21. D, which were isolated from Tanjung Pendam, Belitung Island, and from Cibodas Botanical Garden, Indonesia, respectively. The optimal activity of the enzymes was analyzed using parameters such as incubation time, pH, temperature, carboxymethylcellulose (CMC) concentration. The effect of the addition of some metal ions as activators or inhibitors was also analyzed spectrophotometrically at λ 540 nm. Results demonstrated that the activity of the cellulase enzymes of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21 D reached the optimum level after 2 and 5 days of incubation and at pH values of 8.0 and 6.0, temperatures of 35 °C and 50 °C, and CMC concentrations of 1.75% and 2%, respectively. *S. macrosporeus* BB 32 cellulase was activated by the cations $CuCl_2$, $MgCl_2$, and $ZnCl_2$ but inhibited by NaCl and $CoCl_2$, reducing its activity. The cellulase of *S. macrosporeus* KRC 21.D was activated by the cation NaCl and by the divalent cations $CoCl_2$, $CuCl_2$, $MgCl_2$, and $ZnCl_2$. *S. macrosporeus* BB 32 was deposited at the Indonesian Culture Collection with the collection number InaCC A144.

Keywords: actinomycetes, cellulolytic enzyme, metal ions

Introduction

Cellulase (EC 3.2.1.4) catalyzes the hydrolysis of 1,4-β-d-glucoside bonds in cellulose. This enzyme plays a vital role in nature by recycling polysaccharide, a primary component of the plant cell wall [1]. Cellulase consists of endo-1,4-β-glucanase, exo-1,4-β-glucanase, and β-d-glucosidase. Endo-1,4-β-glucanase cuts the chain bond in cellulose to produce the shorter cellulose molecules, exo-1,4-β-glucanase cuts the chain terminal in cellulose to produce cellobiose, and β-d-glucosidase cuts the molecules of cellobiose to produce two molecules of glucose [2,3]. Every microbial cellulase enzyme has a different composition; therefore, it is necessary to explore the cellulolytic capability of an isolate [4].

Enzymes can be sequestered from various sources such as plants, animal tissues, and microbes [5]. The majority of enzymes that have been used traditionally originate from plants and animals. However, issues such as low availability and high cost of production are limitations in the growth of enzyme industry.

Currently, microbial enzymes have been found to be economically beneficial as microbial culturing can be done in an easier and a more rapid manner than culturing plant or animal cells or tissues. Microbial enzymes produced industrially from actinomycetes bacteria, fungi, and protozoa with desired quantity or quality can be easily manipulated genetically [6,7]. Numerous cellulases are used in industries because of their role as biocatalysts, which efficiently enhances the development of the enzyme. This beneficial aspect can be identified by the continuously increasing commercial value of enzymes [8]. As cellulose has a high commercial demand, it is necessary to produce this enzyme in high quantities for the required consumers [9]. Cellulase has several commercial applications in terms of food processing such as in the coffee industry, wherein it is used for hydrolyzing cellulose during the drying process of coffee beans. In addition, cellulase is generally used in the textile industry and in laundry detergents. It is also used in the pulp and paper industry for various purposes. Cellulases are even used for pharmaceutical applications [10]. Actinomycetes is a soil bacterium with important functions and exhibits high diversity and abundance in the soil [11].

This study was conducted to characterize the cellulase enzyme from actinomycetes bacteria isolated from

Tanjung Pendam, Belitung Island, from a soil sample in the area of Tana Toraja, South Sulawesi, and from a soil sample in Kebun Raya Cibodas, West Java, Indonesia.

Materials and Methods

The Indonesian actinomycetes such as *S. macrosporeus* BB 32 and other actinomycetes strains such as BB 35 and BB 36 isolated from Tanjung Pendam, Belitung Island, were used in this physiological study. TTO 26 and TTO 47 strains were isolated from a soil sample in Tana Toraja, South Sulawesi, Indonesia. *S. macrosporeus* KRC 21.D strain was isolated from Kebun Raya Cibodas, Indonesia. Except BB 32 that was deposited at the InaCC with the collection number InaCC A144, all the other strains were maintained at the Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Indonesia. All microbial strains were grown in slant agar media containing 0.5% CMC.

Cellulolytic actinomycetes were selected qualitatively using media containing 1% CMC with the following composition: 2.0 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$, 1.0 g peptone, and 2.0 g agar dissolved in 100 mL aquadest [12]. Strains that showed a clear zone were grown in media with the following composition: 2.0 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$, 1.0 g peptone, and 2.0 g agar. Then, the microbial cultures were incubated at 37 °C for 6–7 days on slant agar. Cellulolytic enzyme was produced using medium comprising 1% CMC, 2.0 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$, and 1.0 g peptone dissolved in 100 mL aquadest [12]. The enzyme sample was shaken in a shaker incubator at 120 rpm and 37 °C for 5 days. The cellulase activity was analyzed daily using a spectrophotometer at λ 540 nm. Then, before analysis, the microbial culture was centrifuged at 13,000 *g* and 4 °C for 10 min to separate the enzyme solution (supernatant) from the particles of the substrate or the cells. The obtained supernatant was stored in a freezer (−10 °C) for further measurement of cellulase activity [13]. The activity of the cellulolytic enzyme was examined by the DNS method [14] based on the magnitude of sugar reduction achieved by enzyme activity during the hydrolysis of 1% CMC substrate. The activity of the cellulase enzyme was determined qualitatively, wherein each actinomycetes strain was inoculated onto 1% CMC agar media. Each strain was concentrated on the surface of media, incubated for 3–7 days, and observed daily. Growing colony was treated by flooding with the indicator 0.1% Congo red, allowed for 15–30 minutes, and then washed with 2% NaCl [15].

Formation of a clear zone around a colony indicated the presence of cellulolytic enzyme activity. Quantitative examination of the cellulolytic enzyme activity was performed by the following procedure: 0.125 mL of enzyme solution was added to 0.125 mL of the substrate (1% CMC in phosphate buffer solution, pH 7.0) and then incubated at 40 °C for 30 min. Subsequently, 0.25 mL of 3 5-dinitrosalicylic acid (DNS) was added and boiled for 5 min. DNS was added to stop the reaction [16]. Immediately after cooling the sample under flowing water, 2.5 mL of aquadest was added. Measurement was performed using a spectrophotometer at λ 540 nm.

Cellulase activity was calculated using the following formula:

$$\frac{(\text{Glucose content} \times \text{dilution factor}) \times 1000}{(\text{Glucose molecule weight} \times \text{incubation time})} \quad (1)$$

One unit of enzyme activity was defined as the amount of enzyme required to form 1 mmol of product per unit time for each ml of enzyme. Dissolved protein content was determined to evaluate the specific cellulolytic activity. Protein content was examined using Coomassie Brilliant Blue (CBB) G250 stain in an acidic solution, which produced a bluish color [17]. The procedure was as follows: 0.01 mL of enzyme extract was added to 0.5 mL of CBB reactant, which was then vortexed and incubated for 10 min at room temperature; then, the light intensity was measured using a spectrophotometer at λ 595 nm. For enzyme extract blank, 0.15 N NaCl solution was used. The standard was prepared using 0.2 mL of bovine serum albumin solution at different concentrations ranging between 0 and 800 µg/mL.

Because characterization of the cellulase enzyme involves determination of the incubation time, pH, temperature, optimum concentration of CMC substrate, and addition of metal ions [18], this study included all these parameters. The effect of incubation time on enzyme activity was analyzed using an enzymatic reaction solution after 1–5 days of incubation duration. The effect of pH on enzyme activity was assessed based on the reaction with the enzyme solution at a substrate concentration of 1% CMC incubated in 0.05 N acetic buffer (pH 5.0), 0.05 N phosphate buffer at pH values of 6.0, 7.0, and 8.0–9.0, for which Glycine-NaOH Buffer as used. The effect of temperature on enzyme activity was analyzed using the enzymatic reaction solution with CMC at various pH values and temperatures of 35 °C, 40 °C, 45 °C, 50 °C, 60 °C, and 70 °C.

The effect of CMC on enzyme activity was determined using CMC at various concentrations of 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, and 1.75% at pH and optimum temperature which obtained from previous analysis. Furthermore, the effect of metal ions on the cellulase activity was analyzed using the ions Na^+, Co^{2+}, Cu^{2+}, Mg^{2+}, and Zn^{2+} obtained from NaCl, $CoCl_2$, $CuCl_2$, $MgCl_2$, and $ZnCl_2$, respectively, wherein the concentration of each salt was 1 mM for each CMC solution. CMC

solution without the addition of metal ions was used as control treatment.

Results and Discussion

The success of bioconversion of cellulose material depends particularly on the characteristic of the source of cellulose, the source of cellulolytic enzymes, and the optimum condition for catalytic activity and enzyme production [19]. Cellulose is a renewable biological resource found abundantly in agricultural wastes. Cellulose material waste can be hydrolyzed to produce glucose and other soluble sugars using the cellulase enzyme isolated from bacteria and fungi [20]. Qualitative examination using carboxymethyl cellulose based on clear zone formation can be performed to determine whether an isolated microbe can produce cellulase [21, 22].

According to an earlier research [15], cellulolytic microbes could be rapidly filtered out by measuring the clear zone (qualitative). Quantitative filtering can be done for confirmation, although the results are not consistent, exact, and similar to those obtained by filtering the clear zone (qualitative). This indicates that if there is a clear zone formation in the bacterial colony, then the microbes can decompose cellulose to yield glucose with the help of cellulase [21]. The potential index value of bacteria is obtained using a ratio obtained between the diameter of a clear zone and the diameter of the colony [23].

Congo red used in the cellulolytic assay binds with 1,4-β glycoside bonds in cellulose, resulting in a red color, whereas a clear color around the colony of microbes indicates that cellulose has been decomposed to produce a monosaccharide. Since 1,4-β glycoside bonds are released through the action of cellulase produced by microbes, Congo red cannot bind with glucose, resulting in the formation of a clear zone [24]. The pH of Congo red has an influence on the media color. A clear zone is formed around the colony due to the interaction between (1,4)–β-d-glucan and (1,3)-β-d-glucan in the presence of the reaction of cellulose hydrolysis by the fungal strain to produce the enzyme cellulase [25].

The results of this study demonstrated that only two actinomycetes strains, *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D, possessed the ability to make a clear zone around the colony after incubation for 48 h (Figure 1). Furthermore, both strains had the ability to produce cellulolytic enzymes on 1% CMC agar media. The potential of cellulolytic actinomycetes to produce cellulase has been explored earlier [26]. The distribution and abundance of microbial populations such as the actinomycetes *Micromonospora*, *Nocardia*, and *Streptomyces* can support nutrient availability, temperature, and ecology of the habitat, and these strains can also degrade several complex substances such as cellulose and chitin [27] and photosynthetically they can convert sunlight into energy releasing oxygen as a by-product that can used in aerobic processes [28]. *Streptomyces* has the potential to produce antibiotics, vitamins, antimicrobial compounds, and enzymes that are important in both industries and agricultural sector [29, 30]. *Streptomycetes* is one of the genera that is extremely important and represents almost 70% of the soil population among all actinomycetes [31]. It has been reported that *S. macrosporeus* BB 32 produces the enzyme chitinase [32] and *S. macrosporeus* KRC 21.D also possesses a similar property [33]. In addition, an earlier study [34] has demonstrated the antimicrobial activity *S. macrosporus*.

Previous studies [35, 11] have also reported that actinomycetes, especially *Streptomyces*, can produce other cellulase enzymes as cellulose decomposer, which depends on the pH, aeration, temperature, texture, humidity, and also the minimal nutrition content of soil. The results of the present study revealed that the amount of the soluble protein of *S. macrosporeus* BB 32 was 0.0168 µg/mL, whereas that of *S. macrosporeus* KRC 21.D was not detected.

Actinomycetes is a soil microorganism commonly found in several types of soil [36]. Actinomycetes bacteria are saprophytes and actively decompose organic matter, which increases soil fertility. Actinomycetes bacteria are

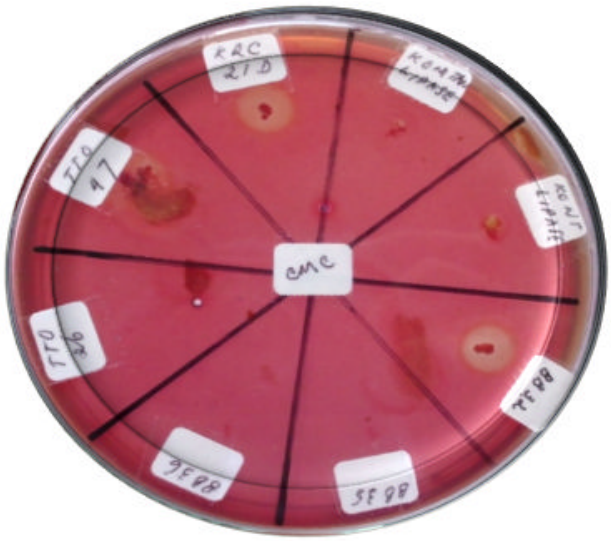

Figure 1. CMC-Degrading Ability of Actinomycetes Strains (BB 32, BB 35, and BB 36 Isolated from the Soil Sample of Post-Tin Mining at Tanjung Pendam, Belitung Island; TTO 26 and TTO 47 Strains Isolated from the Soil Sample in Tana Toraja, South Sulawesi, Indonesia; KRC 21.D Strain Isolated from Cibodas Botanic Garden West Java, Indonesia). Two Strains (BB 32 and KRC 21.D) Indicated by White Arrows Showed the Ability to Degrade CMC

one of the microorganisms that can degrade cellulose other than bacteria, molds, and yeast [37]. Modification of the fermentation medium is an essential step in the design of successful laboratory experiments, pilot-scale development, and manufacturing processes [38].

In the process of enzyme production by using other raw material, microbial strains used and environmental factor are essential. The important parameters those will be considered are incubation time, substrate concentration, temperature, pH, and metal ions addition as activator or inhibitor [12,39,40,41].

In this study, *S. macrosporeus* KRC 21.D exhibited a higher cellulase activity of 4.9 U/mL on the 5th day than *S. macrosporeus* BB 32, whose activity was 4.0 U/mL (Figure 2) on day 2. This result was according to an earlier research that the cellulase enzyme of different isolates by using the same medium would show different optimum activity of the enzyme. This result was according to an earlier research [42] that the cellulase enzyme of different isolates by using the same medium would show different optimum activity of the enzyme.

Regarding the effect of pH on the cellulolytic enzyme activity, the optimum cellulolytic enzyme activities of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D were 5.9 and 6.3 U/mL at pH values of 8.0 and 6.0, respectively (Figure 3). An earlier study [43] had reported that the pH of the fermentation medium affected

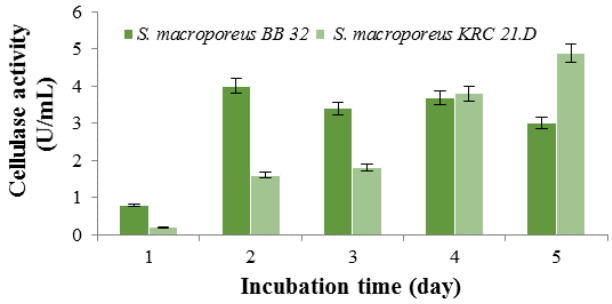

Figure 2. Effect of Incubation Time on Cellulase Activity of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D

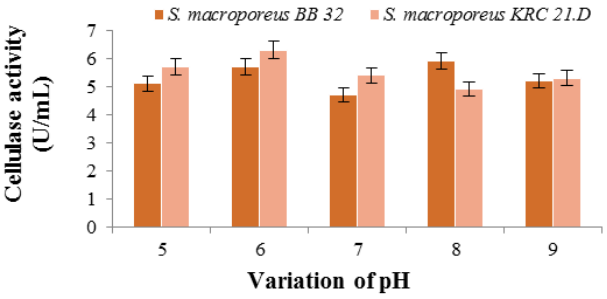

Figure 3. Effect of pH on Cellulase Activity of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D

microbial growth and enzyme production. Several reports have also demonstrated that an optimum pH, especially about 5.0–11, of the fermentation medium used for the production of cellulase by *Streptomyces* sp. could increase the enzyme activity [44,45,46].

The optimum cellulolytic enzyme activities of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D were 7.6 and 7.3 U/mL, which were obtained at 35 °C and 50 °C, respectively (Figure 4). The activity of carboxymethyl cellulase enzyme at low or high temperatures can be considered as a major physiological adaptation on an enzymatic level, such as to compensate the reduction of the chemical reaction rate that was caused by low or high temperatures [47, 48]. It has been reported that *S. griseorubens* AB184139 exhibited the optimum activity of cellulose enzyme at pH 7.0, temperature 45 °C, and 6 days of incubation duration [49]. It is known that *Streptomyces* can use several carbon sources for deriving energy for its growth. A previous study [35] showed that *Streptomyces* has an optimum temperature ranged of 25 °C–35 °C. Several bacterial species that can grow between psychrophilic and thermophilic temperatures are abundant in the soil habitat and compost at an optimum pH of 6.5–8.0, similar to *S. microspores* BB 32 or *S. microspores* KRC 21. D whose optimum temperatures are 35 °C (at pH 6.0) and 50 °C (at pH 8.0), respectively.

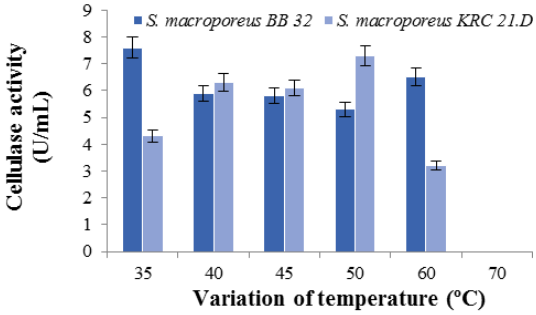

Figure 4. Effect of Temperature on Cellulase Activity of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D

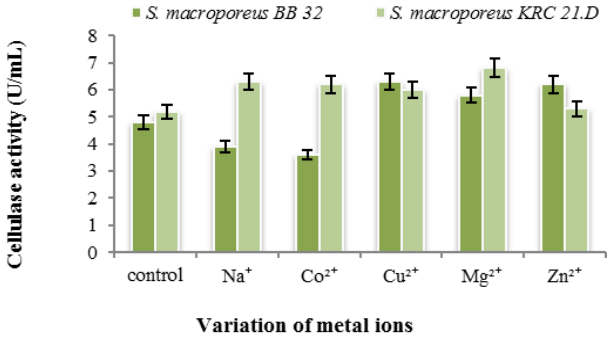

Figure 5. Effect of Concentration of Substrate on Sellulase Activity

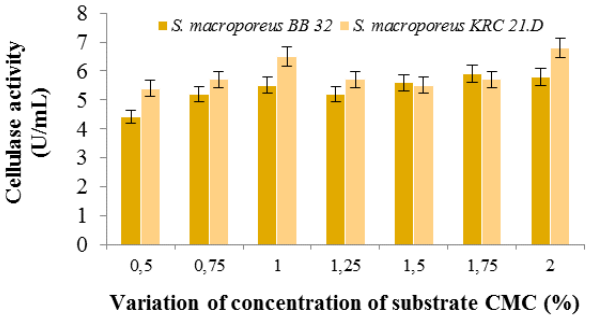

Figure 6. Effect of Addition of Metal Ions on Enzyme Activity

The acidity level of the environment can also be highly affected. *S. griseorubens* have been reported to have a wide range of temperatures and pH values for growth and cellulolytic activity [49]. This strongly indicates that actinomycetes can grow in different habitats with differences in environmental conditions, pH, and temperature. This important capability will be useful not only for achieving better production but also for achieving the end products, including bioethanol and other compounds.

Regarding the effect of the concentration of CMC as a substrate on the enzyme activity, the optimum enzyme activities of *S. macrosporeus* BB 32 and *S. macrosporeus* KRC 21.D were 5.9 and 6.8 U/mL, which were obtained at the CMC concentrations of 1.75% and 2%, respectively (Figure 5). According to an earlier study [50], the reaction of enzymes is basically dependent on the enzyme concentration, the activity of existing enzymes, and the substrate availability. The ability of each microorganism to use carboxymethyl cellulose substrate to produce cellulase enzyme varies according to its environment [49].

There are particular chemical substances that can increase the enzyme activity (activators) or inhibit the activity (inhibitors). This study demonstrated the cellulolytic enzyme activity of *S. macrosporeus* BB 32 was induced by the divalent cations $CuCl_2$, $MgCl_2$, and $ZnCl_2$, whereas the monovalent cation NaCl and the divalent cation $CoCl_2$ inhibited the activity. The cellulolytic enzyme activity of *S. macrosporeus* KRC 21.D was induced by the monovalent cation NaCl and the divalent cations $CoCl_2$, $CuCl_2$, $MgCl_2$, and $ZnCl_2$ (Figure 6). It has been reported that several metals ions such as monovalent, divalent, and trivalent such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, and Fe^{3+} could act as inhibitors and activators of enzyme activity [51].

Conclusions

S. macrosporeus BB 32 and *S. macrosporeus* KRC 21.D possessed the capability of degrading carboxy methyl cellulose (CMC) indicated by the formation of a clear zone. The optimum activities of BB 32 cellulase were as follows: 4 U/mL at an incubation duration of 2 days, 5.9 U/mL at pH 8.0, 7.6 U/mL at a temperature of 35 °C, and 5.9 U/mL at a CMC concentration of 1.75%.

The divalent metal cations $CuCl_2$, $MgCl_2$, and $ZnCl_2$ induced the enzyme activity, whereas NaCl and the divalent cation $CoCl_2$ acted as inhibitors decreasing the enzyme activity. The optimum activities of cellulase isolated from the KRC 21.D strain were as follows: 4.9 U/mL at an incubation duration of 5 days, 6.3 U/mL at pH 6.0, 7.3 U/mL at a temperature of 50 °C, and 6.8 U/mL at a CMC concentration of 2%. In addition, the monovalent cation NaCl and the divalent cations $CoCl_2$, $CuCl_2$, $MgCl_2$, and $ZnCl_2$ induced the enzyme activity.

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References

[1] Morana, A., Maurelli, L., Ionata, E., Cara, F.L., Rossi, M. 2011. Cellulases from fungi and bacteria and their biotechnological applications. In Golan, A.E., Cellulase: Types and Action, Mechanism and Uses. Nova Science Publishers, Inc. USA. pp. 1-79.

[2] Kim, B.J., Alleman, J.E., Quivey, D.M. 1998. Alkaline Hydrolysis/Biodegradation of Nitrocellulose Fines. U.S. Army Construction Engineering Research Laboratories (USACERL). Report Documentation 4A162720D048TE7.

[3] Demers, A., Doane, R., Guzman, S., Pagano, R. 2012. Enzymatic Hydrolysis of Cellulosic Biomass for the Production of Second Generation Biofuels. A Major Qualifying Project Report Submitted to the faculty of Worcester Polytechnic Institute. In partial fulfillment of the requirements for the Degree of Bachelor of Science.

[4] Ram, L., Kaur, K., Sharma, S. 2014. Screening Isolation and Characterization of Cellulase Producing Micro-Organisms from Soil. International Journal of Pharmaceutical Science Invention. 3(3): 12-18.

[5] Acharya, S., Chaudhary, A. 2012. Bioprospecting thermophiles for cellulase production: a review. Brazilian Jurnal Microbiology. 43(3): 844–856, http://dx.doi.org/10.1590/S151783822012000300001.

[6] Demain, A.L., Vaishnav, P. 2009. Production of recombinant proteins by microbes and higher organisms. Biotechnology Advances. 27: 297–306, http://doi.org/10.1016/j.biotechadv.2009.01.008.

[7] Adrio, J.L., Demain, A.L. 2014. Microbial Enzymes: Tools for Biotechnological Processes. Biomolecules. 4(1): 117–139, http://doi.org/10.3390/biom4010117.

[8] Endah, R., Nafisah, Z. 2011. Aktifitas Immobilized β–Amylase dan Free β–Amylase dari Zoogloearamigera ABL 1 dalam Medium Pati Cair dengan Perlakuan Faktor Lingkungan. Biota. 16(1): 95-106. (in Indonesia)

[9] Kuhad, R.C., Gupta,R., Singh, A., 2011. Microbial Cellulases and Their Industrial Applications. Enzyme Research Volume 2011 Article ID 280696, 10 pages, http://dx.doi.org/10.4061/2011/280696.

[10] Abubakar, F.A., Oloyede, O.B. 2013. Production and activity of cellulase from Aspergillus niger using rice bran and orange peel as substrates. International Journal of scientific research and management. (1)5: 285-291.

[11] Nurkanto, A. 2008. Studi Kelimpahan Aktinomisetes Tanah dan Hubungannya Terhadap Enzim Selulase, Amilase, Total Karbon dan Nitrogen Hutan Pasca Kebakaran Bukit bangkirai Kalimantan Timur. Jurnal Biologi Indonesia. 5(1): 81-89, http://dx.doi.org/10.14203/jbi.v5i1.3208.

[12] Sohail, M., Siddiqi, R., Ahmad, A., Khan, S.A. 2009. Cellulase production from Aspergillus niger MS82: effect of temperature and pH. New Biotechnology. 25(6): 437–441, http//doi.org/10.1016/j.nbt.2009.02.002.

[13] Purwadaria, T., Marbun, P.A., Sinurat, A.P., Ketaren, P.P. 2003. Perbandingan Aktivitas Enzim Selulase dari Bakteri dan Kapang Hasil Isolasi dari Rayap. Jurnal Ilmu Ternak and Veteriner. 8(4): 213-219.

[14] Miller, G.L. 1959. Use of dinitrosalicylic acid reagent of determination of reducing sugar. Analytical Biochemistry 31: 246-248, http//doi.org/10.1021/ac60147a030.

[15] Teather, R.M., Wood, P.J. 1982. Use of Congo red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Applied and Environmental Microbiology. 43(4): 777-780.

[16] Kar, S., Mandal, A., Mohapatra, P.K., Mondal, K.C., Pati. B.R. 2006. Production of cellulase-free xylanase by Trichoderma reesei SAF3. Brazilian Journal of Microbiology. 37(4): 462-464, http://dx.doi.org/10.1590/S1517-83822006000400011.

[17] Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microorganisms quantities of protein in utilizing the principle of protein-dye binding. Analytical Biochemistry. 7(72): 248-254.

[18] Kane, S. 2015. The Degradation of Cellulosic Material by Cellulomonas fimi. PhD School of Biological Sciences The University of Edinburgh.

[19] Alam, M.Z., Manchur, M.A., Anwar, M.N. 2004. Isolation Purification, Characterization of cellulolytic Enzymes Produced by the Isolate Streptomyces omiyaensis. Pakistan Journal Biology Sciences. 7: 1647-1653.

[20] Lokhande, S., Musaddiq, M. 2015. Isolation of cellulolytic bacterial strains for bioconversion of municipal solid waste. International Journal of Applied Research. 1(11): 902-905.

[21] Gupta P., Samant, K., Sahu, A. 2012. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential. International Journal of Microbiology. Volume 2012, Article ID 578925, 5 pages, http://dx.doi.org/10.1155/2012/ 578925.

[22] Johnsen, H.R., Krause, K. 2014. Cellulase Activity Screening Using Pure Carboxymethylcellulose: Application to Soluble Cellulolytic Samples and to Plant Tissue Prints. International Journal of Molecular Sciences. 15(1): 830-838, doi: 10.3390/ijms 15010830.

[23] Ruegger, M.J.S., Tauk-Tornisielo, S.M. 2004. Cellulase activity of fungi isolated from soil of the Ecological Station of Juréia-Itatins, São Paulo, Brazil. Brazilian Journal Botany. 27(2): 205–211, http://dx.doi.org/10.1590/S010084042004000200001.

[24] Florencio, C., Couri, S., Farinas, C.S. 2012. Correlation between Agar Plate Screening and Solid-State Fermentation for the Prediction of Cellulase Production by Trichoderma Strains. Enzyme Research. Volume 2012, Article ID 793708, 7 pages, http://dx.doi.org/10.1155/2012/793708.

[25] Vasan, P.T. 2012. Chapter-1. Isolation of Cellulolytic Bacteria. shodhganga.inflibnet. ac.in:8080/jspui/bitstream/.../11/11_chapter%201.p...by P Thirumalai Vasan.

[26] Saini, A., Aggarwal, N.K., Sharma,A., Yadav, A. 2015 Actinomycetes: A Source of Lignocellulolytic Enzymes. Enzyme Research Volume 2015, Article ID 279381, 15 pages, http://dx.doi.org/ 10.1155/2015/279381.

[27] Hayakawa, M. 2008. Studies on the isolation and distribution of rare actinomycetes in soil. Actinomycetologica. 22(1): 12-19, https://doi.org/10. 3209/ saj.SAJ220103.

[28] Singh, J.S., Pandey, V.C., Singh, D. 2011. Efficient soil microorganisms, A new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystems and Environment. 140(4): 339-353, https://doi.org/10.1016/j.agee.2011.01.01 7.

[29] Mc Carthy, A.J., Williams, S.T. 1992. Actinomycetes as agents of biodegradation in environment. Gene. 115 (1-2): 189–192.

[30] Lazzarini, A., Cavaletti, L., Toppo, G., Marinelli, F. 2000. Rare Genera of Actinomycetes As Potential Producers of New Antibiotics. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology. 78: 399-405, https://doi.org/10.1023/A:1010287600557

[31] Malviya, N., Yadav, A.K., Yandigeri, M.S., Arora, D.K. 2011. Diversity of culturable Streptomycetes from wheat cropping system of fertile regions of Indo-Gangetic Plains, India. World Journal of Microbiology and Biotechnology. 27: 1593–1602, doi: 10.1007/s11274-010-0612-3.

[32] Soeka, Y.S., Triana, E. 2016. Pemanfaatan Limbah Kulit Udang untuk menghasilkan Enzim Kitinase dari Streptomyces macrosporeus BB 32. Jurnal Kimia Terapan Indonesia. 18(1): 91-101. (in Indonesian).

[33] Soeka, Y.S. 2015. Karakterisasi enzim kitinase dan identifikasi isolat aktinomisetes KRC 21.D berasal dari Kebun Raya Cibodas. Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia. 1(4): 1156-1161, doi: 10.13057/psnmbi/m010533

[34] Emelda, E.J., Vijayalakshmi, N., Santhanakrishnan, T. 2012. Antimicrobial Activity of Antibiotic Producing Streptomyces macrosporus. IOSR Journal of Pharmacy and Biological Sciences. 2(3): 20-23.

[35] Nurkanto, A. 2007. Identifikasi Aktinomisetes Tanah Hutan Pasca Kebakaran Bukit Bangkirai Kalimantan Timur dan Potensinya Sebagai Pendegradasi Selulosa dan Pelarut Fosfat. Biodiversitas. 8(4): 314-319, doi:1013057/biodiv/d080414. (in Indonesian).

[36] Elberson, M.A., Malekzadeh, F., Yazdi, M.T., Kameranpour, N., Noori-Daloii, N.R., Matte, M.H., Shahamat, M., Colwell, R.R., Sowers, K.R. 2000. Cellulomonas persica sp. nov. and Cellulomonas iranensis sp. nov" mesophilic cellulose-degrading bacteria isolated from forest soils. International Journal of Systematic and Evolutionary Microbiology. 50: 993-996, doi:10.1099/00207713-50-3-993.

[37] Xu, G.H., Chen, J.C., Liu, O.H., Zhang, Y.H., Jiang, P., Ye, X.Q. 2008. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. Journal of Food Science. 73: 11-18, doi: 10.1111/j.1750-3841.2007.00546.x.

[38] Stanbury, P.F., Whitaker, A., Hall, S.J. 2000. Principles of Fermentation Technology. Butterworth-Heinmann, Heinmann. Oxford.

[39] Bakare, M., Adewale, I., Ajayi, A., Shonukan, O. 2005. Purification and characterization of cellulose from the wild-type and two improved mutants of Pseudomonas fluorescens. African Journal of Biotechnology. 4: 898-904.

[40] Immanual, G., Dhanusa,R., Prema, P., Palavesam, A. 2006. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. International Journal of Environmental Science & Technology. 3(1):25–34, doi: 10.1007/BF03325904.

[41] Yopi, N. Rahmani, Izzaty, F., Putri, C.E., Suparto, I.H. 2016. Optimization of Cellulase Production from Marine Bacterium Bacillus cereus C9 by Submerged Fermentation. Jurnal Teknologi Indonesia. 39(1): 15–21, http://dx.doi.org/10.14203/jti.v39 i1.238.

[42] Prasanna, H.N., Ramanjaneyulu, G., Reddy, B.R. 2016. Optimization of cellulase production by Penicillium sp. 3 Biotech. 6(2): 162, http://doi.org/10.1007/ s13205-016-0483-x.

[43] Odeniyi, O.A., Onilude, A.A., Ayodele, M.A. 2009. Production characteristics and properties of cellulase/polygalacturonase by a Bacillus coagulans strain from a fermenting palm-fruit industrial residue. Africa Journal Microbiology Research. 3(8): 407-417.

[44] Jaradat, Z.W., Dawagreh, A., Ababneh, Q., Saadoun, I. 2008. Influence of culture conditions on cellulose production by Streptomyces sp. (Strain J2). Jordan Journal of Biological Science. 1(4): 141–146, DOI: 10.5963/LSMR0304002.

[45] Solingen, P., Meijer, D., Kleij, W.A., Barnett, C., Bolle, R., Power, S.D., Jones, B.E. 2001. Cloning and expression of an endocellulase gene from a novel streptomyces isolated from an East African soda lake. Extremophiles. 5(5): 333-341.

[46] Yassien, A.A.M., Jiman-Fatani, A., Hani, Z.A. 2014. Production, purification and characterization of cellulase from streptomyces sp. African Journal of Microbiology Research. 8(4): 348-354, DOI: 10.5897/AJMR2013.6500.

[47] Feller, G., Gerday, C. 2003. Psychrophilic enzymes: hot topics in cold adaptation. Nature Reviews Microbiology. 1(3): 200-208, DOI:10.1038/nrmicro773.

[48] Carrard, G., Koivula, A., Söderlund, H., Béguin, P. 2000. Cellulose-binding domains promote hydrolysis of different sites on crystalline cellulose. Proceedings of the National Academy of Sciences United State of America. 97(19): 10342–10347, DOI:10.1073/pnas.160216697.

[49] Prasad, P., Singh, T., Bedi, S. 2013. Characterization of the cellulolytic enzyme produced by Streptomyces griseorubens (Accession No. AB184139) isolated from Indian soil. Journal of King Saud University – Science. 25: 245–250, https://doi.org/10.1016/j.jksus.2013.03.003.

[50] Vepsäläinen, M. 2012. Functional biodiversity in soils. Monographs of the Boreal Environment Research. Edita Prima Ltd. Helsinki.

[51] Pereira, J.C., Giese, E.C., Moretti, M.M.S., Gomes, A.C.S., Perrone, O.M., Boscolo, M., Silva, R., Gomes, E., Martins, D.A.B. 2017. Effect of Metal Ions, Chemical Agents and Organic Compounds on Lignocellulolytic Enzymes Activities. Chapter 6. Pp. 139-164, http://dx.doi.or g/10.5772/65934.