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# Utilizing rice husks by moderately halophilic Bacillus spp. isolated from Sawa Lake as a carbon source

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## ABSTRACT

Rice husk is a sustainable, plentiful, and cheap resource with great potency for bioconversion to important bioproducts. This process required suitable employment of active pretreatment and hydrolysis enzymes to produce reduced sugars. Five isolates (AE1, AE2, AE3, AE4, and AE5) of moderately halophilic Bacillus were isolated from Sawa Lake, which is resistant to 12% sodium chloride. Results showed that isolates (AE1, AE3, and AE4) produce cellulase enzymes. AE3 isolate chooses to produce cellulase after being cultured in nutrient broth supplemented with 1% of pretreatment rice husk and incubated for (4, 7, 11, 18, and 21) days at 30°C with rotation 150rpm, with activity (48.08, 109.38, 68.77, 68.16 and 41.42) U/ml, the concentration of cellulose residue (125, 97.4, 106.4, 122.7 and 142.9) µg/ml, respectively. These results prove that the cellulose component of pretreatment rice husk was consumed and increased up to 7days of incubation period at 30°C with a rotation of 150rpm.

**Keywords:** *Bacillus, cellulase production, rice husk pretreatment, moderately halophilic bacteria.*

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## INTRODUCTION

Plants are considered to be the most abundant organic matter on earth, producing cellulose (40-50) % (Saranraj et al., 2012). In addition, rice husks are biomass obtained from rice grain with low mass intensity and high silica and mineral content (Jain et al., 2015). Chemical component were, cellulose (25-35) %, hemicellulose (18-21) %, lignin (26-31) %, silica (15-17) %, soluble solids (2-5) % and (7.5) % moisture (Luduena et al., 2011). Rice husk has good chemical stability and strength due to its insolubility in water and high silica content, respectively (Lee et al., 1999).

Proper biotechnological utilization of these wastes in the environment will reduce pollution and convert them into beneficial byproducts (Milala et al., 2005). Biodegradation is the application of the biological system to convert foodstuff into more palatable nutritional or necessary food or improve and increase the nutritional value of fibrous agricultural byproducts (Larry, 1995). Enzymatic hydrolysis of cellulose proceeds with specific enzymes (Weiss et al., 2013).

Pretreatment of rice husk is a process that allows the cracking of cellulose, hemicellulose, and lignin into small pieces. This process aims to support enzymes by improving accessibility to cellulose fractions (Ravindran and Jaiswal, 2016). Physical, chemical, and biological techniques suggest for rapid and efficient degradation of rice husk for diverse biotechnology applications, such as biofuel production, biorefinery, bioproducts, feedstock for lipids, chemicals, and lignocellulose enzyme production (Sweeney and Xu, 2012; Nurul Atika et al., 2014; Ghaffar et al., 2015; Ravindran and Jaiswal, 2016).

Cellulase is the enzymatic saccharification of the cellulosic biomass, including endoglucanases, exoglucanases, and β-glucosidases enzymes. Cellulase performs synergistically to develop hydrolysis of cellulose in sugars (Sadhu and Maiti, 2013). Enormous microorganisms, such as bacteria, fungi, and actinomycetes, were recognized as cellulase producers (Wilson, 2011).

Therefore, the research goal is to obtain isolates belonging to the genus Bacillus from Sawa lake, which possesses the ability to produce cellulase after utilizing rice husk as a carbon source.

## MATERIALS AND METHODS

### *Pretreatment of raw material*

Rice husk is treated with acidified sodium chlorite by utilizing sodium chlorite and acetic acid at 80ºC, according to a modified method (Hubble and Ragauskas, 2010).

### *Isolation of bacterial isolates*

Sawa Lake water samples were heat-treated at 80ºC for 10min, and subcultured on nutrient agar dishes, colonies were recovered and purified by streaking on fresh nutrient agar after incubating for 24hr at 30ºC.

### *Screening of moderate halophilic bacterial isolates*

Ten concentrations of sodium chloride (1, 3, 5, 7, 10, 12, 14, 16, 18, and 20) % with nutrient agar were intended and inoculated by Sawa Lake bacterial isolates and incubated for 24hr at 30ºC.

### *Solid media for detection of cellulose*

Bacterial isolates improve their potency to produce cellulase by making spots on supplement nutrient agar containing 1% each of cellulose, CMC, and pretreatment rice husk. The plates were treated with an aqueous solution of iodine reagent and HCl (0.1N) (Yeoh et al., 1985), after incubating for 24hr at 30ºC, cellulase producers were surrounded by a clear zone in the medium.

### *Assay of cellulase activity*

Two percent of bacterial suspension was inoculated nutrient broth supplemented with 1% of pretreatment rice husk and incubated in a shaker incubator with 150rpm at 30°C for (4, 7, 11, 18, and 21) days, supernatant was collected after centrifuged at 10000rpm for 15min. According to Miller (1959), enzyme efficacy was examined using the 3,5 dinitrosalicylic acid (DNS) method. The mix of the reaction consisted of a 1ml substrate (CMC in phosphate buffer 0.05M pH 5.9) and 1ml crude enzyme (supernatant), then incubated at 40°C for 30min (Atlas et al., 1995). The reaction was finished by adding 1ml of DNS solution to each tube and incubating tubes in boiling water for 5min to cool with tap water directly. Finally, optical density at 575nm has measured the activity of the enzyme.

One unit of cellulase efficacy was determined as the amount of enzyme which releases 1µg of reducing sugars/min at 40°C (Ariffin et al., 2006).

***Determination of cellulose residue by anthrone reagent***

Rice husk is collected after incubating samples in a shaker incubator with 150rpm at 30°C for (4, 7, 11, 18, and 21) days. Twenty ml of sulfuric acid (67%) was added and permitted to stand for 60min, then diluted 1ml of the solution up to 100ml. Four ml of anthrone reagent, prepared according to Scott and Melvin (1953), was added to 1ml of the diluted solution and mixed well. Tubes were cooled and measured for absorbance at 620nm after heating in a boiling water bath for 10min. Set a blank with anthrone reagent and distilled water. Calculate the amount of cellulose in the sample according to the standard curve of cellulose (Fig. 4) (Updegroff, 1969).

## RESULTS AND DISCUSSIONS

***Delignification of rice husk by acidified sodium chlorite and sodium bicarbonate***

This study included removing a lot of lignin after adding sodium chlorite and sodium bicarbonate (Fig. 1). Procedure belong to Hubbell and Ragauskas (2010) were reported that the acidified sodium chlorite method should be adequate to separate lignin from cellulose samples with lignin content below 30% when the reaction was duplicated two times.

**FIGURE 1:** Rice husk, (Left) before pretreatment, (Right) after pretreatment

For biofuel and enzyme production, cellulose and hemicellulose must be converted to reducing sugar after the breakdown of lignin material. Extraction and removal of lignin by microbial treatment methods could facilitate and accelerate the penetration of enzymes to carbohydrates, such as cellulose and hemicellulose (Stella, 2016).

***Collection of isolates***

Sawa Lake water sample (TDS 20800ppm) was heat-treated to destroy all vegetative cells, then cultured on nutrient agar to permit germination of heat-resistant spores. Morphological diagnoses of five isolates belonging to the genus Bacillus (AE1, AE2, AE3, AE4, and AE5) were characteristics specified by microscopy (Gram and spore staining) after being incubated for 24hr at 30ºC.

***Estimating of cellulase production***

***A. Qualitative assay***

Five moderate halophilic Sawa Lake Bacillus isolates (AE1, AE2, AE3, AE4, and AE5) possess the ability to tolerate concentration of sodium chloride up to 12% inoculated as spots on nutrient agar after supplementing with each of cellulose, CMC, and pretreated rice husk, as well as, Adnan et al. (2018) showed that Bacillus isolates (Saw2 and Saw3) tolerate to various concentrations of NaCl up to 14%, and (Saw1 and Saw4), resisted to 12% of NaCl, after incubation for 24hr at 30ºC.

Results demonstrated that isolates (AE1, AE3, and AE4) produced cellulase (Table 1) after incubating plates for 24hr at 30ºC and flooded with an aqueous solution of iodine reagent and HCl (0.1N) (Yeoh et al., 1985). The clear zone surrounding the spot referred to cellulase production bacteria (Fig. 2).

**TABLE 1:** Inhibition clear zone of cellulose material against of moderately halophilic Bacillus isolates

| **Materials** | **Inhibition clear zone (mm)** | | |
|---|---|---|---|
| **Isolates** | **Cellulose agar** | **CMC agar** | **Pretreatment rice husk agar** |
| AE1 | 23 | 25 | 25 |
| AE2 | Zero | Zero | Zero |
| AE3 | 28 | 30 | 26 |
| AE4 | 20 | 22 | 25 |
| AE5 | Zero | Zero | Zero |

**FIGURE 2:** Cellulase production on nutrient agar content with each of (left) cellulose, (Middle) CMC, (Right) pretreatment rice husk, after incubating for 24hr at 30ºC

***B. Quantitative assay***

Moderately halophilic Bacillus isolate (AE3) created cellulase after being cultured in nutrient broth supplemented with 1% of pretreatment rice husk and incubated for (4, 7, 11, 18, and 21) days at 30°C with rotation 150rpm. Results appeared that cellulase activity according to reducing sugar production were (48.08, 109.38, 68.77, 68.16, and 41.42) U/ml, respectively (Fig. 3), the best incubation period of cellulase production was up to 7days, then after that results were diminished down to 21days of the incubation period. In recent years, methods were determined cellulase activity through DNS base, and reducing nitro of DNS to amino by reducing sugar, through forming a reddish-brown color due to amino synthesis (Vancov and Keen, 2009), and present a positive correlation between reducing sugar and the brown color, which can be recognized by the spectrophotometric method.

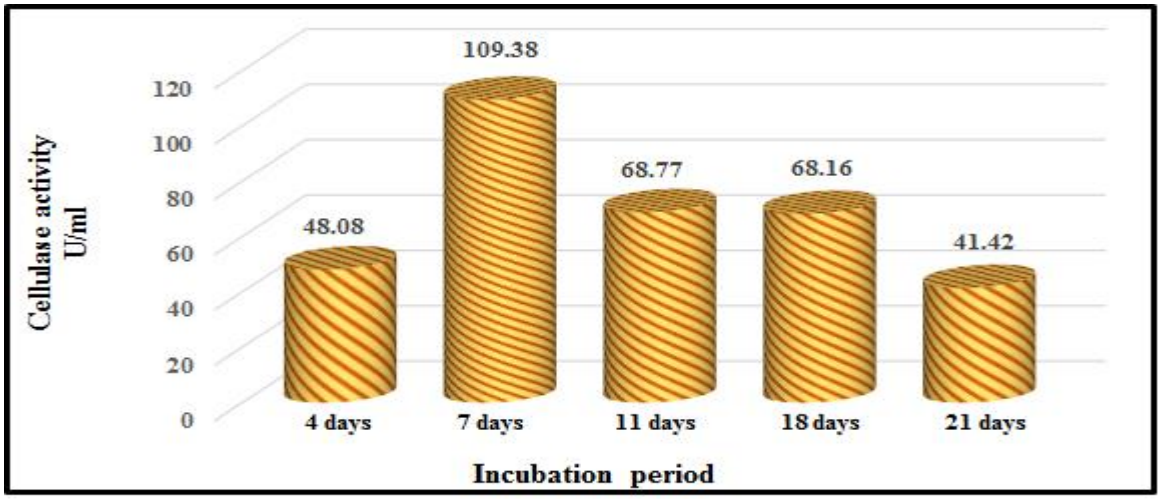


**FIGURE 3:** Cellulase activity producing by AE3 isolate after incubation for (4, 7, 11, 18 and 21) days at 30°C with rotation150rpm

### *Measurement of cellulose residue*

According to a standard curve of cellulose (Scott and Melvin, 1953) (Fig. 4), results appeared that optical density at 620nm of culture after incubation for (4, 7, 11, 18, and 21) days at 30°C with rotation 150rpm, were (0.675, 0.526, 0.575, 0.663 and 0.772) nm, with a concentration of cellulose residue (125, 97.4, 106.4, 122.7 and 142.9) µg/ml, respectively, by using AE3 isolate and pretreatment rice husk as cellulose source (Fig. 5). These results indicated that consuming cellulose expulsion from pretreatment rice husk was increased up to 7days of incubation period at 30°C with rotation 150rpm.

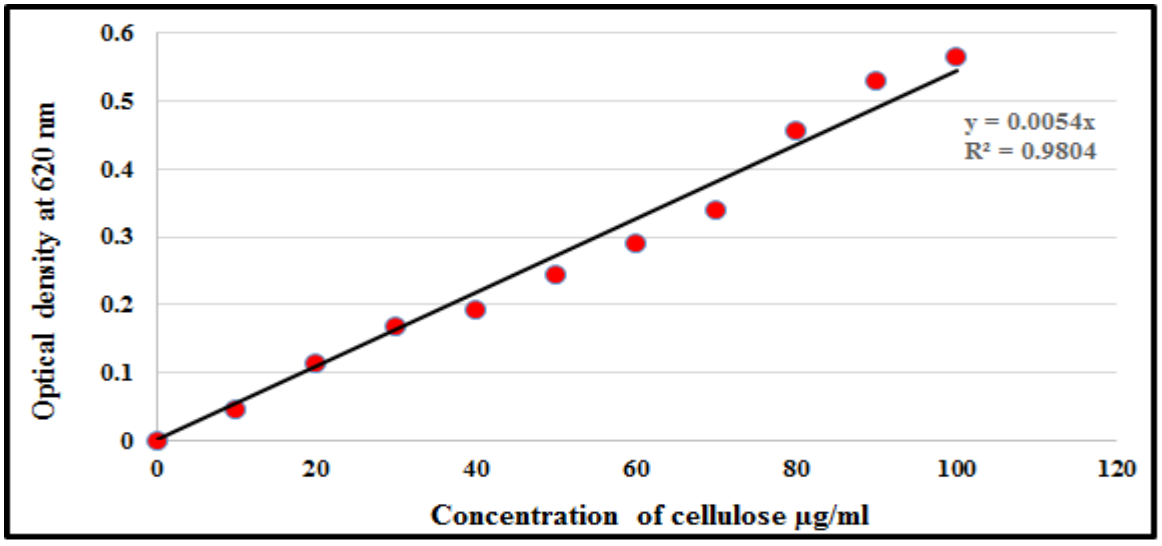


**FIGURE 4:** Standard curve of cellulose (10-100) µg/ml

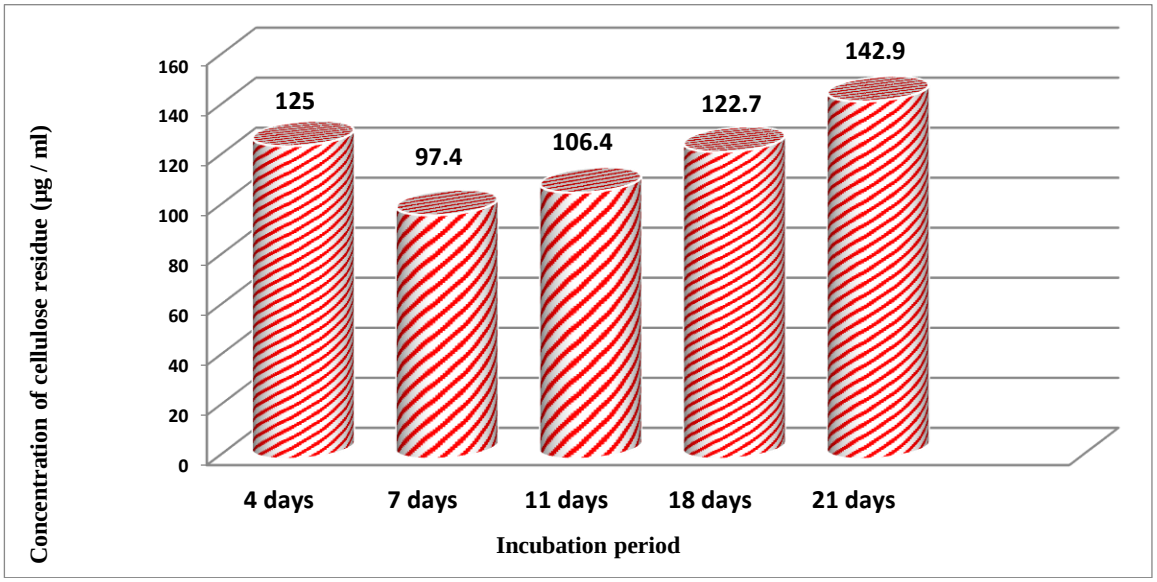


**FIGURE 5:** Concentration of cellulose residue after AE3 isolate incubation for (4, 7, 11, 18 and 21) days at 30°C with rotation 150rpm

## CONCLUSIONS

This search demonstrated that moderately halophilic Bacillus isolates of Sawa Lake were able to develop and resist at 12% NaCl concentration, which could diminish the risk of microbial pollution and utilize the cellulose of pretreated rice husk up to 7days of incubation period at 30°C, The availability of moderately halophilic bacteria manufacturing halotolerant cellulase provided an interest in employing agricultural wastes processes through and creation of environmentally friendly materials.

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## REFERENCES

1. Adnan H. A., Eman H.A. and Seham M.M. 2018. Efficiency of Moderately Halophilic Bacillus spp. Isolated From Hypersaline Environments In Producing Levansucrase. Australian Journal of Basic and Applied Sciences. 12(1): 28-31.
2. Ariffin, H., Abdullah, N., Kalsom, M.S.U., Shirai, Y., Hassan, M.A. 2006. Production and characterization of cellulase by Bacillus pumilus EB3. International Journal of Engineering and Technology, 3(1): 47-53.
3. Atlas, R.M., Brown, A.E., Parks, L.C. 1995. Laboratory Manual of Experimental Microbiology. Mosby-Year Book, Inc. St. Louis, Baltimore.
4. Ghaffar, S.H., Fan, M., McVicar, B. 2015. Bioengineering for utilization and bioconversion of straw biomass into bio-products. Industrial Crops and Products, 77: 262-274.
5. Hubbell, C.A., Ragauskas, A.J. 2010. Effect of acid-chlorite delignification on cellulose degree of polymerization. Bioresour. Technol., 101: 7410-7415.
6. Jain, R.K., Ghosh, D., Agrawal, D., Suman, S.K., Pandey, D., Vadde, V.T., Dixit, A.K., Adhikari, D.K., Dasgupta, D. 2015. Ethanol production from rice straw using thermotolerant Kluyveromyces sp. IIPE453. Biomass Convers Biorefin., 5: 331-337.
7. Larry, R.B. 1995. Application of biotechnology to indigenous foods. Food Technology, January. 86-90.
8. Lee, C.K., Low, K.S., Liew, S.C., Choo, C.S. 1999. Removal of arsenic (V) from aque-ous solution by quaternized rice husk. Environ. Technol., 20: 971-978.
9. Luduena, L., Fasce, D., Alvarez, V.A., Stefani, P.M. 2011. Nanocellulose from rice husk following alkaline treatment to remove silica. Bioresources, 6(2): 1440-1453.
10. Milala, M.A., Shugaba, A., Gidado, A. 2005. Studies on the use of agricultural wastes for cellulase enzyme production by Aspergillus niger. Res. J. Agric. Biol. Sci., 1(4): 325-328.
11. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3): 426-428.

12. Nurul Atika, M.R., Umi Kalsom, M.S., Rosfarizan, M., Suraini, A.A. 2014. Effects of chemical and thermal pretreatments on the enzymatic saccaharification of rice straw for sugars production. Bioresources, 9(1): 510-522.
13. Ravindran, R., Jaiswal, A.K. 2016. A comprehensive review on pre-treatment strategy for lignocellulose food industry waste: Challenges and opportunity. Bioresource Technology, 199: 92-102.
14. Sadhu, S., Maiti, T.K. 2013. Cellulase production by bacteria: a review. Br. Microbiol. Res. J., 3: 235-258.
15. Saranraj, P., Stella, D., Reetha, D. 2012. Microbial Cellulases and Its Applictions. International Journal of Biochemistry and Biotechnology Science, 1: 1-12.
16. Scott, T.A., Melvin, E.H. 1953. Determination of dextran with anthrone. Analyt. Chem., 25: 1656-1661.
17. Stella, M. 2016. Structural Changes of Rice Straw Pre-Treated with Paenibacillus and Aspergillus fumigatus. International Journal of Agricultural and Food Research, 5(4): 1-8.
18. Sweeney, M.D., Xu, F. 2012. Biomass converting enzymes as industrial biocatalysis for fuels and chemicals: Recent developments. Catalysts, 2(2): 244-263.
19. Updegroff, D.M. 1969. Semimicro determination of cellulose in biological materials. Anal. Biochem., 32: 420-424.
20. Vancov, T., Keen, B. 2009. Rapid isolation and high-throughput determination of cellulase and laminarinase activity in soils. J. Microbiol. Method, 79: 174-177.
21. Weiss, N., Börjesson, J., Pedersen, L.S. 2013. Enzymatic lignocellulose hydrolysis: Improved cellulase productivity by insoluble solids recycling. Biotechnology for biofuels, 6(1): 5.
22. Wilson, D.B. 2011. Microbial diversity of cellulose hydrolysis (Review). Curr. Opin. Microbiol., 14(3): 259-63.
23. Yeoh, H.H., Khew, E., Lim, G. 1985. A simple method for screening cellulytic fungi. Mycologia, 77: 161-162.

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