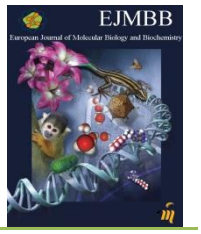
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



ISOLATION, SCREENING, IDENTIFICATION OF POTENTIAL LIGNINASE PRODUCING BACTERIA FROM VEGETABLE WASTE OF DINDIGUL DISTRICT, TAMIL NADU, INDIA AND ITS GROWTH CHARACTERIZATION

Esther Motaro[1*], T. Selvin Jebaraj Norman[2]

Centre for Futures studies, Gandhigram Rural Institute, Dindigul.,Tamil Nadu, India.

Article Info
Received 23/01/2017
Revised 16/02/2017
Accepted 19/02/2017

Key words:-
Acinetobacter beijerincki; Dindigul; Ligninase; Production; Optimization.

ABSTRACT
A potential ligninase producing bacterium was isolated from vegetable waste samples of Dindigul district, Tamil Nadu, India. The potential strain showed highest activity of 77.7 units/ml enzyme in biochemical assay and it was identified as *Acinetobacter beijerincki* based on 16S rRNA sequencing method. The potential strain was examined for enhanced growth factor in fermentor study which revealed maximum growth with pH 7, 37°C temperature, 2% carboxy methyl cellulose and 0.75% tannic acid. From the above observations, the isolated strain showed the possibilities for the promising production of ligninase enzyme with a general growth characteristic applicable for easy lab fermentations.

INTRODUCTION

Lignocellulose, also known as woody cellulose, is the close association of lignin and cellulose, which is the primary component of plant cell walls. Woody cellulose also contains a number of other polysaccharides, such as hemicelluloses. The lignocellulose from plant roots, stems and leaves is the major constituent of the waste products derived from crops and trees [1]. These biomass wastes are abundant in nature, and only a few of them are exploited by humans. In nature, lignocellulose is mainly degraded by fungi and bacteria. There are mainly three groups of fungi that can degrade lignocellulose: white-rot fungi, brown-rot fungi and soft-rot fungi [2]. Among these fungi, white-rot fungi have the ability to secrete extracellular ligninolytic enzymes with the best ability to degrade lignocellulose biomass, and therefore have the best prospects for development and utilization [3]. White - rot fungi are invade wood cells through the decomposition of lignocellulose, leading to a characteristic white spongy saprophytic flamentous fungi that generally colonize wood in nature. They release lignin-degrading enzymes and clump called white rot. There are many kinds of white-rot fungi, including many higher basidiomycetes, most of which belong to Aphyllophorales, Homobasidiomycetes [4]. The typical species of white-rot fungi is *Phanerochaete chrysosporium* Burdsall, which mainly distributed in North America.

At present, thousands of white-rot fungi have been characterized, but only more than ten of them can be utilized to degrade lignocellulose; these fungi mainly belong to *Trametes, Bjerkandera, Phanerochaete, Pleurotus, Lentinula* and *Ganoderma* [5]. Given the unique metabolism and extracellular ligninolytic enzymes of white-rot fungi, they can potentially be employed to decompose large amounts of organic pollution; therefore, these fungi can likely be utilized in the future disposal of waste water, gas, solids, as well as the disposal and environmental remediation of lignocellulosic wastes [6]. However, bacteria of producing ligninase enzyme were rarely reported. This study presents an isolation, screening and identification ligninolytic enzymes from bacterial species of vegetable waste and cultivation conditions were optimized for the enhanced growth of these potential bacteria.

Corresponding Author

Esther Motaro
Email: - master.maniji@gmail.com

MATERIALS AND METHODS

Isolation of bacterial strain

Vegetable samples were collected from two different locations of Dindigul district, Tamil Nadu, India. During collection, aseptic methods were strictly applied and the samples were transferred to pre-sterilized 250 ml screw type bottle containers which were kept maintained at 4˚C in an ice box and were processed immediately in the lab. One gram of the central portion of the collected samples was serially diluted on saline water (0.9% NaCl) and spread plated on nutrient agar plates. After 48 hours incubation, the enumeration of bacterial strains was carried out and morphological district colonies were pure cultured on fresh nutrient agar plates. All the strains were individually maintained as nutrient agar slant at 0°C refrigerated conditions.

Screening of potential ligninase bacteria

The axenic cultures were freshly broth cultured on nutrient broth medium using 10ml in 30ml screw cap tubes. After incubation period, the broths were centrifuged at 3000 rpm for 15 min and supernatant was used for the following studies. Ligninolytic or ligninase activity was measured periodically by determining the rate of oxidation of veratryl alcohol to veratraldehyde. Reaction mixtures contained 275 µl of extracellular broth, 2 mM veratryl alcohol, 0.4 mM H_2O_2 and 0.1 mM sodium tartrate, pH 2.5 in a final volume of 0.5 ml. The reactions were started by H_2O_2 addition immediately after buffer was added and were monitored at 310 nm [7]. The extracellular broth from flask cultures was obtained by centrifuging the cultures at 10 000g for 5 min at 4°C.

Molecular identification of the potential bacterium

Molecular identification of the potential strain was referred using 16S rRNA gene sequence analysis using the bacterial universal primer set of Eubac 27F (5′- AGAG TTTG ATCM TGGC TCAG -3′) and 1492R (5′- GGTT ACCT TGTT ACGA CTT -3′). The PCR product was purified using the QIAGEN PCR purification kit for sequencing and sequenced on an ABI Prism 377 automatic sequencer (Applied Biosystems, CA, USA). Sequence homologies were examined using BLAST version 2.2.12 of the National Center for Biotechnology Information [8]. The evolutionary analysis was performed using the Maximum Likelihood method based on the Tamura-Nei model [9].

Optimization of fermentation conditions

The potential strain was standardized for optimal growth conditions, i.e., varying one parameter at a time. The fermentation process was carried out in a 3 L laboratory fermentor (Scigenics, India) with 2.1 L working volume using nutrient broth as the basal medium with the basal culture conditions of pH 8.0, 37°C temperature, 34ppt salinity, agitation at 150 rpm and aeration at 1.0 VVM. The inoculum was prepared with the exponential phase culture of the potential strain using nutrient broth where the optical density (OD 620nm) of the inoculum culture was adjusted to 0.1 as per the procedure of McFarland turbidity 0.5 standard which was equivalents to the bacterial concentration of 1×10^8 CFU/ml. At 48 hours for bacterial strains, broth medium were withdrawn and centrifuged at 2700 ×*g* for 15 min and the cell pellets were used for estimation of cell biomass on dry weight basis [10]. The growth parameters studied for the isolates were different pH conditions at 6, 7 and 8, different carboxy methyl cellulose concentration at 0.5%, 1%, 1.5%, 2 %and 2.5%, three different temperatures at 28°C, 37°C and 45°C and different concentration of tannic acid ranged within 0.5 – 2.5ml/L with the intervals of 0.5 ml/L was determined.

RESULTS AND DISCUSSION

Screening and identification for potential ligninase producing bacteria

The procured vegetable samples were serially diluted and spread plated on nutrient agar plates. After incubation, the plates have the total heterotrophic bacteria count ranged between 187×10^5 - 81×10^6 CFU. Further, the plates were observed for distinct morphological colonies which were isolated and pure cultured on fresh nutrient agar plates. There were 34 morphological distinct bacterial strains were obtained which were named as DGLB1 – 34. Among the isolates, the strain DGLB19 showed maximum activity of 77.7 units/ml ligninase enzyme. The potential strain was identified as based on 16S rRNA molecular identification and the phylogenetic position of the strain was examined by BLAST analysis. The results revealed that the strain belongs to the genus *Acinetobacter* of the family Moraxellaceae. Based on the sequence homology, the strain DGLB19 showed more than 100% similarity with the available sequences of NCBI database. The phylogenetic position of the strain DGLB19 is shown in the fig. 1. Following these comparisons, the strain DGLB19 was identified as *Acinetobacter beijerincki*. This strain was chosen for the further characterization based on its maximum enzyme activity. Similar to this investigation, an earlier study also isolated a wild type strain, *Phanerochaete chrysosporium* which also showed promising production of ligninase enzyme [11].

Growth characterization of *Acinetobacter beijerincki*

Four different growth conditions were chosen for the examination of enhanced growth of this strain. The important characteristic of most organisms is their strong dependence on the pH for cell growth. The isolated strain showed the highest growth at pH 7.0 with 2.296 g/L cell biomass (Fig. 2), further, the isolated strain showed lowest growth at acidic and basic conditions. Regarding optimization for determining optimum temperature, the strain showed a strong dependence on growth with varying temperature conditions and the highest growth was achieved in 37˚C with 3.03 g/L cell biomass (Fig. 3). In this investigation, different CMC

concentrations were studied for their enhanced growth of the potential isolate. The results showed that maximum growth was revealed at 2% CMC with 4.23 g/L cell biomass, followed by this, the strain showed 4.19 g/L cell biomass 2.5% CMC concentration (Fig. 4). Similarly, this study extend its characterization using different concentration of tannic acid, maximum growth of 4.57 g/L cell biomass was obtained using 0.75% tannic acid, next to this, 4.44 g/L cell biomass was achieved when 0.5% tannic acid was used (Fig. 5). Similar to this study, s *Ganoderma* howed similar growth characterization while optimizing its biotic and abiotic growth conditions [12]. The above observations proved the potential production of ligninase enzyme and its growth characterization could be used as a baseline data for many commercial applications.

on the 16S rRNA gene sequences. This analysis was done based on Maximum-likelihood method using MEGA 7 [9]. Numbers at nodes indicate levels of bootstrap support (%) and the black dot indicates the potential strain of this study.

Figure 1. Neighbor joining phylogenetic tree [8] was constructed for the potential strain *Acinetobacter beijerincki* DGLB19 with their closest NCBI (BLASTn) strains based.

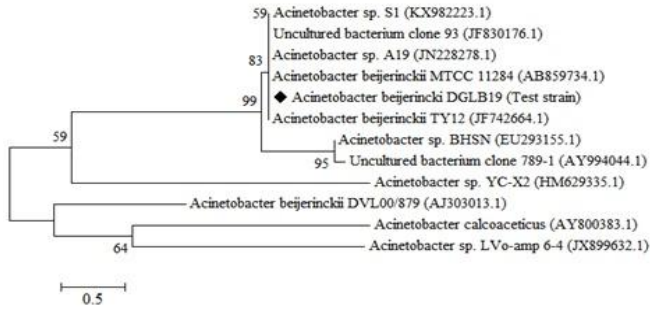


Figure 2. Growth characteristics of *A. beijerincki* at various pH conditions

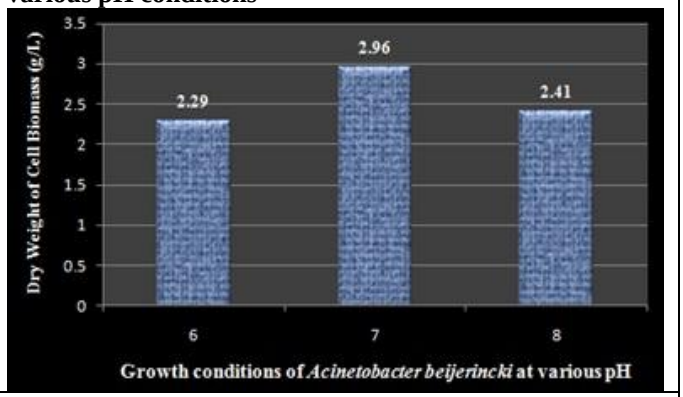


Figure 3. Growth characteristics of *A. beijerincki* at different temperature conditions

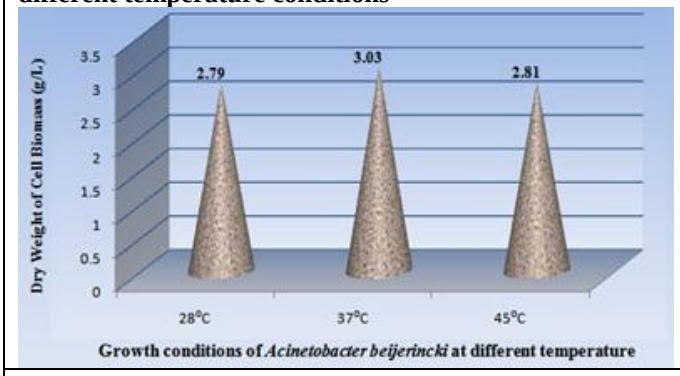


Figure 4. Growth characteristics of *A. beijerincki* at different carboxy methyl cellulose concentrations

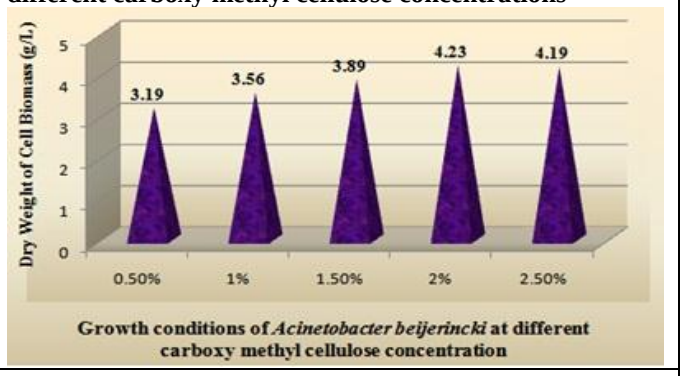
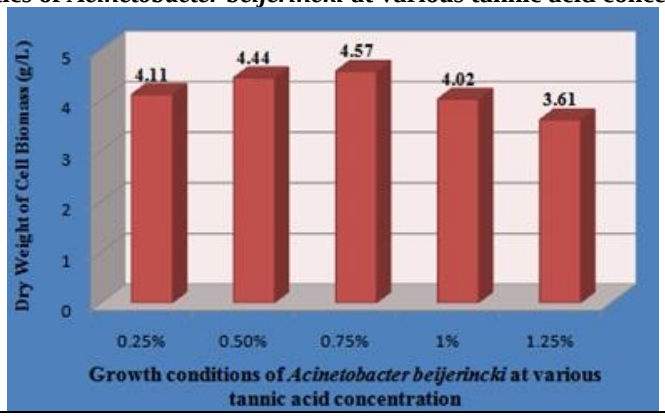


Figure 5. Growth characteristics of *Acinetobacter beijerincki* at various tannic acid concentrations



Acknowledgement: None

Conflict of interest: The authors declare that they have no conflict of interest

REFERENCES

1. Adaskaveg JE, Gilbertson RL. (1986). In vitro decay studies of selective delignification and simultaneous decay by the white rot fungi *Ganoderma lucidum* and *G. tsugae*. *Can J Bot*, 64, 1611–1619.
2. Asgher M, Sharif Y, Bhatti HN. (2010). Enhanced production of ligninolytic enzymes by *Ganoderma lucidum* IBL-06 using lignocellulosic agricultural wastes. *Int J Chem React Eng*, 8, 59–77
3. Blanchette RA. (1984). Selective delignification of eastern hemlock by *Ganoderma tsugae*. *Phytopathology*, 74, 153–160.
4. Hatakka A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiol Rev*, 13, 125–135.
5. Lin L, Lin ZW, Guo WN, Yang J, Wang SH. (2009a). Screening and cloning ligninolytic strain and cellulase gene expression. *Chin J Eco-Agr*, 17, 938–943.
6. Liu SX, Lai H. (2001). Advances of lignin-degrading enzyme in molecular biology. *J Chongqing Coll Educ*, 14, 64–67.
7. Kirk TK, Croan S, Tien M, Murtagh KE, Farrell RL. (1986). Production of multiple ligninases by *Phanerochaete chrysosporium*: effect of selected growth conditions and use of mutant strain. *Enzyme Microb Technol*, 8, 27–32.
8. Tamura K, Nei M, Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA), 101, 11030-11035.
9. Kumar S, Stecher G, Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870-1874.
10. Mani P, Dineshkumar G, Jayaseelan T, Deepalakshmi K, Kumar CG, Balan SS. (2016). Antimicrobial activities of a promising glycolipid biosurfactant from a novel marine *Staphylococcus saprophyticus* SBPS 15. 3 Biotech, 6, 163, http://dx.doi.org/10.1007/s13205-016-0478-7.
11. Reddy CA. (1993). An overview of the recent advances on the physiology and molecular biology of lignin peroxidases of *Phanerochaete chrysosporium*. *J Biotechnol*, 30, 91–107.
12. Zhang JP, Wang JW, Jiang JM, Yang WD. (2005). Efficient strains screening of ligninolytic *Ganoderma*. Forest Res (China), 18, 106–108.