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Microbial cellulases – Diversity & biotechnology with reference to mangrove environment: A review

B.C. Behera [a], B.K. Sethi [b], R.R. Mishra [b], S.K. Dutta [c], H.N. Thatoi [a,*]

[a] *Department of Biotechnology, North Orissa University, Baripada 757003, Odisha, India*
[b] *Department of Biotechnology, MITS School of Biotechnology, Bhubaneswar 751024, India*
[c] *Department of Zoology, North Orissa University, Baripada 757003, Odisha, India*

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Abstract Cellulose is an abundant natural biopolymer on earth, found as a major constituent of plant cell wall in lignocellulosic form. Unlike other compounds cellulose is not easily soluble in water hence enzymatic conversion of cellulose has become a key technology for biodegradation of lignocellulosic materials. Microorganisms such as aerobic bacteria, fungi, yeast and actinomycetes produce cellulase that degrade cellulose by hydrolysing the β-1, 4-glycosidic linkages of cellulose. In contrast to aerobic bacteria, anaerobic bacteria lack the ability to effectively penetrate into the cellulosic material which leads to the development of complexed cellulase systems called cellulosome. Among the different environments, the sediments of mangrove forests are suitable for exploring cellulose degrading microorganisms because of continuous input of cellulosic carbon in the form of litter which then acts as a substrate for decomposition by microbe. Understanding the importance of cellulase, the present article overviews the diversity of cellulolytic microbes from different mangrove environments around the world. The molecular mechanism related to cellulase gene regulation, expression and various biotechnological application of cellulase is also discussed.

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* Corresponding author.
E-mail address: thatoinou@gmail.com (H.N. Thatoi).

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1. Introduction

Cellulose is the most abundant component of plant biomass on the earth [1]. In the terrestrial environments, it is the primary product of photosynthesis and the most abundant renewable bio-resource (100 billions dry tons/year) produced in the biosphere [2]. Although produced by some animals (e.g. tunicates) and few bacteria, it is found exclusively in all plant cell walls. Cellulose is present in pure state, in most cases and primarily associated with hemicelluloses and lignin. It comprises about 35–50% of plant dry weight while hemicelluloses and lignin comprise 20–35% and 5–30% of plant dry weight respectively. Cellulose is a linear polysaccharide composed of monomers of the glucose unit, bound together by β-1, 4-glycosidic linkage.

Mangrove communities are highly ecosystem which provides large quantities of organic matter to the adjacent coastal water in the form of detritus. Hence it is rich in energy and contains a large active microbial population both attached and living free [3]. Roughly 30–50% of the organic matters in mangrove leaves are leachable, water-soluble compounds, such as tannins and sugars, the remaining fraction of the organic matter presumably consists of plant structural polymers commonly referred to as lignocelluloses [4]. As cellulose is not soluble like other substance, bacterial and fungal degradation occurs exocellularly and the products of cellulose hydrolysis are available as carbon and energy sources for other microbes that inhabit in these environments. Mangrove is composed of thick organic matter mixed with sediment hence it is anaerobic except the sediment surface. In such anaerobic environments, decomposition of cellulose such as mangrove leaves and woods are brought about by complex communities of interacting microorganisms by hydrolysing the β-1, 4-glycosidic linkages of cellulose [5]. Biodegradation of β-1, 4-glycosidic linkages of cellulosic biomass is usually done by enzymes such as cellulase and cellulosome, produced by numerous microorganisms. Decomposition of cellulose produces large quantity of detritus thus makes a mangrove a sink of organic matter. Flow of carbon from this fixed cellulosic sinks to atmospheric CO_2 is very important for waste treatment processes. Thus, cellulose availability forms the basis of many microbial interactions that occurs in the anaerobic environments like mangroves [6] and the cellulase enzyme produced by these microorganisms that degrade cellulose have attracted much interest because of the diversity of their application. The major industrial application of cellulases are biopolishing, bio-stoning, bio-finishing, etc. in textile industry, starch processing, grain alcohol fermentation, malting and brewing in beer and wine industry, extraction and processing of fruit and vegetable juices, etc. in food industry, in pulp and paper industry [7], controlling plant pathogen and disease in agriculture, as well as in house hold laundry detergents for improving fabrics softness and brightness, etc. [8]. Considering the importance of bioconversion of cellulosic biomass and role of cellulases in this bioconversion process, the present review highlights the diversity of cellulose degrading microorganisms from mangrove environment and their mode of action in degrading cellulosic biomass. Further the review also addresses genetics and the molecular mechanisms involved in cellulase gene expression along with various biotechnological applications of cellulase enzyme.

2. Screening methods for cellulase producing microorganisms

Cellulose degrading microorganisms from soil are generally isolated on CMC agar medium [9] containing: (g/l): Carboxymethylcellulose (CMC), 10; KH_2PO_4, 4; Na_2HPO_4, 4; Tryptone, 2; $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.001; $FeSO_4{\cdot}7H_2O$, 0.004; Agar,15 and pH adjusted to 7.0. The plates are incubated at 37 °C for 24–48 h. Zone of hydrolysis are visualised by flooding the plates with 0.1% Congo red solution and washed the plate with 1 M NaCl.

Another method is also available for screening of cellulose degrading microorganisms, in which strains are grown on ISP-4 media (carboxymethyl cellulose-10 g, K_2HPO_4-10 g, $MgSO_4$-10 g, NaCl-3 or 4%, $CaCO_3$-a pinch, $(NH_4)_2SO_4$-2 g, $MnCl_2$-1 mg, $ZnSO_4$-1 mg, $FeSO_4$-1 mg, agar-20 g) for 3–4 days and then flooded with 5 ml of 1.0% iodine solution along with 1 ml mercuric iodide. Colony produced clear yellow zones are considered to be cellulase positive [10]. Carboxymethyl cellulose hydrolysis capacity (HC value) of the isolates can be estimated by calculating the ratio of diameter of clearing zone and colony following the method of Lu et al. [11].