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Isolation and characterization of cellulose hydrolyzing bacteria for bioethanol production

B. M. Chandrarathne*

Department of Chemistry, Faculty of Science, University of Kelaniya, Sri Lanka
*chandraratne.bhagya@gmail.com

Bioethanol is a renewable and cleaner liquid fuel alternative to fossil fuels. Bioethanol has gained growing interest over time, because it can provide an economical and environment friendly sustainable energy source. First generation bioethanol production used simple sugars produced by the sugar crops. However, utilization of sugar crops leads to a competition between food supply and energy production. Therefore, second generation bioethanol production has evolved, which uses cellulosic materials from agricultural biomass instead of sugar crops. However, cellulosic materials have to be hydrolysed prior to the fermentation. Enzymatic hydrolysis of cellulose into reducing sugars is catalysed by cellulases. This enzyme is produced by cellulose hydrolysing microorganisms. Therefore, the main objective of this study was to isolate and characterize cellulose hydrolysing bacteria that can be used in bioethanol production. Soil samples were collected from compost sites and from the top of the mat of fibrous roots at the center of the Bird's nest ferns (*Asplenium nidus*). Bacterial strains were isolated from soil, using basal salt medium with filter papers as the only source of cellulose carbon. Streak plate technique was used to isolate single bacterial colonies. Isolated bacterial strain was characterized by colony morphology and Gram staining. Functional characterization was done by calculating the ratio between diameter of the clear zone and the diameter of bacterial colony in Congo red agar medium (cellulolytic index). Using DNS assay, the amount of reducing sugars generated during cellulosic material hydrolysis by bacteria-derived cellulase enzyme was measured. Co-culture system, which included isolated bacterial strain and dry baker yeast was optimized to ferment those reducing sugars to bioethanol. Production of bioethanol was measured by an Ebullio meter. Isolated bacterial strain produced 1.37 mg mL⁻¹ concentration of reducing sugars and 23.67 mg mL⁻¹ concentration of bioethanol. Further research is in progress to optimize isolated bacterial strain in bioethanol production.

Keywords: Cellulose hydrolyzing bacteria, Cellulases