CHARACTERIZATION OF THERMOPHILIC ANAEROBIC CELLULOLYTIC BACTERIA & THEIR CELLULASES

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Palabras clave: thermophilic anaerobes, cellulolytic bacteria, cellulases

Introduction. Anaerobic cellulose degradation is a complex process. Although only 5-10% of the cellulose is degraded under anaerobic conditions, it plays a key role in the carbon cycle and is one of the hall marks of evolution. Of the anaerobic cellulolytic bacteria, thermophilic bacteria have gained importance due to their potential industrial applications *viz*., production of liquid fuels, organic acids etc. However, very little attention is paid to study the diversity of microbes from various habitats, their cellulase complex and role in bioconversion[1].
In this paper, Characterization of thermophilic anaerobic bacterial strains isolated from three different habitats and their enzyme systems are discussed.

Methodology. Cellulolytic anaerobes were isolated from anaerobic digester fed with animal manure slurry, compost beds and marine sediments using modified Hungate´s medium, following roll tube technique[2] and under incubation at 55°C. Purified isolates were characterized by following standard biochemical tests and G+C content of DNA. Growth and enzyme activities[3] on different substrates, *viz*., crystalline cellulose (CC), avicel, cotton, filter paper (FP), swollen cellulose (SC), precipitated cellulose (PC) carboxymethylcellulose (CMC), cellobiose and glucose were studied. Extracellular and cell bound enzyme complex of the isolates were fractionated and purified using the Fast Performance Liquid Chromatography (Pharmacia LKB, Uppsala, Sweden) with Superose-6 column.

Results & discussion. Three strains, one from each habitat showing higher growth and activity was identified as *Clostridium thermocopriae* (digester slurry), *Clostridium* strain NC (compost beds) and *Clostridium* strain NMS (marine sediments). Strain NC recorded the maximum growth with insoluble substrates, modified cellulosics and soluble substrates (Fig. 1). In case of enzyme activities, endo- and exo- glucanase activities of all the three strains showed an increase until 10th day of growth, whereas increased cellobiase activity was recorded beyond that period of growth. Between strains, strain NMS recorded higher endoglucanase activity and *C.thermocopriae* showed maximum exoglucanase (34.8 U) and cellobiase (38.2 U) activities. Among the different substrates tested, modified cellulosics *viz*., swollen- and precipitated- cellulose recorded maximum endo (29.3 U) and exoglucanase (34.8 U) activities respectively for *C.thermocopriae.* It was further observed in this study that sporulation reduced the endo- and exo- glucanase activities of all the strains. Similarly, the presence of cellobiose at 1.7 mM level repressed both endo- and exo- glucanase activities, but was derepressed after 10 d of growth. Maximum endoglucanase activity was recorded at a pH of 7.0 for *C.thermocopriae* and pH 7.5 for strains NC and NMS. The optimum temperature was found to be 60°C for both endo- and exo- glucanase activities for all the three strains. Acetic acid was the major product of cellulose degradation by all strains (88.5%) and the rate of production by strain NMS was 0.3 $g.g^{-1}$ of substrate degraded.

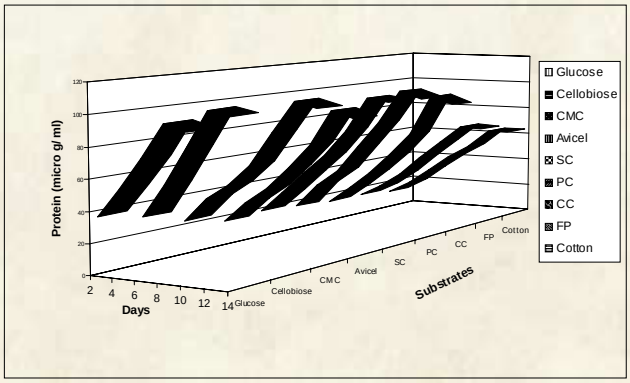

Fig. 1. Growth of Clostridium strain (NC) on different substrates

Cell bound fractions of all the three strains grown on insoluble substrates showed higher endo- and exo-glucanase activities than extracellular fractions. Both extracellular and cell bound fractions of cellobiose grown cells of all strains showed the presence of high molecular weight protein (2 x 10^6 Da), with different enzyme activities. The high MW proteins present in extracellular fractions showed higher cellobiase activity, whereas cell bound fractions showed high endo- and exo- glucanase activities. The maximum endo- and exo- glucanase activities were restricted to low molecular weight proteins (30-37 kDa) when the cells were grown on insoluble substrates and high MW proteins (>2 x 10^6) when grown on soluble substrates. Presence of high MW proteins represented the organization of the enzyme complex as cellulosomes[4]

Conclusions. Insoluble substrates elicited higher exo- and endo- glucanase activities in all strains tested and the presence of cellobiose repressed the activity of these enzymes. Physico-chemical parameters for obtaining higher enzyme activities were optimized. Higher production of acetic acid by the strain NMS showed their potential for bioconversion of solid residues rich in cellulose.

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