ISOLATION OF ENDPOLYGALACTURONASE HYPERPRODUCING MUTANTS OF ASPERGILLUS SP. CH-Y-1043

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SUMMARY

With the aim of obtaining hyperproducing strains of pectinases, Aspergillus sp. CH-Y-1043 was mutated with NTG and mutants resistant to glycerol catabolic repression were selected. Among the mutants obtained, CH-SS/M63 produced an endo-PG activity 400% higher than the wild type, using lemon peel as the sole carbon source.

INTRODUCTION

Microbial pectinases, especially the endo-type, play an important role within food industry since they favour the extraction, clarification and reduction in viscosity of fruit juices[1,2]. Production of these enzymes in most microorganisms is limited by mechanisms which regulate their synthesis; most pectinases are induced by pectin and are subjects to repression due to the presence of repressor substances or of products associated with the degradation of pectin as reported for polygalacturonase of A. niger[26,4], of Pyrenochaeta terrestris[5] and polygalacturonate lyase of Aeromonas liquefaciens[6]. Presently, the microbial synthesis of enzymes at industrial level requires highly productive strains to reduce production costs. The use of microorganisms resistant to catabolic repression could therefore diminish enzyme repression and thus increase production yields in microbial fermentation. Extracellular enzymes such as cellulases[7], invertases[10], amylases and proteases[12], are produced on a major scale by hyperproducing mutants obtained through selection methods. Some of them are resistant to catabolic repression[7,12]. Considering the increasing demand of pectinases for industrial processes, it is surprising to find relatively few studies on the isolation of hyperproducing mutants to improve production of these enzymes. As far as practical applications go, fungi are preferred in the production of industrial pectinases, since these may be excreted into the culture medium in as much as 90%[14]. Mutagenesis of Penicillium 7/4B with ethyleneimine and UV light produced a 2-fold increase in polygalacturonase activity and
a 0.5 fold increase in pectinesterase activity\textsuperscript{16}; in \textit{Rhizopus sp.}, pectinolytic activity was increased 3 times\textsuperscript{15} and 2.5 times in \textit{A. niger} \textsuperscript{36}18. However, as far as we know, hyperproducing mutants are not used for industrial production of pectinases.

The strain \textit{Aspergillus sp.} CH-Y-1043 is a wild fungus isolated in our laboratory\textsuperscript{19}, which shows few nutritional requirements and produces extracellular pectinases at 37°C on pectin or on agroindustrial waste products containing pectin as the sole carbon source\textsuperscript{19,22}. The activity of its enzymatic filtrates in the clarification of apple juice is comparable to that of some commercial products. Enzyme production in this fungus is subject to catabolic repression by the degradation products of pectin\textsuperscript{23}.

Because of the practical applicability of pectinases produced by \textit{Aspergillus sp.} CH-Y-1043, mutagenesis with NTG was performed to obtain hyperproducing mutants resistant to catabolic repression.

**MATERIALS AND METHODS**

**Microorganism.** \textit{Aspergillus sp.} CH-Y-1043 was previously isolated in our laboratory\textsuperscript{19}. Stock cultures were grown on potato-dextrose-agar slants at 37°C and kept at 4°C.

**Basal Media.** It contained (°/o) (NH\textsubscript{4})\textsubscript{2} SO\textsubscript{4} 0.2, KH\textsubscript{2}PO\textsubscript{4} 0.2, K\textsubscript{2}HPO\textsubscript{4} 0.2 and was supplemented with 1°/o pectin (medium A), 1°/o pectin plus 1°/o galacturonic acid (medium D), 1°/o pectin plus 1°/o glucose (medium B), or 1°/o pectin plus 1°/o glycerol (medium C) all added with 2.0°/o agar. The initial pH for all media was 4.5.

**Medium E.** To detect clearance zones produced by the strains, a medium containing 0.25°/o pectin, 2.0°/o agar and 250 µg/ml cycloheximide was used.

**Medium CP.** The complete medium contained 2.0°/o glucose, 1.0°/o yeast extract and 2°/o agar.

**Screening method.** The wild type spores were spread on PDA plates. Once single colonies had appeared after 72h incubation at 37°C, the colonies were streaked on medium A. The colonies grown after 72h incubation at 37°C were cut out with a cork borer (3 mm) and placed in petri dishes containing medium E and incubated for 48h at 37°C. After this time 2°/o iodine solution was added to detect clearance zones (SCHEME 1).

**Mutant isolation.** Mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) was carried out according to Martinelli and Clutterbuck\textsuperscript{26}. NTG (1mg/ml) was added to suspension of spores with optical density of 0.2 at 540nm in 0.1M