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# PRODUCTION OF CELLULASE AND β-GLUCOSIDASE ACTIVITIES FOLLOWING GROWTH OF *STREPTOMYCES HYGROSCOPICUS* ON CELLULOSE CONTAINING MEDIA.

L. Spear, J. Gallagher, L. McHale & A.P.McHale.
Dept. of Biological and Biomedical Sciences,
University of Ulster,
Coleraine, Co. Londerry, Northern Ireland.

## ABSTRACT

The actinomycete, *Streptomyces hygroscopicus* was shown to be capable of producing extracellular cellulase and cell associated β-glucosidase activity during growth on cellulose containing media. Cell homogenates were shown to contain a β-glucosidase fraction which was stable for up to 24h. at 30°C and had half-lives of 480min. and 220min. at 40 and 50°C, respectively. The enzyme fraction was also shown to be optimally active at pH 4.0 suggesting that it might represent a suitable supplement for fungal cellulase systems, deficient in β-glucosidase activity.

## INTRODUCTION

To date, a large variety of microorganisms have been shown to produce enzymatic systems capable of degrading cellulosic materials (Wood, 1992). In comparison with studies on fungal cellulase systems, relatively little is currently known about cellulase systems produced by actinomycetes. Many members of this group of microorganisms grow in mycelial form and as such may be viewed as being relatively invasive, similar in many ways to filamentous fungi. The advantages associated with this form of growth, particularly with respect to increasing available substrate area on solid/semi-solid substrates such as cellulosics and chitins are abundantly clear (Irhuma *et al.*, 1991;).

In a recent study in our laboratory it was demonstrated that the actinomycete *Streptomyces hygroscopicus* had the ability to produce chitinase activity during growth on chitin containing media (Irhuma *et al.*, 1991). During that study it was coincidentally discovered that the organism had the ability to grow on filter paper, dampened with salt solutions. Here we report on the production of an extracellular cellulase system by the mesophilic actinomycete, *S. hygroscopicus*. We also report on the partial characterisation of a cell associated β-glucosidase fraction produced by that organism, which is capable of functioning at a relatively low pH. Since a lack of β-glucosidase in fungal cellulase systems has been reported to be rate-limiting with respect to cellulose degradation (Sternberg, 1976; Shewale, 1982; Stoppok *et al.*, 1982), the *S. hygroscopicus* β-glucosidase may prove to be a suitable enzyme supplement to those systems as a result of its fortuitous enzymatic properties.

## MATERIALS AND METHODS

*Organism:* *Streptomyces hygroscopicus* 9628, obtained from the National Collection of Industrial and Marine Bacteria (UK) was maintained on nutrient agar plates at 30°C. 1 $cm^2$ portions were utilised to

inoculate flasks containing 50ml aliquots of nutrient broth, supplemented with 2% (w/v) Solka Floc (BW40; purified ball-milled spruce cellulose from Brown & Co., Berlin, NH, U.S.A.). Flasks were incubated at 30°C in an orbital shaker at 150rpm for times indicated.

*Preparation of enzyme extract:* Cells were harvested and washed in 0.2M sodium phosphate buffer. pH 7.0 by centrifugation at 10,000 x g for 30min. at 4°C. The cell pellet was frozen to -70°C followed by rapid thawing (x3) and finally ground using a pestle and mortal. The ground material was then resuspended in 0.2M sodium phosphate buffer, pH 5.0 and centrifuged at 20,000 x g for 40 min. at 4°C. The crude extract could be stored at - 70°C for extended periods of time without significant loss of activity.

*Cellulase assay:* Cellulase activity was determined by measuring the release of reducing sugars from filter paper as described previously by McHale & Coughlan (1980), except that assays were performed at 40°C.

*β-glucosidase assay:* Activity was determined by spectrophotometrically measuring the release of p-nitrophenol from the substrate, p-nitrophenyl-β-D-glucoside (Sigma, UK) at 430nm. The assays were carried out in 5ml reaction volumes containing substrate, 0.2M sodium phosphate buffer, pH 5.0 and enzyme. These were incubated at 40°C for the required time and reactions were terminated by the addition of 2mls of 1M glycine/NaOH, pH 10.0.

## RESULTS AND DISCUSSION

*Production of cellulase and β-glucosidase following growth of S. hygroscopicus on cellulose containing media:* As stated above it had previously been determined that the actinomycete, S. hygroscopicus had the ability to grow on filter paper which had simply been moistened in salt solutions (data not shown). In order to determine whether or not the organism was capable of producing a cellulase system, it was decided to culture the organism on cellulose containing media and to assay culture filtrates for both extracellular cellulase and β-glucosidase activities. It was found that, while significant amounts of extracellular cellulase activity could be detected, the amount of extracellular β-glucosidase activity was extremely low (Fig.1). Production of cellulase was found to reach a maximum following 75h growth, at which time the amount of β-glucosidase activity was almost un-detectable.

Since only low amounts of β-glucosidase were detected in the extracellular culture filtrates, it was decided to determine whether or not more significant quantities of that activity were associated with the cells. To this end samples of cells were taken from the fermentations at various stages of growth and homogenized. The extracts from the disrupted cells were assayed for β-glucosidase activity and the results obtained are shown in Fig.1. It was found that a very large proportion of the activity produced during growth was cell associated, with maximum levels being produced within 75h growth. This coincided with production of maximum amounts of overall cellulase activity in the extracellular culture filtrates.

Previous studies have demonstrated that β-glucosidase activity produced by a variety of *Streptomyces* strains appears to be cell associated (Moldoveanum & Kluepfel, 1983; Mihoc & Kluepfel, 1990). It has been reported that, while the action of extracellular fungal cellulases on cellulose results in the production of large quantities of glucose (McHale & Coughlan, 1982) the major end product resulting from the action of bacterial cellulases on cellulose is the disaccharide cellobiose (Katayeva *et al.*, 1992).

The virtual absence of extracellular β-glucosidase activity following growth of *S. hygroscopicus* on cellulose containing media, suggests a mechanism for assimilation of cellulose which may involve the initial extracellular conversion to cellobiose, followed by a cell associated conversion of that cellobiose to glucose. Whether or not the β-glucosidase activity produced by this organism is associted with the cell internally or attached to the cell wall externally has yet to be determined.

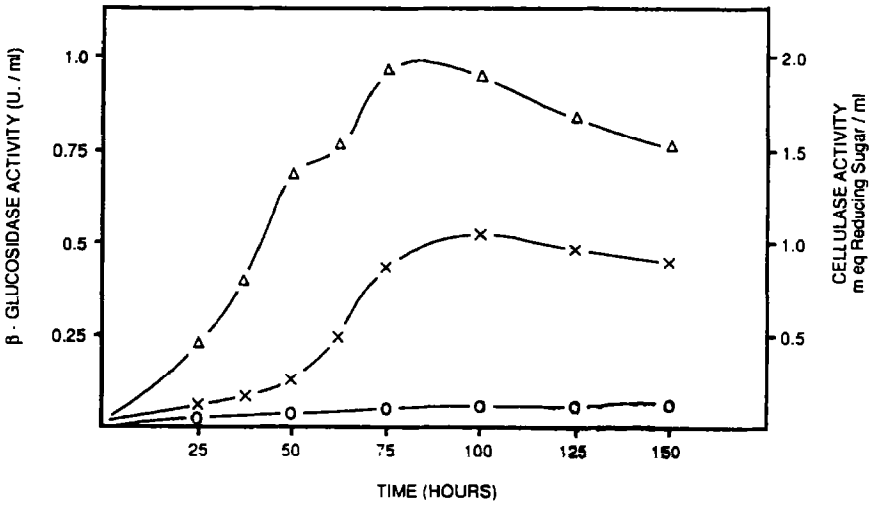


Fig. 1 Production of cellulase and β-glucosidase activities during growth of *S. hygroscopicus* on cellulose containing media. Assays for cellulase (X-X), extracellular β-glucosidase (o-o) and intracellular β-glucosidase (Δ-Δ) activities were carried out as described in the Methods section.

Partial characterization of the cell-associated β-glucosidase activity produced by S. hygroscopicus following growth on cellulose containing media: Since it was previously reported that the addition of exogenous β-glucosidase to fungal cellulase systems would reduce inhibition of endo- and exoglucanases by cellobiose and thereby enhance overall cellulose degradation (Stemberg, 1976), it was decided to further characterise the enzyme fraction produced by *S. hygroscopicus*. Properties such as pH optimum, temperature optimum and thermal stability of the enzyme fraction produced by *S. hygroscopicus* were examined. The results are shown in Table.1. The enzyme was shown to have a temperature optimum of 45-50°C. In comparison with similar enzymes produced by other *Streptomyces* species including *Streptomyces sp.* CB-12, *S. lividans* and *S. flavogriseus* , this operating temperature is considerably higher (Moldoveanu & Kluepfel, 1983; Michoc & Kluepfel, 1990). Indeed it is more comparable with operating temperatures of fungal cellulases and β-glucosidases (McHale & Coughlan, 1982; Berg & Pettersson, 1977).

TABLE 1: Enzymatic properties of a cell associated β-glucosidase produced by *S. hygroscopicus*

| PROPERTY | |
|---|---|
| Temp. Optimum | 45 -50°C |
| $T_{1/2}$ @ 30°C | Stable up to 24 h. |
| $T_{1/2}$ @ 40°C | 480 (min.) |
| $T_{1/2}$ @ 50°C | 220 (min) |
| pH Optimum | 4.0 |

As shown in Table 1, the $T_{1/2}$ at 50°C of 220 min. demonstrates that the enzyme is more stable than that produced by organisms such as *Cellumonas uda* (Stoppok *et al.*, 1982), *S. flavogriseus* and *Streptomyces sp.* CB-12 (Moldoveanu & Kleupfel, 1983). With respect to many β-glucosidases produced by both bacteria and fungi, the enzyme produced by *S. hygroscopicus* was unique in that it had an optimum operating pH of 4.0.

As a result of the above described, fortuitous properties, it is believed that the *S. hygroscopicus* β-glucosidase may represent a suitable candidate for supplementation of fungal cellulase systems found to be lacking in that component, thereby increasing the overall efficiency of conversion of cellulose to glucose.

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