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Short Communication: Effect of water activity on cell growth and phenol oxidase production by *Streptomyces cyaneus* var. *viridochromogenes* in surface culture. *World Journal of Microbiology & Biotechnology* 12, 1, pp. 101–2.

Below we have reprinted a corrected version of pages 101 and 102 of the above manuscript. We apologise that the original pages were printed incorrectly. The microorganism used in the short communication is a bacteria (actinomycetes) rather than a fungus as mentioned in the text. The text has been corrected accordingly.

Short Communication: Effect of water activity on cell growth and phenol oxidase production by *Streptomyces cyaneus* var. *viridochromogenes* in surface culture

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In surface cultures of *Streptomyces cyaneus* var. *viridochromogenes*, NaCl depressed water activity (a_w) without supporting growth. Reducing a_w from 0.987 to 0.951 led to 3- and 4-fold increases in intracellular and extracellular phenol oxidase activities, respectively.

Key words: Phenol oxidase, *Streptomyces cyaneus*, surface culture.

Lignin-related enzymes such as phenol oxidase and peroxidase could be used to detoxify paper-mill effluents. In order to scale-up the production of phenol oxidase for this and other biotechnological processes, the present study, on the effect of water activity (a_w) on the cell growth and enzyme production of *Streptomyces cyaneus* var. *viridochromogenes* in surface culture, was carried out. The strain used produces significant phenol oxidase activity in solid-state fermentations (SSF) (Berrocal *et al.* 1993). Although water activity (a_w) is known to be one of the main factors affecting cell growth and enzyme production in SSF, there have been very few reports of its effects on surface cultures (Acuña-Argüelles *et al.* 1994).

Materials and Methods

Microorganism and Media

Streptomyces cyaneus var. *viridochromogenes* was grown on mineral salt medium (Crawford 1978) supplemented with 3 g yeast extract, 1 ml trace-element solution, 15 g agar and 40 g (40-mesh) wheat straw/l. NaCl (at 8.7, 35 or 70 g/l), glycerol (at 26, 101 or 202 g/l) or sucrose (at 92, 342 or 649 g/l) was added as a depressor of water activity, to give a_w values, measured over 2 h at 28°C with a meter (Aw30001; Ebro), of 0.91 to 0.985. The inoculum (10^7 c.f.u./ml) was produced by adding 5 ml 0.01% (w/v) Tween-80 to cultures grown for 6 days at 28°C on plates of Bennett medium.

Cell Growth

Cell growth was measured as colony area and as dry wt. Inoculum

(20 μ l) was added to 25 ml culture medium in a Petri dish and incubated at 28°C. Colony area was measured on days 1 and 5 and the difference in the two values was taken as the colony-area increment. To measure dry wt, a disc of Cellophane was placed over the medium in each of several, similar dishes of culture medium before 100 μ l inoculum were placed on each disc. After 6 days' incubation at 28°C, the mycelium was removed from each disc, dried on filter paper overnight at 60°C and then weighed.

Phenol Oxidase

Samples of fresh mycelium in 5 ml distilled water with 5 μ l 0.01% (w/v) Tween-80 were stirred for 1 h at 28°C. Extracellular enzyme activity was determined by assaying the supernatant produced by centrifuging this suspension for 10 min at $21,000 \times g$. Mycelial samples were also washed three times with distilled water, centrifuged ($21,000 \times g$, 10 min) and the pellets each resuspended in 2 ml distilled water and disrupted ultrasonically. After centrifuging the sonicate ($28,000 \times g$, 35 min), intracellular activity was determined by assaying the supernatant.

Phenol oxidase activities were determined in the supernatants using 5 mM 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) in 0.2 M acetate buffer, pH 5.6, A_{436} values being read after incubation for 3 min at 60°C (Werner *et al.* 1970). One unit of activity was defined as that required to liberate 1 μ mol product per min. Total activities were converted to specific activities (U/mg protein) after determining protein in the supernatants by the Bradford method, with BSA as standard. Phenol oxidase activity on the agar plates used to determine colony weights, after mycelium and cellophane were removed, was detected qualitatively by pouring a mix of guaiacol, α -naftol and 0.1 M ABTS in 96% ethanol over the agar (Stalper 1978).

Results

Cell Growth

The maximum increment in colony area was observed when glycerol was used to depress a_w to 0.951. Although

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Figure 1. Effect of water activity on colony dry wt (■, □) and intracellular (●, ○) and extracellular (▲, △) phenol oxidase activity of *Streptomyces cyaneus*, after using sucrose (■, ●, ▲) or NaCl (□, ○, △) as water-activity depressors.

NaCl and sucrose gave smaller but similar increments, it was obvious that colony densities with NaCl were less than those with sucrose. The dry weights of colonies grown with sucrose or NaCl were therefore determined (Figure 1). Colonies grown with NaCl decreased in dry wt as a_w decreased. In contrast, the colonies grown with sucrose had higher dry wt at an a_w of 0.967 than at one of 0.985, although dry weights then fell as a_w was reduced further.

Phenol Oxidase

Intra- and extra-cellular, phenol oxidase activities were only determined after using NaCl or sucrose to depress a_w . The two depressors had similar effects on the activities (Figure 1), both intra- and extra-cellular activities increasing as a_w decreased from 0.987 to 0.951 (by 3- and 4-fold, respectively). Reducing a_w further caused a sharp reduction in both activities. In submerged, shake-flask cultures, in contrast, extracellular, phenol oxidase activity increased 3-fold as NaCl concentration was increased from 0% to 7% (w/v) (data not shown). Attempts to demonstrate phenol oxidase activity on agar plates were largely unsuccessful; positive results were only obtained with an a_w of 0.951.

Discussion

The wide colonies obtained with glycerol and the relatively dense colonies generally obtained with sucrose probably reflect use of these two depressors by the actinomycetes, as carbon sources (see Arias & Hardisson 1982). The effect of water-activity depressors on the growth of cells of some microorganisms has already been described (Marshall et al. 1971). In the present study, the a_w giving maximum phenol oxidase activities did not correspond to that giving maximum cell growth. Similar results were obtained for the production of cellulase activity by *Trichoderma reesei* QM9414 and *Sporotrichum cellulosophilum* growing on wheat

bran (Kim et al. 1985). However, cell growth of and glucoamylase production by *Aspergillus niger* seem to be similarly correlated to a_w (Pandey et al. 1994). Troller & Stinson (1978) noted that decreasing a_w also had a varied effect on the production of enzyme activities by bacteria, some activities increasing and some decreasing.

The limited success with demonstrating phenol oxidase activity in the agar plates probably indicates that much of the extra-cellular activity produced by the fungus in surface culture is associated with the cell wall and little diffuses through the cellophane into the agar. The only positive plates were those with optimal a_w for enzyme production.

The experimental methodology used in the present study may prove useful in the study of phenol oxidase from other bacteria and fungi and of other enzymes.

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