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Short Communication: Isolation of mutants of *Penicillium purpurogenum* with enhanced xylanase and β-xylosidase production

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***Penicillium purpurogenum* was mutated with u.v. light to increase xylanase production. The best mutant, UV-64, was treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and a second generation of mutants was obtained (NG-188 and NG-737). NG-737 produced 125 U of xylanase/ml when grown on oat spelts xylan supplemented with wheat bran compared with 69 U/ml for the wild-type strain. The mutants also showed a 2.2-fold increase in β-xylosidase as compared with the wild-type.**

Key words: mutants, *Pencillium purpurogenum*, xylanase, β-xylosidase.

Xylanases are a set of enzymes responsible for xylan biodegradation. Due to their industrial potential, much investigation has been directed towards obtaining sources of highly active enzymes, among them the isolation of hyperxylanolytic mutants of fungi and bacteria (Singh *et al*. 1995). This paper describes the isolation of mutants with improved endoxylanase and β-xylosidase activities from a native strain of *Penicillium purpurogenum*.

Materials and Methods

Microorganism
The *Penicillium purpurogenum* strain isolated from soil has been described by Steiner *et al*. (1994).

Production of enzymes
Erlenmeyer flasks (250 ml) containing 100 ml of Mandels' medium (Mandels & Weber 1969) plus carbon sources at 1% (wheat straw, oat spelts xylan, birchwood xylan or wheat bran) were inoculated with 1×10^7 spores/ml. Flasks were shaken at 200 rev/min at 28 °C for 10 days. Samples were taken periodically, centrifuged and the supernatants kept at −20 °C until analysed.

Analytical methods
Xylanase and β-xylosidase were estimated as described by Belancic *et al*. 1995. Endoglucanase, β-glucosidase and filter-paper-hydrolysing activity were assayed as in Steiner *et al*. (1994). Acetyl-esterase was estimated according to Poutanen & Sundberg (1988). One unit of enzyme activity was defined as the amount of enzyme which releases 1 μmol of product/min.

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Mutagenesis
P. purpurogenum was grown for 7 days on potato dextrose agar (PDA) plates at 28 °C. Spores, 2×10^7 ml, were suspended in 0.05 M acetate buer pH 5.5 and exposed for 75 s to short wave u.v. light at a distance of 18 cm (to obtain a 1% survival). Irradiated spores were plated on PDA containing 0.1% Triton X-100 as colony growth restrictor (PDA-T medium) and incubated for 4 days at 28 °C. UV mutants were exposed to N-methyl-N′-nitro-N-nitrosoguanidine (NG). Spores, 2×10^7/ml, were suspended in the buer described above and treated for 2 h with NG (400 mg/ml final concentration) (to obtain a 5% survival). After mutation, spores were diluted in buer, plated on PDA-T and incubated for 4 days at 28 °C.

Selection Media and Screening of Mutants
Mutant colonies obtained on PDA-T were transferred to a medium containing Mandels' solution (Mandels & Weber 1969), 2% oat spelts xylan, 0.1% Triton X-100 and 2% agar, (MXT medium). MXT plates were inoculated in duplicate with six colonies (five mutants plus the parental strain) and incubated for 60 h at 28 °C. One plate was stained with Congo Red (Bartley *et al*. 1984) and the hydrolysis halo diameter/colony diameter ratio (HD/CD) was measured. The duplicate plate was kept at 4 °C.

Results and Discussion

Approximately 550 colonies were screened after mutagenesis with u.v. light. Colonies with the highest HD/CD ratios were chosen. The selected mutants were grown in Mandels' media with 1% wheat straw as carbon source. Best activity was obtained with mutant UV-64 that produced 67.8 U xylanase/ml after 5 days of culture compared with 37.7 U/ml at day 7 for the wild-type strain. UV-64 maintained its high activity after repeated transfers, so it was subjected to further mutation using NG. 800 colonies were screened and two mutants with the best HD/CD ratios were selected: NG-188 and NG-737.

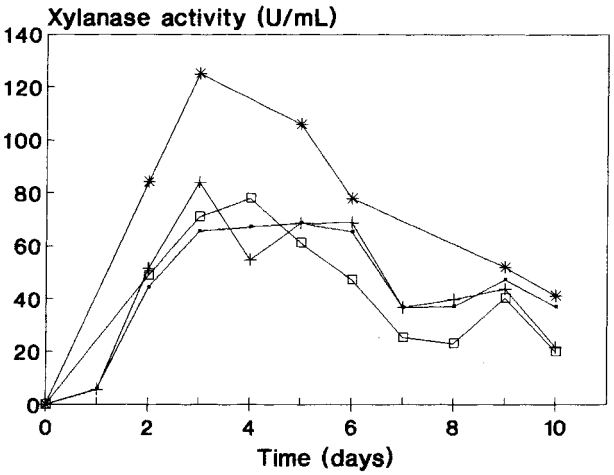

Figure 1. Kinetics of xylanase production by *penicillium purpurogenum* wild-type (•) and mutants UV-64 (+), NG-737 (∗) and NG-188 (□) grown in liquid medium with oat spelts xylan (0.75%) plus wheat bran (0.25%) as carbon sources.

In order to optimize enzyme production the three mutants were grown on the different carbon sources listed above. The best medium for xylanase production was oat spelts xylan (0.75%) supplemented with wheat bran (0.25%). Figure 1 shows the kinetics of enzyme production in this medium. Mutant NG-737 produced the highest activity (124.9 U/ml) after 3 days of culture, compared with 67 U/ml after 5 days on 1% wheat straw. Enzyme activities did not vary significantly in repeated experiments. The addition of 1% Tween-80 or grapeseed oil to the growth medium did not stimulate xylanase production.

Production of β-xylosidase was also improved. When grown on 1% wheat straw, mutant UV-64 produced 1.23 U/ml compared with 0.54 and U/ml for the wild-type. Birchwood xylan (1%) was the best medium for β-xylosidase production, UV-64 produced 4.85 U/ml compared with 2.2 U/ml for the wild-type.

When grown on wheat straw, the three mutants showed also some increase in endoglucanase (8 versus 5 U/ml) and acetyl esterase (0.7 versus 0.46) activities. No difference was detected for filter-paper-hydrolysing activity (0.15 U/ml), and the mutants produced less β-glucosidase activity than the wild type (0.3 versus 0.5).

Thermal stability of xylanase was studied at 50 °C. The supernatants were diluted in 0.05 M acetate buer pH 5.3, incubated in the absence of substrate for 32 h and the activity was measured. Best stability was shown by NG-188 with a half-life of 14 h as compared with 6 h for the wild-type. These values compare fovourably with those reported by Biswas *et al*. (1990) and Deshpande *et al*. (1987).

There are few reports on the isolation of hyper-xylanolytic mutants. Singh *et al*. (1995) selected mutants of *Fusarium oxysporum* producing 35.6 and 15.0 U of xylanase ml when grown on xylan and wheat straw respectively. Biswas *et al*. (1990) using a mutant of *Aspergillus ochraceus* obtained best xylanase on wheat bran: 28 U/ml in shake flasks and 112 U/ml of xylanase in solid state cultures. In our case, when wheat bran was the sole carbon source enzyme production was very low (data not shown).

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