World Journal of Microbiology & Biotechnology **15**: 751–752, 1999.
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Short Communication

Extracellular production of L-glutaminase by alkalophilic *Beauveria bassiana* BTMF S10 isolated from marine sediment

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Received in revised form 25 May 1999; accepted 6 August 1999

Keywords: Beauveria bassiana, fermentation, L-glutaminase

Summary

Beauveria sp. BTMF S10 isolated from marine sediment produced extracellular L-glutaminase. Maximal L-glutaminase yield (46.9 U/ml) was obtained in a medium supplemented with 1% (w/v) yeast extract and sorbitol, 9% (w/v) sodium chloride and 0.2% (w/v) methionine, initial pH 9.0 and at 27 °C after 108 h. This enzyme was inducible and growth-associated.

Introduction

L-Glutaminase has received significant attention recently owing to its potential applications in medicine as an anticancer agent and in food industries (Yano *et al.* 1988; Prabhu & Chandrasekaran 1997). Production of extracellular L-glutaminase by marine bacteria has been reported (Renu & Chandrasekaran 1992; Prabhu & Chandrasekaran 1997). Whereas, except for the reports on terrestrial *Aspergillus oryzae* (Yano *et al.* 1988), which is the present source of this enzyme, no reports are available for any marine fungi. In the present study we report the extracellular production L-glutaminase by *Beauveria* sp. isolated from marine sediment.

Materials and Methods

Microorganism and growth conditions

Beauveria bassiana BTMF S10 (Suresh & Chandrasekaran 1998) was grown in minimal medium composed of (g/l): K_2HPO_4, 10; KH_2PO_4, 5; $MgSO_4 \cdot 7H_2O$, 10; NaCl, 90; L-glutamine 10 and pH 8.0. After inoculation with a 4% (v/v) conidial suspension (12×10^6 c.f.u./ml, 12 days old), incubated at 27 °C on a rotary shaker at 150 rev/min. After cultivation the mycelia were removed from the broth by centrifugation and the supernatant was used for enzyme assays.

Optimization of process parameters for L-glutaminase production

The medium described above was taken as a basal medium and the different process parameters including pH (6–13); temperature (22–42 °C); NaCl (0–15% w/v); additional nitrogen sources (1% w/v) viz. peptone, yeast extract, beef extract, malt extract, calcium nitrate and potassium nitrate; additional carbon sources (1% w/v) viz. glucose, maltose, manitol, mannose, sucrose and sorbitol; amino acids (1% w/v) viz. L-glutamine, L-glutamic acid, L-asparagine, arginine, methionine, proline and lysine were optimized independently. Finally the time course of production was evaluated under the optimized conditions. All experiments were conducted in triplicate and the mean values are reported.

Analytical methods

Enzyme assay. L-Glutaminase was assayed using L-glutamine as substrate (Imada *et al.* 1973).

Growth. The biomass was estimated as total cell protein (Herbert *et al.* 1971) and the growth was expressed in terms of total cell protein gram per liter (g/l).

Results and Discussion

B. bassiana BTMF S10 isolated from marine sediment, could grow in high alkaline media and produce extracellular chitinase (Suresh & Chandrasekaran 1998,

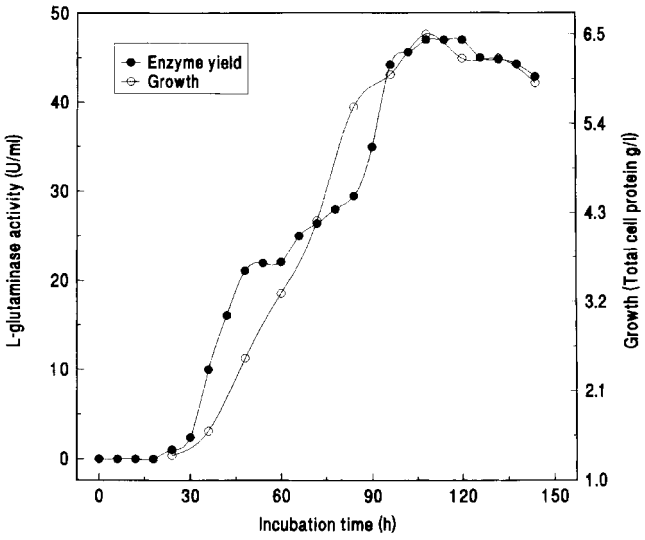

Figure 1. Time course of L-glutaminase production by *B. bassiana* BTMF S10 at optimized conditions: pH 9.0; temperature 27 °C; NaCl 9% (w/v); yeast extract and sorbitol 1% (w/v) and methionine 0.2% (w/v).

1999). It produced L-glutaminase (7.8 U/ml) extracellularly in an arbitrarily selected medium containing 1% (w/v) L-glutamine after 48 h before optimization of process parameters. The optimum pH and temperature that promoted maximal L-glutaminase production were 9.0 (7.8 U/ml) and 27 °C (14.1 U/ml) respectively. Nevertheless, L-glutaminase activity was detectable over a range of pH from 7.0 (4.0 U/ml) to 12.0 (3.5 U/ml), and from 22 (8.4 U/ml) to 37 °C (5.9 U/ml). Comparable results were reported with the same strain under solid state fermentation for extracellular chitinase production (Suresh & Chandrasekaran 1998, 1999). Supplementation of the medium with 9% (w/v) of NaCl supported maximal glutaminase yield (13.5 U/ml) compared with control (0% NaCl, 7.2 U/ml) indicating a halotolerant property of the strain. Among the different nitrogen sources tested, yeast extract (14.6 U/ml) and potassium nitrate (14.1 U/ml) contributed enhanced enzyme yield when compared with the control (7.6 U/ml). Among the different carbon sources tested, sorbitol not only promoted maximal yield but also led to a double fold increase (15.1 U/ml), in enzyme yield compared to the control (7.8 U/ml). These results were similar to that observed with *Vibrio* sp. (Prabhu & Chandrasekaran 1997). The enhanced production of L-glutaminase might be due to rapid growth accomplished by the easy availability of additional carbon sources along with L-glutamine. Among the amino acids tested, except asparagine (2.3 U/ml), all other amino acid enhanced L-glutaminase yield. Methionine supported maximal production (15.9 U/ml). On further optimization of methionine concentration the L-glutaminase yield increased to a maximal yield of 17.4 U/ml with increase in the concentration (0.2% w/v). However, further increase in concentration above 0.2% did not enhance yield. *B. bassiana* elaborates extracellular L-glutaminase only on induction by an amino acid since there was no enzyme production in the absence of any amino acid.

During the time course production of L-glutaminase, the maximal enzyme production (46.9 U/ml) was recorded at 108 h of incubation (Figure 1). Results indicate that L-glutaminase synthesis in *B. bassiana* BTMF S10 is growth-associated. Further, the maximal L-glutaminase yield (46.9 U/ml) recorded with this fungus is at an appreciable level when compared to earlier reports for fungi (Yano *et al.* 1988) and bacteria (Renu & Chandrasekaran 1992; Prabhu & Chandrasekaran 1997). The present study indicates scope for the use of *B. bassiana* BTMF S10 as an ideal organism for the industrial production of extracellular L-glutaminase.

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