Optimization and Purification of L-Asparaginase Produced by *Streptomyces tendae* TK-VL_333

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Cultural factors affecting the production of L-asparaginase by *Streptomyces tendae* isolated from laterite soil samples of Guntur region were investigated on glycerol-asparagine-salts (modified ISP-5) broth. Optimal yields of L-asparaginase were recorded in the culture medium with the initial pH 7.0 incubated at 30 °C for 72 h. The strain utilized sucrose (2%) and yeast (2%) extract as carbon and nitrogen sources for L-asparaginase production. The productivity of L-asparaginase was slightly enhanced when the strain was treated with cell-disrupting agents like EDTA. The crude enzyme was purified to homogeneity by ammonium sulfate precipitation, Sephadex G-100 and CM-Sephadex G-50 gel filtration. By employing sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the molecular weight of the enzyme was recorded as 97.4 kDa. This is the first report on production and purification of L-asparaginase from *S. tendae*.

Key words: Actinomycetes, *Streptomyces tendae*, L-Asparaginase

Introduction

L-Asparaginase has been extensively used for the treatment of acute lymphoblastic leukaemia and tumour cells. Due to its potent activity as antitumour agent, microbes capable of producing this enzyme have attained special importance. The enzyme has been isolated and characterized from *Escherichia coli* (Derst *et al*., 1994; Mercado and Arenas, 1999), *Erwinia* spp. (Maladkar *et al*., 1993; Aghaiypour *et al*., 2001), *Enterobacter aerogenes* (Mukherjee *et al*., 2000), and *Thermus thermophilus* (Prista and Kyridio, 2001). Among these, enzymes obtained from *E. coli* and *Erwinia* spp. have commercial interest and are widely employed as antileukaemic agent in humans. Besides, actinomycetes such as *Streptomyces griseus* (De Jong, 1972), *S. karnatakensis*, *S. venezuelae* (Mostafa, 1979), *Nocardia asteroides* (Gunasekaran *et al*., 1995), *S. longsporusflavus* F-15 (Abdel-Fatah, 1996), and *S. phaeochromogenes* FS-39 (Abdel-Fatah *et al*., 1998) are likely to produce this enzyme and provide a clue to search for potent sources of actinomycetal origin. As a part of our ongoing search for L-asparaginase-producing strains, *S. tendae* TK-VL_333 showing positive response on glycerol-asparagine-salts agar for L-asparaginase production was isolated from laterite soils of Guntur region. The present study briefly describes the production, optimization, and purification of L-asparaginase produced by *S. tendae*.

Material and Methods

Microorganism

Streptomyces tendae TK-VL_333 was isolated from laterite soil samples of Guntur region, and the 16S rRNA gene sequence of the strain has been deposited in NCBI genbank with the accession number FJ877150.

Production profile of L-asparaginase

For determining the production profile of L-asparaginase, a culture suspension prepared from an one-week-old culture of the strain maintained on yeast extract-malt extract-dextrose agar medium was inoculated into glycerol-asparagine-salts (modified ISP-5) broth containing 1% glycerol, 1% asparagine, 0.1% K_2HPO_4, 0.1% trace salt solution (0.01% $FeSO_4 \cdot 7H_2O$, 0.01% $MnCl_2 \cdot 4H_2O$, 0.01% $ZnSO_4 \cdot 7H_2O$) with initial pH 7.2 (Abdel-Fatah, 1996). The inoculated flasks were incubated at 35 °C for 6 d in order to estimate the cell growth of the strain as well as the L-asparaginase production at a 24-h interval.

L-Asparaginase assay

The enzyme assay was performed according to the procedure described by Peterson and Ciegler (1969) with slight modifications. Cells harvested by centrifuging the culture broth at 7,800 × *g* for 15 min were ground in Tris [tris-(hydroxymethyl)-aminomethane]-HCl buffer using a homogenizer. Later, they were again centrifuged, and the cell-free extract (0.2 ml) was mixed with 0.8 ml of 0.05 M Tris-HCl buffer and 1 ml of 0.04 M L-asparagine. After incubating the reaction mixture for 15 min at 37 °C in a water bath shaker, the reaction

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was terminated by the addition of 0.5 ml of 15% (w/v) trichloroacetic acid. Precipitated proteins were removed by centrifugation, and the liberated ammonia was determined spectrometrically at 500 nm using Nessler's reagent. Tubes kept at zero time incubation served as control. The enzyme activity was determined on the basis of liberation of ammonia calculated with reference to a standard curve of ammonium sulfate. One L-asparaginase unit (U) equals to that amount of enzyme which releases 1 μM of ammonia (ammonium sulfate as standard) in 1 min at 37 °C. The cell dry weight (mg/10 ml) was recorded simultaneously by drying the cell debris collected after centrifugation in an oven at 90 °C for 24 h.

Optimization of L-asparaginase production

The influence of different cultural and nutritional conditions, such as initial pH (7–10), incubation temperature (20–40 °C), carbon (arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, sorbitol, starch, sucrose, and xylose) and nitrogen (L-asparagine, beef extract, L-glutamine, malt extract, peptone, tryptone, and yeast extract) sources, on the production of L-asparaginase was determined by growing the strain in modified ISP-5 broth for 72 h. Optimal levels of the preferred carbon and nitrogen sources for the enzyme production were also recorded (Khamna *et al*., 2009). Besides, the effect of cell-disrupting agents like EDTA, lysozyme, SDS, and penicillin-G on the release of L-asparaginase from the 72-h-old culture was tested (Mostafa and Ali, 1983).

Purification of L-asparaginase

The purification of L-asparaginase from the crude extract was carried out at 4 °C according to the method of El-Bessoumy *et al*. (2004). The purity of the enzyme preparation was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970), and the molecular masses of the bands obtained on the gels were estimated by comparing them with those of standard proteins (Bio-Rad, Richmond, CA, USA).

Results and Discussion

The production of L-asparaginase was monitored during the growth of *S. tendae* in modified ISP-5 broth. The enzyme assay was carried out for 6 days at an interval of 24 h. L-Asparaginase production was initiated after 24 h of growth, and a gradual rise was noticed up to 72 h of incubation. Maximum yield of biomass [(34.3 ± 1.58) mg/10 ml] as well as enzyme production [(4.13 ± 0.181) U/mg of cell dry weight] was obtained with a 3-day-old culture. A positive correlation between cell growth and L-asparaginase productivity was reported in *S. karnatakensis* (Mostafa, 1979) and *S. albidoflavus* (Narayana *et al*., 2008). In *Amycolatopsis* CMU-H002, maximum yields of L-asparaginase and biomass were obtained from a 72-h-old culture (Khamna *et al*., 2009).

Initial pH levels of the culture medium had a profound effect on the production of L-asparaginase by the strain. A high yield [(4.14 ± 0.244) U/mg of cell dry weight] of the enzyme production was found at pH 7.0. These findings are consistent with the results of Abdel-All *et al*. (1998) for *S. phaeochromogenes* FS-39 where pH 7.0 was optimum for L-asparaginase production. Sahu *et al*. (2007) also noted optimal L-asparaginase production by *S. aureofasciculus*, *S. chattanoogenesis*, *S. hawaiiensis*, *S. orientalis*, *S. canus*, and *S. olivoviridis* at pH levels between 7 and 8.

The production of L-asparaginase was high [(4.27 ± 0.264) U/mg of cell dry weight] when the strain was cultivated in modified ISP-5 broth at 30 °C. In *S. collinus* (Mostafa and Salma, 1979) and *S. longsporusflavus* F-15 (Abdel-Fatah, 1996), the optimum temperature for L-asparaginase production was 30 °C. The present study revealed that L-asparaginase production by the strain was high when it was grown in modified ISP-5 broth with initial pH 7.0 for 72 h at 30 °C.

Modified ISP-5 broth amended with different carbon and nitrogen sources was used to study the impact of nutrients on the L-asparaginase production by the strain. Among the carbon sources tested, culture medium supplemented with sucrose [(5.27 ± 0.173) U/mg of cell dry weight] followed by sorbitol [(5.02 ± 0.077) U/mg of cell dry weight] stimulated the enzyme yields. Biosynthesis of L-asparaginase was greatly suppressed in media supplemented with carbon sources like lactose [(0.09 ± 0.01) U/mg of cell dry weight] and starch [(0.48 ± 0.028) U/mg of cell dry weight)] when compared to that of the control [(4.27 ± 0.264) U/mg of cell dry weight]. Sahu *et al*. (2007) noticed optimal yields of L-asparaginase production from *S. aureofasciculus* LA-2, *S. chattanoogenesis* LA-8, *S. hawaiiensis* LA-15, *S. orientalis* LA-20,

S. canus LA-29, and *S. olivoviridis* LA-35 with sucrose as carbon source. Krishna Reddy and Reddy (1990) reported sorbitol as one of the best carbon sources for L-asparaginase production by *S. albus*.

The final pH value of the fermentation broths of *S. tendae* containing sucrose, sorbitol or glycerol was in the alkaline region. The pH value of media amended with other carbon sources was in the acidic region which may be the reason for low productivity of L-asparaginase; this is evident from reports stating that acidity of the fermentation medium could inhibit the production of L-asparaginase (Geckil *et al*., 2006; Narayana *et al*., 2008).

Studies on the effect of different levels of sucrose (1–4% w/v) revealed that optimal yields of L-asparaginase [(5.56 ± 0.272) U/mg of cell dry weight] were recorded in medium amended with 2% sucrose. The optimal level of carbon source for L-asparaginase production by *S. phaeochromogenes* FS-39 was 2% glycerol (Abdel-All *et al*., 1998), while it was 1.5% starch for *S. longsporusflavus* F-15 (Abdel-Fatah, 1997). In the present work, optimal yields of L-asparaginase were recorded by culturing the strain in modified ISP-5 broth containing 2% sucrose with initial pH 7.0 at 30 °C for 72 h.

L-Asparaginase production by the strain was found to vary with different nitrogen compounds tested. Among them, yeast extract favoured high yields of L-asparaginase [(6.05 ± 0.1) U/mg of cell dry weight] followed by L-asparagine. Yeast extract was reported as an excellent nitrogen source for L-asparaginase production by *Erwinia aroideae* and *E. carotovora* EC-113 (Liu and Zajic, 1972; Maladkar *et al*., 1993), respectively, while L-asparagine favoured high levels of L-asparaginase productivity in *S. karnatakensis*, *S. venezuelae* (Mostafa, 1979), and *S. collinus* (Mostafa and Salma, 1979).

Besides, the study of different levels of yeast extract (1–3% w/v) revealed that the production of L-asparaginase by the strain was high [(7.01 ± 0.2) U/mg of cell dry weight] in medium containing 2% yeast extract. In *S. albidoflavus*, Narayana *et al*. (2008) recorded 2% yeast extract as optimal content for L-asparaginase production. In the present study, the optimal conditions for the synthesis of L-asparaginase by *S. tendae* included modified ISP-5 broth (initial pH 7.0) supplemented with 2% yeast extract and 2% sucrose maintained at 30 °C for 72 h.

The intracellular nature of L-asparaginase was reported in *S. griseus* (De Jong, 1972) and *S. albidoflavus* (Narayana *et al*., 2008). As the enzyme L-asparaginase was found only in the cell extracts of *S. tendae*, the effect of several cell-disrupting agents on its release from the cells was determined. EDTA was effective for the release of L-asparaginase [(7.55 ± 0.225] U/mg of cell dry weight] from the cells of *S. tendae*.

Intracellular L-asparaginase of *S. tendae* was purified by employing ammonium sulfate precipitation, Sephadex G-100 and CM-Sephadex G-50 gel filtration. The crude enzyme extract obtained from a 72-h-old culture was subjected to fractional ammonium sulfate precipitation (60–80%). Most of the enzyme activity was found in the precipitate. The specific activity of the enzyme precipitate purified through a Sephadex G-100 column was 51.7 U/mg with a purity of 17.23-fold and the recovery was 30.5%. The active enzyme fractions were pooled and further purified by CM-Sephadex C-50 column chromatography. The purity of the enzyme was raised to 87.2-fold with 25.7% recovery.

Abdel-Fatah (1997) purified the intracelluar L-asparaginase of *S. longsporusflavus* (F-15) up to 30.5-fold with 19.1% recovery. With Sephadex G-200 gel filtration, L-asparaginase of *Streptomyces* sp. PDK2 showed a purity of 83-fold and

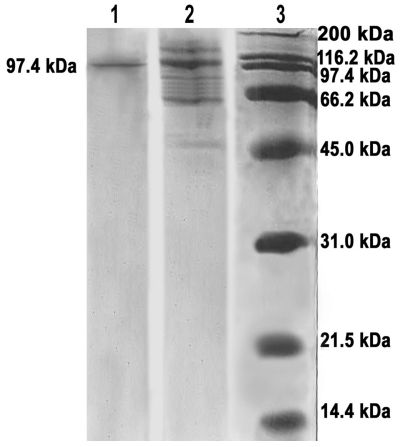

Fig. 1. SDS-PAGE analysis of L-asparaginase produced by *Streptomyces tendae* TK-VL_333. Lane 1, CM-Sephadex C-50 purified enzyme concentrate of L-asparaginase; lane 2, dialyzed enzyme concentrate of ammonium sulfate precipitate (80%) of crude enzyme extract; lane 3, standard protein marker.

2.18% recovery (Dhevagi and Poorani, 2006) while L-asparaginase obtained from *S. albidoflavus* was purified to 99.3-fold with a final recovery of 20% (Narayana *et al*., 2008).

L-Asparaginase purified from different microorganisms showed different molecular weights like 80 kDa in *Corynebacterium glutamicum* (Savitri *et al*., 2003), 140 kDa in *Streptomyces* sp. PDK2 (Dhevagi and Poorani, 2006), and 116 kDa in *S. albidoflavus* (Narayana *et al*., 2008). In the present study, the homogeneity of purified L-asparaginase from *S. tendae* checked by SDS-PAGE revealed a distinct protein band near 97.4 kDa (Fig. 1). This is the first report of L-asparaginase purification from *S. tendae*.

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