Encapsulation of resting cells of *Nocardia globerula* NHB-2 in alginate gel beads and optimization of process parameters for their acyltransferase activity

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Introduction

Amidase, an amide-hydrolyzing enzyme also shows acyltransferase activity.The use of acyltransferase or bacteria with microbial enzymes with acyltransferase activity may be used to convert amides to HAs (as shown in reaction below) (Fournand *et al.* 1998). In acyltransferase catalyzed biotransformation, amides acts as acyl-group donors and hydroxylamine as acyl-group acceptors.

$$\underset{\text{(amide)}}{R{-}CONH_2} + \underset{\text{(hydroxylamine)}}{NH_2OH} \xrightarrow{\text{Acyltransferase}} \underset{\text{(hydroxamic acid)}}{R{-}CONHOH} + \underset{\text{(ammonia)}}{NH_3}$$

Moreover, acyltransferase activity of amidase can be used for the synthesis of pharmaceutically active hydroxamic acids, which have been reported as tumor inhibitors, anti-HIV and anticancerous (Fournand *et al.* 1998, Ramakrishna *et al.* 1999). Some hydroxamic acids can conjugate with metal ions and thus find their use to eliminate metal ions in wastewater treatment and nuclear technology (Fournand *et al.* 1998). Some other hydroxamic acids (α-aminohydroxamic acid, acetohydroxamic acid etc.) have also been investigated as anti-human immunodeficiency virus agents or antimalarial agents or have been recommended for treatment of ureaplasma infections and anemia (Fournand *et al*. 1998, Holmes 1996.).

Here, we describe, encapsulation of whole resting cells of *Nocardia globerula* in alginate gel beads and process optimization for their acyltransferase activity which could be used for the synthesis of various hydroxamic acids.

Materials and methods

Chemicals

The nitriles, amides, acetohydroxamic acid and hydroxylamine hydrochloride used in the present study were purchased from Lancaster Synthesis, England. The media components were obtained from HiMedia, Mumbai, India. All other reagents and chemicals used were of highest analytical grade available.

Microorganism, culture conditions and preparation of resting cells

The bacterial isolate *Nocardia globerula* NHB-2 has been procured from the culture collection of the Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla-5, India. It had been isolated earlier from the soils of Himachal Pradesh as a nitrile-metabolizing bacterium (Bhalla *et al.* 2005). The bacterium was routinely subcultured on nutrient agar slants at 30 ^{0}C and maintained at 4 ^{0}C. Preculture was prepared by inoculating a loop full of culture from the slant to 2 ml of seed medium containing 0.5% peptone, 0.3% beef extract, 0.1% yeast extract and 1% glucose

(pH 7.5) at 30 ^{0}C, 160 rpm for 24 h. This 2 ml of seed culture was added to 50 ml of seed medium and grown under the same conditions. These 24 h preculture were added to 50 ml of production medium containing 3% Tryptone, 1.5% yeast extract, 0.5% NaCl, pH 8.5 (Piotraschke *et al.* 1994) and 0.2% acetonitrile (v/v) as an inducer, followed by incubation at 30 ^{0}C for 24 h in an incubator shaker at 180 rpm.

Cells were harvested by centrifuging the cultures at 5000 x g for 20 min at 0-4 ^{0}C. The pellets were suspended and washed twice with glycine-NaOH buffer (pH 8.5) and finally re-suspended in the same buffer and were referred to as 'whole resting cells'. The whole resting cells were assayed for acyltransferase activity and used for further investigations.

Acyltransferase assay

The acyltransferase activity was determined spectrophotometrically measured by the method described by Brammar and Clarke (1964). If not stated otherwise, the assay was performed in 2 ml of reaction mixture containing resting cells, acetamide solution and hydroxylamine hydrochloride solutions (adjusted to pH required with 10N NaOH) in 100mM glycine-NaOH buffer (pH 8.5) at 55^O C for 15 min. The absorption was measured at 500 nm and related to the amount of acetohydroxamate formed in reaction by comparison with a standard.

Immobilization of Nocardia globerula in alginate gel beads

Whole resting cells were immobilized by the method as described by Kierstan and Bucke (1977). Known amount of whole resting cells of *Nocardia globerula* NHB-2 were added to the 2% sodium alginate solution and mixed well. The mixture was extruded drop wise via a 10 ml syringe from a height of about 20 cm into 1 L of chilled, stirred 0.2 M calcium chloride ($CaCl_2$) solution. The generated beads were kept in 0.2M $CaCl_2$ solution for 1 h for proper hardening and then the beads were placed in 10mM $CaCl_2$ solution till further use.

Optimization of process parameters for gel entrapped resting cells

The conversion of acetamide to acetohydroxamic acid was carried out using alginate gel entrapped resting cells of *Nocardia globerula* in selected 100 mM glycine-NaOH buffer at different pH value from 5.0 to 10, at temperature between 30 to 70 ^{0}C and varied concentrations of acetamide and hydroxylamine hydrochloride from 150 μmoles to 950 μmoles. Substrate affinity of acyltransferase activity of immobilized cells was tested using a number of substrates. Gel beads were kept at 4^{O}C and room temperature for 20 days to check shelf life of immobilized enzyme. Enzyme activity was estimated on every single day. Thermostability and reusability of the immobilized cells were also studied

Results and Discussion

Optimization of reaction parameters for gel entrapped resting cells

The maximum turnover of acetamide to AHA was obtained in 100 mM glycine-NaOH buffer at pH 8.5 and a temperature of 55 ^{0}C. Gel beads with 0.8 mg resting cells (dcw) showed a maximal acyltransferase activity when concentrations of acetamide and hydroxylamine HCL were 500 mM and 1000 mM respectively. The maximum AHA production was observed at 50 ^{0}C in 2 h of reaction (Fig. 2).

Gel beads entrapped cells exhibited broad substrate affinity with greater turnover of aliphatic

amides as compared to aromatic amides to their respective hydroxamic acids. Similar broad-spectrum amide specificity was reported for acyltransferase activity of amidase of *Rhodococcus* sp. R312 (Fournand *et al*. 1998).

Alginate gel beads containing resting cells of *Nocardia globerula* were recycled for ten cycles and it was observed that there was no loss of activity occurred .Minimum or negligible loss of enzyme activity was noticed in alginate gel entrapment.

Thermostability of alginate gel beads was also investigated by preincubating them at different temperatures in the range of 35^{O}C to 85^{O}C for 120 minutes (Fig. 3). The entrapped resting cells retained very good activity up to 55^{O}C. In comparison to free cells, alginate gel immobilized cells exhibited higher thermostability probably due to inert nature of alginate and gelling reaction is endothermic.

Shelf life of alginate immobilized enzyme was studied at both room temperature and 4^{O}C by preincubation of resting cells for 20 days. The results obtained have been shown in Fig. 4. Only 5% loss of activity was observed on storage at 4^{O}C, whereas at room temperature 83% residual activity was retained.

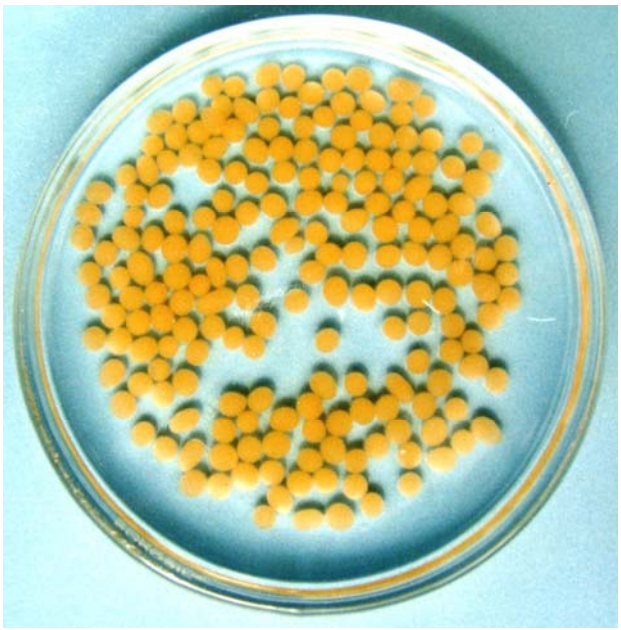

Fig. 1 Alginate beads containing resting cells of *Nocardia globerula.*

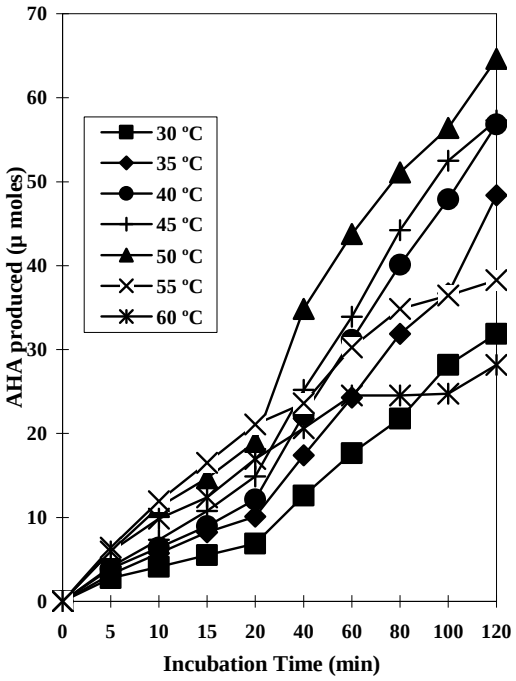

Fig. 2 Time course of acyltransferase reaction at different temperatures.

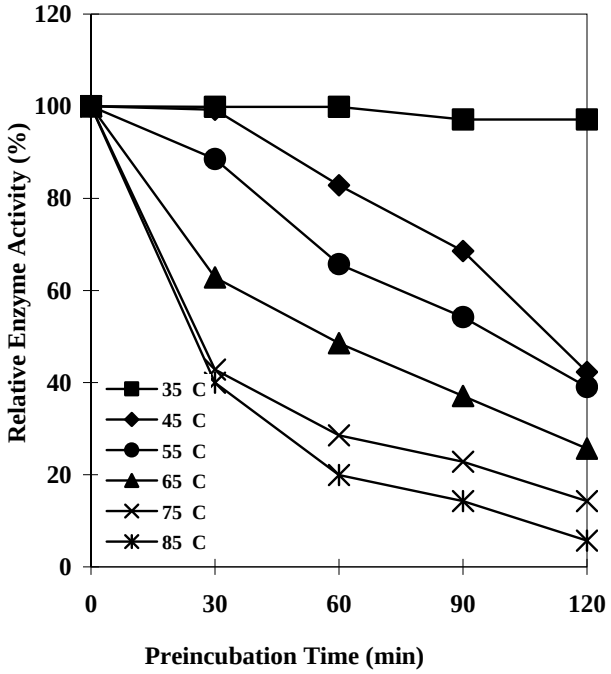

Fig. 3 Thermostability of acyltransferase of *Nocardia globerula.*

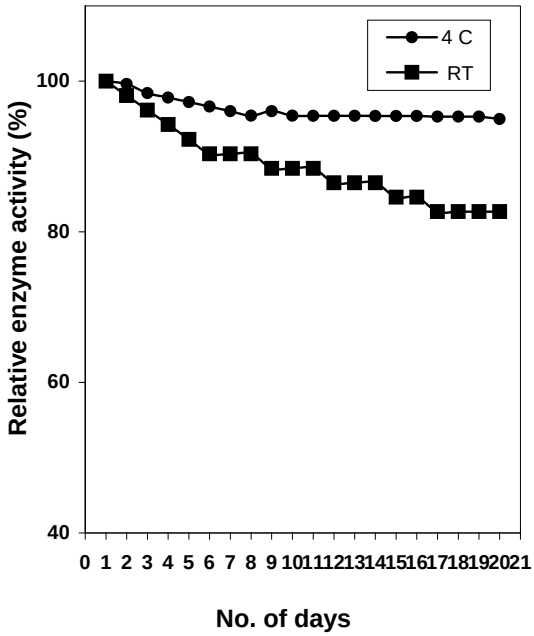

Fig. 4. Shelf life of enzyme

Conclusions

The results obtained in this investigation show that *Nocardia globerula* entrapped in alginate gel beads express thermostable acyltransferase activity that may be used for the synthesis of AHA.

References

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