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ORIGINAL PAPER

Biotransformation of amide using *Bacillus* sp.: isolation strategy, strain characteristics and enzyme immobilization

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Abstract Amidase hydrolases/acyltransferases are of considerable industrial interest due to potential applications in the production of useful hydroxamic acids. The test strain, capable of acetamide degradation, was isolated by an enrichment technique with acetamide as sole source of nitrogen. Based on morphology, physiological tests and biochemical tests, this isolate was identified as *Bacillus* sp. and on the basis of 16S Ribosomal ribonucleic acid sequence, a phylogenetic tree was drawn and was identified as *Bacillus megaterium*. Resting cells containing active acyltransferase enzyme were prepared and immobilized in the gel beads of sodium alginate, agar, polyacrylamide and polyvinyl alcohol–alginate. The beads were tested for acyltransferase using Iron (III) chloride reagents at 55°C and were found to be affected by substrate concentration, type of buffer, buffer pH and reaction temperature. These factors were optimized using sodium alginate immobilized beads. This study proved useful in understanding the technique of immobilization of acyltransferase enzyme, its operational stability and its importance in the synthesis of hydroxamic acid.

Keywords Acetamide · Acyltransferase · Biodegradation · Biotransformation · Hydroxamic acid · Immobilized cells

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Introduction

Biocatalysis is an established method for the synthesis of organic compounds. It is estimated that two-third of total research in the field of biotransformation has been performed using hydrolases (Martinkova et al. 2008). Nitrile degrading enzymes namely nitrilase, nitrile hydratase and amidase play important roles in the branch of biotransformation (Kubac et al. 2008). Amidase catalyze the hydrolysis of amide to free carboxylic acid and ammonia, which also involves nitrile hydrolysis: the carboxyl group of amide undergoes a nucleophilic attack, resulting in the formation of a tetrahedral intermediate, which is converted to acyl-enzyme with the removal of ammonia and subsequently hydrolyzed to acid (Kohyama et al. 2007). All of the different amidases also exhibit acyl transfer activity in the presence of hydroxyl amine (Fournand et al. 1998). Microbial degradation of amide compounds has been reported by various group of researchers using different strains such as *Rhodococcus rhodochrous* IFO 15564 (Kashiwagi et al. 2004), *Rhodococcus erythropolis* A4 (Brandao et al. 2003), *Arthobacter* sp. J1 (Asano et al. 1982), *Bevibacterium* sp. R312 (Mayaux et al. 1990), *Klebsiella* oxytoca (Kao et al. 2006), *Rhodococcus erythropolis* MP50 (Hirrlinger et al. 1996), *Rhodococcus rhodochrous* M8 (Kotlova et al. 1999). The present study was undertaken to determine the ability of the immobilized *Bacillus* sp. isolated from the soil contaminated of dye and pharmaceutical industrial drainage supporting the vegetation growth for the biotransformation of acetamide to acetohydroxamic acid. The wide spectrum amidase (acetamide amidohydrolase) from *Bacillus* sp. was able to hydrolyze acetamide into corresponding acid. Further, it was also able to transfer acyl group of short chain amide to hydroxylamine forming corresponding hydroxamate. In

order to simplify handling of the amidase and to improve its stability, a suitable immobilization method (Giorno and Drioli 2000) was searched. The immobilization technique using different matrices like agar, polyacrylamide and alginate were successfully applied to a number of different enzymes (Banerjee et al. 2002). A modification of alginate method using a milder cross linking agent, polyvinyl alcohol, was also suitable for the immobilization of relatively labile enzyme like amidases (Bhalla et al. 1997; Schoevaart et al. 2004; Kumar et al. 2006). Thus, the suitability of this PVA–alginate method for immobilization of the amidase from *Bacillus* was examined. The soil samples were collected in the year 2008 during months of May and June from Sanganer and Sitapura areas of Jaipur, Rajasthan, India.

Materials and methods

Sampling sites

Soil samples were collected from effluent sites of dye and pharmaceutical industries located in urban Sanganer and Sitapura areas of Jaipur, Rajasthan, India.

Dye industry

Sanganer town, district Jaipur (Rajasthan, India), is famous worldwide for its textile dyeing and printing industries. There are large number of industries involved in textile printing processes, which use a variety of chemicals and dyes during processing and finishing of raw materials. Many textile dyes are amide compounds in nature which are known carcinogens and mutagens (Jeanne 1998). Further, untreated and sometimes even treated effluents from these industries are released into surface waters of Amani Shah Drainage (the major drainage channel of Jaipur City) which is then utilized for irrigating nearby agricultural fields. Soil sample was collected from such sludge farm.

Pharmaceutical industry

Amol Pharmaceuticals Pvt. Ltd., Sitapura Industrial Area, Jaipur, (Rajasthan, India) is a leading and the largest manufacturer in the nutraceutical and pharmaceutical products. Many drugs produced and solvents used are amide compounds in nature (Jeanne 1998). Due to wide occurrence and addition of various toxic amides through industrial effluents in natural habitats, microbes in such soils have evolved the metabolic capabilities to degrade these chemicals. Soil sample was collected from soil nearby of drainage supporting the vegetation growth.

Chemicals

Acetamide, hydroxylamine and acetohydroxamic acid were obtained from Hi Media Chemicals Ltd, India. Peptone and Yeast extract were purchased from Suyog Diagnostics Private Ltd (New Delhi, India). All other chemicals used were of analytical reagent grade purity and were obtained from Merck, India.

Isolation of microorganisms from soil by use of acetamide enriched medium

The soil samples collected from sampling sites were pooled and transported in sterile containers and stored at 4°C. Bacteria were isolated from soil sample by an enrichment culture procedure (Layh et al. 1992). Enrichments were carried out in the nitrogen-free minimal medium containing 2 g of acetamide as nitrogen source (Pal and Samanta 1999). The soil sample (1 g) was suspended in 10 mL of saline solution (0.85%), and a 2-mL aliquot of the suspension was introduced into an Erlenmeyer flask containing 28 mL of minimal medium. The cultures were incubated at 30°C on a rotary shaker at 150 rpm for 5 days. The cultures were then transferred to the fresh medium (1:15, v/v). After repeating this procedure four times, the resulting cultures were diluted and plated onto minimal medium plates to isolate pure colonies. For the inoculation procedures, dilutions 10^{-3}–10^{-5} were chosen. Aliquots (0.1 mL) were spread evenly onto the NFMM agar with 0.2 g acetamide per 100 mL of the medium as a nitrogen source to induce the amidase/acyltransferase enzyme activity. Duplicates were done for each inoculation. For isolation of a single colony, the well defined isolated colonies were streaked onto fresh NFMM agar and incubated at 37°C for 48 h. The Nitrogen Free Minimal Medium contained (g/L): acetamide, 2; glycerol, 4; KH_2PO_4, 1; K_2HPO_4, 1; NaCl, 1; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.01; pH 7.2.

Characterization and identification of the isolated strain bacterium

Biochemical characterization of the isolate includes the test for fermentation reaction (lactose) and enzyme activity (catalase, citrate, methyl red, oxidase, urease and Vogues Proskauer) were carried out as described by Mata et al. (2002).

Analysis of 16S rRNA sequence

Genomic DNA of the isolate was extracted with a GenElute DNA extraction kit from Sigma. The 16S rRNA gene of isolate was amplified using the universal primers 8F

(5′-AGAGTTTGATC CTGGCTCAG) and 1541R (50-AA GGAGGTG ATCCAGCCGCA-3′). The amplification was done by initial denaturation at 95°C for 5 min followed by 10 cycles of 93°C for 1 min, 63°C for 1 min, 71°C for 1.5 min; 20 cycles of 93°C for 1 min, 67°C for 1 min, 71°C for 2 min and final extension at 71°C for 5 min. The purified PCR product was sequenced in both directions using an automated sequencer at NCCS (National Centre for Cell Science), Pune (India).

Transmission electron microscopy (TEM)

Cell morphology of the selected isolate F-8 from culture plates of 48 h was studied using Gram-staining technique and then the organism was observed by a transmission electron microscope. Washed cells were suspended in distilled water. The suspension (10 μL) was applied on a carbon-coated grid (3,000 mesh, 3 mm i.d.) followed by air drying. The adherent bacteria were negatively stained with 92% (w/v) phosphotungstic acid and examined under a transmission electron microscope at an accelerating voltage of 80 kV.

Preparation of resting cells

Bacillus was inoculated into 50 mL of modified nutrient broth containing 5 g peptone, 1 g yeast extract, 1 g beef extract, and 10 g glucose per litre of distilled water pH 7.2 and incubated at 30°C for 24 h in an incubator shaker at 175 rpm (Chand et al. 2004). These 24-h preculture were added to 50 mL of production medium containing 30 g tryptone, 15 g yeast extract, 5 g NaCl per litre of water, pH 7.5 followed by incubation at 30°C for 24 h in an incubator shaker at 175 rpm. Then culture was centrifuged at 5,000*g* for 15 min at 0–4°C. The cell pellets were suspended in 0.1 M solution phosphate buffer (pH 7.0) after washing twice with the same buffer. These cell suspensions were referred to as 'resting cells' having concentration of 300 mg/mL (wet weight). They were assayed for amidase and acyltransferase activity and used for further investigations.

Colorimetric screening method based on amidase catalyzed acyl transfer activity

The colorimetric screening method was based on amidase catalyzed synthesis of hydroxamic acid. When an acidic ferric chloride solution was added to the reaction mixture, color changed from light yellow to magenta which indicated the occurrence of a positive reaction. The colorimetric screening method was performed in a similar manner to the assay of acyl transfer activity established by Fournand et al. (1998). The reaction medium consisted of an aqueous solution of acetamide adjusted to pH 7 with 10 M NaOH (2 mL; 400 mM); hydroxyl amine hydrochloride, adjusted to pH 7 with 10 M NaOH (2 mL; 2 M); sodium phosphate buffer pH 7 (2 mL; 100 mM); and resting cells (2 mL). The reaction was performed at 30°C. One unit of acyl transfer activity was defined as the quantity of enzyme required for production of 1 μmol/min of acetohydroxamic acid at 30°C and pH 7.0.

Acetohydroxamic acid was assayed using the method developed by Brammar and Clarke (1964) based on the colorimetric determination of the resulting red brown complexes with Fe(III).

Samples of 0.5 mL reaction medium were taken at regular intervals (every 4 min) and immediately assayed by adding 1 mL of iron chloride solution (21 mL $FeCl_3$ 27.5% (w/v), 5.3 mL (12.5 M) HCl, to 100 mL distilled water). This stopped the reaction and yielded a stained complex. Absorbance was measured at 500 nm. The molar absorptivity of the iron (III)/2,2 dimethylcyclopropane hydroxamic acid complex was 3.34×10^2 mol^{-1} cm^{-1}. Reaction mixture without resting cells was also tested to exclude any possible spontaneous chemical synthesis of hydroxamic acid.

Immobilization of cells of *Bacillus*

10 mL of resting cells (200 mg/mL) were mixed thoroughly with sodium alginate solution (15 mL, 3.3 w/v). The resultant suspension was dropped into a magnetically stirred solution of $CaCl_2$ (0.2 M) to obtain spherical beads. The beads were stirred further for 1 h, washed and stored at 4°C (Kierstan and Bucke 1977).

In case of immobilization in agar, 1-mL resting cells (200 mg/mL) were added to 9 mL sodium phosphate buffer (100 mM, pH 7) and thoroughly mixed with 2.5% of 100 mL agar solution. The mixture was immediately poured into a petridish and kept at 50°C for solidifying. The gel was cut into beads of 1 cm diameter and stored in sodium phosphate buffer at 4°C for further use (Kierstan and Coughlan 1985).

For immobilization in polyacrylamide to 8 mL of sodium phosphate buffer (pH 7.0), 1.5 g of acrylamide, 0.75 g of bis-acrylamide and 5 mg of ammonium persulfate were added thoroughly mixed and heated. 50 μL of TEMED was added to this test tube. In another test tube, 2 mL of resting cell suspension (200 mg/mL) was thoroughly mixed with 3 mL chilled sodium phosphate buffer. The contents of the two test tubes were mixed properly and immediately poured in petridish and covered. Polymerization was allowed to proceed for 1 h. The solidified gel was cut into beads of 1 cm diameter and stored in the same buffer at 4°C for further use (Kierstan and Coughlan 1985).

For immobilization in polyvinyl alcohol–alginate (Pattanapipitpaisal et al. 2001), 20% (w/v) PVA and 15% (w/v)

alginate were mixed in 20 mL of distilled water and heated to 60°C to completely dissolve the PVA. The solution was then cooled to 35°C and thoroughly mixed with 5 mL resting cell suspension (200 mg/mL). The resultant suspension was dropped into a magnetically stirred solution of $CaCl_2$ (0.2 M) and immersed for about 2 h to form spherical PVA–alginate beads.

Assay of acyl transferase activity of immobilized cells

According to Brammar and Clarke (1964) and Fournand et al. (1997), immobilized cells were incubated with amide and hydroxyl amine and sodium phosphate buffer at 37°C for 1 h. After incubation, the beads were removed by filtration. The filtrate was centrifuged at 10,000*g* for 30 min to remove the fine particles. The clear supernatant was then assayed for acyl transferase activity.

Reusability of alginate, agar, polyacrylamide and PVA alginate immobilized cells for acyl transferase activity was determined.

The immobilized beads were used repeatedly as biocatalysts in 10 reactions to test their reusability potential. Numbers of beads containing an equivalent enzyme as present in given resting cell suspension were added in each reaction mixture and removing the beads from the reaction mixture (instead of using $FeCl_3$ reagent) stopped the reaction (Mateo et al. 2004).

Retention of acyl transferase activity by immobilized cells in different matrices in comparison to free resting cells.

Cells (400 mg) immobilized in different matrices and an equivalent amount of free cells were incubated separately with acetamide and hydroxyl amine in sodium phosphate buffer at 37°C for 1 h.

Optimization of reaction conditions

Reaction conditions such as pH, temperature, substrate concentration and type of buffer for free resting cells and alginate immobilized cells were optimized (Nawaz et al. 1998).

Comparative study of degradation pattern of acetamide by free and immobilized bacteria

Reaction mixture (100 mL) for acetohydroxamic acid production contained 125 mM of acetamide, 250 mM of hydroxyl amine hydrochloride, 100 mM sodium phosphate buffer (pH 7.0) and 2.65 mL of resting cell suspension (equivalent to 50 mg/mL dry wt. cells) in both immobilized and non-immobilized forms. The reaction mixture was incubated at 55°C for 5 h in a rotary water bath shaker. Percent conversion of acetamide to acetohydroxamic acid is estimated using HPLC. (High Performance Liquid Chromatography, Agilent Model no. 1050, Hewlett Packard Series 1050).

Results and discussion

Isolation of acetamide degrading bacteria

About three colonies were isolated from soil that were capable of utilizing acetamide as sole carbon and nitrogen source, out of these colonies, two bacterial isolates were A*grobacterium* sp. and the third was *Bacillus* sp. (Biochemical tests have been shown here only for the isolate chosen for further study). *Bacillus* sp. F-8 was selected as the most prominent utilizer of amide being able to grow profusely on NFMM agar containing acetamide and also showed better acyl transferase activity as compared to all other isolates and so was chosen for further study. The difference in enzyme activity could be due to their differences in microbial metabolism that attributed to genetic differences and their responses to changes in their environment as suggested by Landis and Ming-Ho (2003). Based on these observations, further studies were performed with isolate F-8.

F-8 was rod shaped facultative anaerobic bacterium which was motile by means of one or two subpolar flagella. The isolate was catalase and methyl red positive, urease negative and unable to ferment lactose. For the colony morphology, the colonies were smooth, circular, white-cream, entire, opaque and approximately 2 mm in diameter after 2 days at 37°C on NFMM agar.

Therefore, based on the morphological and biochemical properties, F-8 isolate was tentatively classified as *Bacillus* sp.

The partial sequences of 16S rRNA of F-8 (Fig. 1) obtained from NCCS, Pune were aligned with reference sequences obtained from BLASTN search of GenBank using multiple sequence alignment software. The phylogenetic tree was constructed using the neighbor joining method (Ventosa et al. 1982) which showed *Bacillus megaterium* was the closest relative of F-8 with 100% similarity (Fig. 2). The isolate F-8 was redesignated as *B. megaterium* F-8. The isolate F-8 was deposited into the GenBank under the accession number HQ909050.

Many studies on contaminated soils undergoing bioremediation were approved by *Pseudomonas* sp. (Nawaz et al. 1998), but very few papers were reported on the roles of *Bacillus* sp. in nitrile bioremediation (Yousefi et al. 2009). Wiegand et al. (1999) reported the isolation of bacteria from various environments such as freshwater, saltwater, arable land, woodland and compost that could grow on the polyester amide BAK 1095 polymer. The mechanism of polymer degradation was examined using

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GAGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTC
ATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGT
GCCCAACTAAATGCTGGCAACTAAGATCAAGGGTTGCGCTTTG
CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACC
ATGCACCACCTGTCACTCTGTCCCCCGAAGGGGAACGCTCTATC
TCTAGAGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTTCGCG
TTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC
CGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGG
CGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGCGGAAACCC
TCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGT
ATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGT
TACAGACCAAAAAGCCGCCTTCGCCCTGTGTTCCTCCACATCTC
TACGCATTTCACCGCTACACGTGGAATTCCGCTTTTCTCTTCTGC
ACTCAAGTTCCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGT
GGGCTTTCACATCAGACTAAGAACCGCC
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Fig. 1 Partial sequence of 16S rRNA of isolate F-8

the selected isolates. It was discovered that the bacteria selectively cleaved the polymer at its ester bonds releasing low-molecular water-soluble oligoamides.

The isolates were also tested for their ability to break down other ester-containing polymers such as Degranil W 50, a linear polyester urethane urea. 8 of the 12 strains examined by Wiegand et al. (1999) were also able to break down this polymer. These isolated microorganisms predominantly belonged to the genus *Bacillus*. The present study also supports the earlier findings that *Bacillus* sp. are more tolerant to high levels of toxic amides due to their resistant endospores.

Bacterial identification

According to the Bergey's manual of systematic bacteriology and considering the physiological and biochemical tests performed, the strain was tentatively named as *Bacillus* sp. strain F-8. To confirm the identity of the isolate, PCR amplification and sequencing of the 16S rRNA gene were done. 16S rRNA gene sequence analysis of strain F-8 with respect to *Bacillus* sp. showed that the closest relative of isolate F-8 was *B. megaterium.* Figure 3 shows the electron micro photographic profile of strain F-8.

The results of experiments on morphological, cultural and physiological properties of F-8 are present in Table 1.

Immobilization of cells and their reusability study

Resting cells of *Bacillus* sp. were efficiently immobilized by entrapment in agar, polyacrylamide, polyvinyl

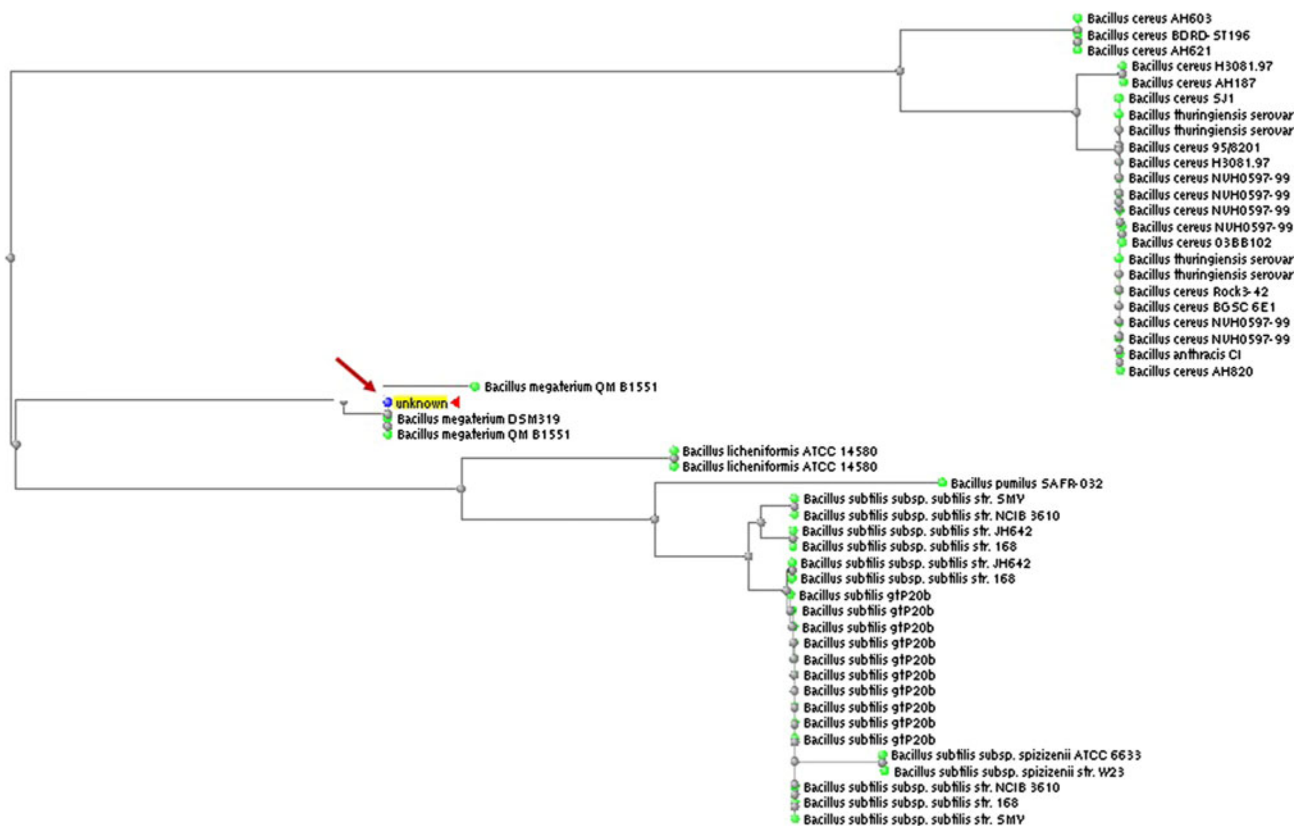

Fig. 2 Phylogenetic tree for the taxonomic location of strain F-8 obtained from neighbor joining method

Fig. 3 Electron micro photographic profile of strain F-8

alcohol–alginate and alginate gel. On entrapment of 5 mg dry weight of resting cells that contained 125 enzyme activity units, there was no change in reaction pH, temperature, substrate concentration and type of buffer with respect to free cells.

The level of recovery of enzyme activities after cell entrapment were 75, 65 and 80% for agar, polyacrylamide and polyvinyl alcohol alginate gel immobilization, respectively, whereas no loss of enzyme activity was observed in case of alginate gel immobilization. Similarly, agar and polyacrylamide gel entrapped cells showed lower temperature stability as compared to free resting cells.

Interestingly, in comparison to free resting cells, PVA alginate gel and alginate gel immobilized cells showed higher thermal stability, probably due to inert nature of alginate; moreover, the gelling reaction was also endothermic.

When immobilized cells were recycled, up to the fifth recycle, a gradual increase in enzyme activity was observed both in agar and polyacrylamide gel and afterwards activity remained constant till the tenth recycle. Initially, entrapped resting cells exhibited about 75, 65 and 80% residual enzyme activity in agar, polyacrylamide and PVA alginate gel respectively, however, it was increased to about 92, 85 and 98% after the fifth recycle. It might be possible that after repeated use of immobilized biocatalyst, a certain level of substrate and product is maintained within the immobilized system which favors the reaction, however, gradual swelling of the gel discs on each recycle was noticed which might facilitate substrate permeability through the compact gels. In conclusion, PVA alginate and alginate gel discs showed better reusability in comparison to agar and acrylamide gel beads.

Table 1 Morphological, biochemical and physiological characteristics of F-8 isolate

Feature	F-8
Shape	Rod
Spore	+
Gram reaction	+
Catalase	+
Oxidase	–
Motility	+
pH range for growth	6–9
Acid from	
D-Mannose	–
D-Fructose	+
Maltose	+
D-Glucose	+
Lactose	–
Galactose	–
D-Mannitol	–
Sucrose	+
Arabinose	+
Hydrolysis of	
Starch	+
Gelatin	+
Urease	–
Indole production	–
MR	+
VP	+
Utilization	
Inulin	–
H_2S production	–
Nitrate reduction	+

Optimization of reaction condition for assay of acyl transferase activity

Assay of acyl transferase activity of immobilized bacterial cells showed that there was no significant change in type of buffer, pH, temperature and substrate concentration with respect to free cells.

The highest acyl transferase activity of resting cells was around at pH 7.7 while alginate entrapped cells showed pH optima at 7.0 (Fig. 4). This slight shift of pH optimum to a lower pH value may be due to the influence of matrix. The matrix limits the diffusion of ions including the reaction products i.e. acetohydroxamic acid which alters the pH value locally within the matrix.

Sodium phosphate buffer showed maximum enzyme activity with immobilized cells while potassium phosphate proved to be slight better to sodium phosphate buffer with free cells (Fig. 5).

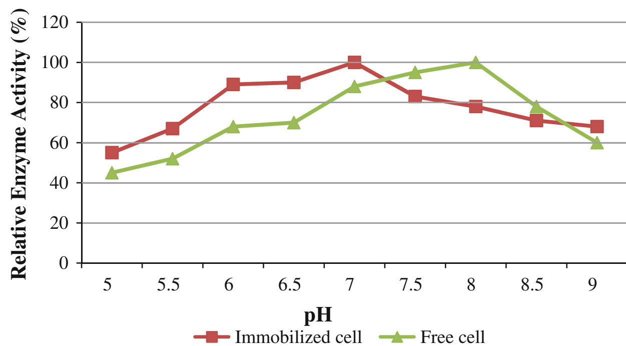

Fig. 4 Effect of Buffer pH on acyltransferase activity immobilized cells versus free cells of F-8

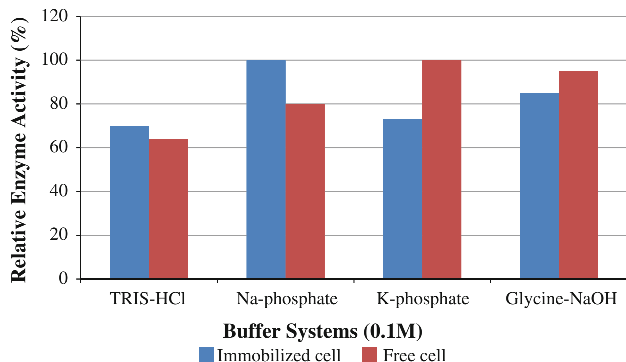

Fig. 5 Effect of different buffer systems on acyltransferase activity of immobilized cells versus free cells of F-8

Potassium phosphate was not compatible with alginate because there occurs a preferential exchange of calcium ions from the alginate to the phosphate in potassium phosphate buffer. Consequently, after the exchange reaction, the resultant potassium alginate was highly water soluble.

Immobilized cells showed a maximum activity at concentration of acetamide equivalent to 600–700 mM in comparison to free cells which showed maximum enzyme activity at lower concentration of acetamide since the growth of the free cells was completely inhibited by higher concentration (Fig. 6).

The abrupt decrease of enzyme activity above 700 mM resulted from product inhibition due to limited diffusion in matrix. However, limitation by diffusion did not appear to be of significant importance at lower acetamide concentration because the turnover of acetamide by free cells was almost similar to immobilized cells.

Maximum acyl transferase activity was obtained at temperature ranging from 45 to 55°C for both free and immobilized cells (Fig. 7).

However alginate gel immobilized cells showed higher thermal stability in comparison to free cells probably due to inert nature of alginate, moreover the gelling reaction was also endothermic.

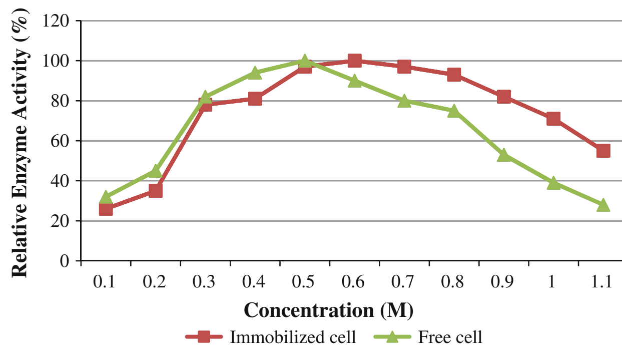

Fig. 6 Effect of acetamide concentration on acyltransferase activity of immobilized cells versus free cells of F-8

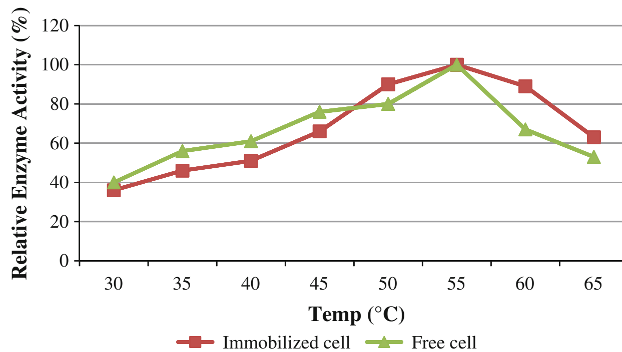

Fig. 7 Effect of temperature on acyltransferase activity of immobilized cells versus free cells of F-8

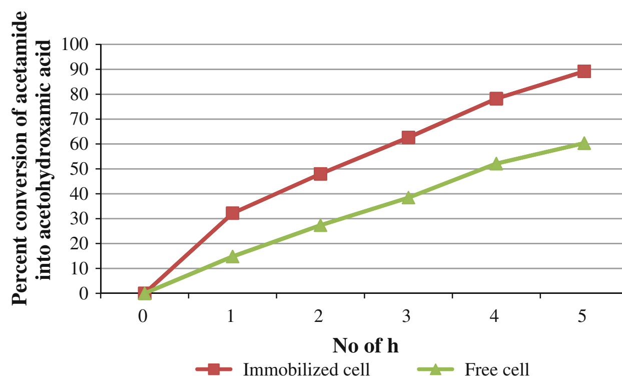

Fig. 8 Percent conversion of acetamide to acetohydroxamic acid by alginate gel entrapped resting cells and free cells of F-8 strain

Degradation pattern of acetamide

The rate of hydroxamic acid synthesis gradually increased in both free and immobilized cells with incubation time. Immobilized cells could easily degrade approximately 90% of the xenobiotic after just 5 h of incubation at 55°C whereas free cells grow poorly at 55°C and degraded less than 60% of the xenobiotic (Fig. 8).

This data indicated that immobilized cells of *Bacillus* are faster than free cells in degrading acetamide to

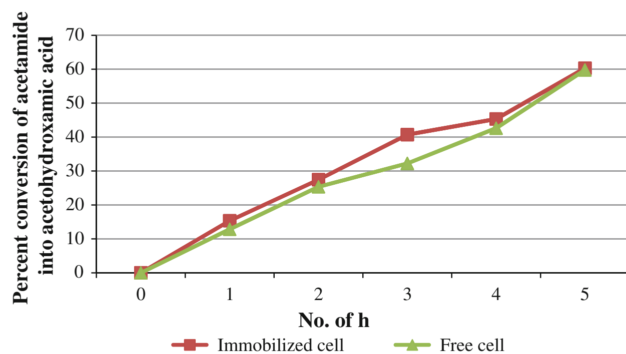

Fig. 9 Percent conversion of acetamide to acetohydroxamic acid by polyvinyl alcohol alginate gel entrapped resting cells and free cells of F-8 strain

acetohydroxamic acid and this rapid transformation is due to the fact that immobilized cells contain a high concentration of cells at their peak catabolic activity whereas free cells take time to multiply. Also, the alginate matrix of the immobilized cells protects the cells from the substrate toxicity and offers a protective shield against adverse physical conditions. The PVA alginate immobilized cells degraded 60% of the xenobiotic after 5 h of incubation at 55°C (Fig. 9).

Therefore, this PVA–alginate method is suitable and can be successfully applied for immobilization of the amidase from *Bacillus*.

Maximum acyltransferase activity of *B. megaterium* F-8 was recorded at 55°C after incubating the reaction mixture for 20 min. Enzyme activity increased gradually from 30 to 55°C, above this temperature loss in activity occurred. Nawaz et al. (1994) reported maximum acyltransferase activity for amidase of *Rhodococcus* sp. R312 at 30°C. Majority of the acyltransferase enzymes optimally catalyse acetamide degradation in the mesophilic range (23–37°C), but Cramp et al. (1997) recorded that the acyl transferase activity of *Bacillus* strain is thermophilic in nature. This is similar to current finding of incubation temperature for maximum acyltransferase activity of *B. megaterium* F-8. Biocatalyst thermostability is of paramount importance for any bioprocess (Banerjee et al. 2002). In the present process, the bioconversion yield determined for the biosynthesis of acetohydroxamic acid is almost 90% which is higher to 86% synthesised using the amidase of *Rhodococcus* sp. R312 as reported earlier (Fournand et al. 1998).

Conclusion

Due to the efficient degradation of amide up to 90%, it is believed that immobilized cells of isolate F-8, characterized and identified as *B. megaterium* F-8 has high potential for bioremediation of waste water containing toxic amides. The results obtained in this investigation show that *B. megaterium* F-8 entrapped in alginate gel discs express thermostable acyl transferase activity that may be used for the synthesis of hydroxamic acid. Biotransformation at commercial scale has a very high potential in contrast to chemical processes for the synthesis of this commodity compound which is a key compound in medicine, analytical chemistry, phytochemistry, agronomy, nuclear technology and waste water treatment studies (Fournand et al. 1997).

The amidase used here exhibits unusual characteristics such as excellent thermo stability and high bioconversion rates and also, the process reported here is simple and less expensive as compared to previously reported process as it uses only whole cells and not the purified enzymes, which implies that it may be novel amidase and is worthy of further studies.

Therefore, it is concluded that *Bacillus* or the immobilized cells of this microbe could be successfully used for the treatment of industrial waste water containing toxic amides. In light of such wide applications of hydroxamic acid, further application oriented studies are required to fully exploit biotechnological potential of such versatile biocatalyst.

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