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ISOLATION, IDENTIFICATION AND SUBSTRATE SPECIFICITY OF A NITRILASE PRODUCING BACTERIA, *Acidovorax* sp. SK1

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ABSTRACT

The process of biocatalysis or biotransformation remains the core of industrial biotechnology owing to its importance in the synthesis of high-value products in a cost effective manner. Nitrile biotransformation has also achieved considerable attention in last few decades due to its widespread biotechnological and industrial applications. In the present study, a versatile, potent nitrile-degrading bacterium, *Acidovorax* sp. SK1 was isolated from the cracker waste dumping site of Sivakasi, Tamilnadu and characterized for its biocatalytic potential, nitrilase production and enzyme activity. A pH sensitive indicator-based assay was performed to identify the nitrile degrading ability of the isolated samples. Semi quantitative High performance thin layer chromatographic (HPTLC) method was performed for quantitative measurement of mandelic acid produced from degradation of nitrile compound, mandelonitrile. The optimization of medium and nutritional parameters were studied for the improvement of nitrilase activity, which indicated that maximum nitrilase activity was observed at an optimum pH of 7.0, agitation at 100 rpm, glucose as best carbon source (10 g/L) and yeast extract (0.1 g/L) as principal nitrogen source. Biomass is also a critical parameter in the biocatalysis of mandelonitrile to mandelic acid and at a biomass of 100 mg/L, maximum nitrilase activity of 0.026 I.U was observed. The versatility of *Acidovorax* sp. in the hydrolysis of mandelonitrile to pharmaceutically valuable mandelic acid makes it a potential biocatalyst in organic synthesis. The present work demonstrates the production of nitrilase and the biotransformation of mandelonitrile to mandelic acid using the newly isolated strain of *Acidovorax* sp. SK1 and also suggests that this strain open avenues for biotechnological application as an effective green catalyst.

Keywords: *Acidovorax* sp., Biocatalysis, HPTLC, Mandelonitrile, Mandelic acid, Nitrilase

INTRODUCTION

The enzymatic biocatalysis or biotransformation process offers an efficient catalytic strategy and remains the core of industrial biotechnology for the synthesis of valuable products such as mandelic acid and nicotinic acid of industrial importance (**Choi *et al.*, 2015**). Nitrile biotransformation has been the subject of attention for a couple of years due to the interest for industrial production of high value chemicals i.e. acids and amides. Nitrilase, nitrile amino hydrolases which hydrolyze nitriles and forms corresponding carboxylic acids, ammonia and also amide as intermediate are commercially important enzymes. A wide variety of microorganisms like bacteria, Actinomycetes, filamentous fungi, yeasts and also plants have the inherent ability to metabolize nitrile compounds by virtue of the presence of nitrilases (**Gong *et al.*, 2012**). Nitrile hydrolyzing enzymes such as nitrilase, nitrile hydratase, and amidases have the inherent properties to carry out selective hydrolysis at specific temperature and pressure which allow their importance in industrial biocatalysis process (**Howden and Preston, 2009**).

Nitriles are a group of highly toxic organic compounds, commonly found in nature. Among the different nitriles, cyanoglycosides and cyanolipids are formed by a number of plants especially among the members of family *Brassicaceae.* These nitriles are highly toxic and carcinogenic and nitrile degrading microorganisms seems to be promising method for eliminating these pollutants without forming any toxic byproducts (**Fang *et al.*, 2015**). Nitrilase (3.5.5.1) hydrolyzes carbon nitrogen triple bond of nitriles to form corresponding acid and liberate ammonia. The inherent properties of high substrate specificity, enantioselectivity, and regio-selectivity make nitrilases attractive biocatalysts for the production of fine chemicals and industrially important pharmaceutical intermediates such as nicotinic acid, isonicotinic acid, mandelic acid, glycolic acid, benzoic acid, hydroxybenzoic acid etc. (**Bhalla *et al.*, 2018**). Based on the above facts and also due to its commercial importance the present study using the microbial route for the synthesis of mandelic acid by enzymatic degradation of mandelonitrile was undertaken in the present study. Mandelic acid is an important intermediate for the preparation of semisynthetic penicillin, cephalosporin, and other antibiotics. Though a number of enzymatic methods were developed for the synthesis of mandelic acid; the nitrilase-catalyzed hydrolysis gains considerable interest. The properties such as mild reaction conditions, high enantioselectivity, independence of expensive cofactor, and most importantly, this bioprocess can afford 100% theoretical yield with greater industrial importance makes the nitrilase-catalyzed hydrolysis process of significant interest (**He *et al.*, 2007; Zhang *et al.*, 2015**).

The conventional methods of screening are more tedious and time consuming. So that high throughput calorimetric methods were used for nitrilase producing organisms. It's a simple pH responsive calorimetric method where pH indicators like bromothymol blue, neutral red, phenol red or cresol red can be used. These pH indicators detect a decrease in pH on formation of the corresponding acid from nitriles during the biotransformation process. Most of these screening methods are quantitative in nature and are readily available. Reaction products are separated and analyzed by chromatographic methods or calculated in coupled reactions. Liquid chromatography-mass spectrometry (LC-MS) or Gas chromatography-mass spectrometry (GC-MS) is mainly used for precise analysis of the reaction products and specifically for enantioselectivity. Thin layer chromatography or high performance thin layer chromatography (HPTLC) have been often employed for semi quantitative and qualitative detection of nitrilase enzyme activity (**Gong *et al.*, 2012**).

Enzyme or biocatalyst which helps organisms to carry out metabolism is also used for the commercial production of natural and non-natural compounds. In the present study, a versatile, potent nitrile-degrading bacterium, *Acidovorax* sp. SK1 was isolated and its biocatalytic potential, nitrilase production and enzyme activity was evaluated for synthesis of industrially important mandelic acid by enzymatic degradation of mandelonitrile amply supported by GC-MS and HPTLC analysis.

MATERIALS AND METHODS

Chemicals and culture media

Analytical grade media components, DNA isolation kit, 16S rRNA amplification chemicals, reagents for ammonia assay, organic solvents for HPTLC and materials for immobilization were purchased from Hi-Media laboratory, Mumbai, India. All nitriles, isobutyronitrile, mandelonitrile, 3-phenyl propionitrile, 4-methoxy 2-nitrobenzonitrile and mandelic acid were obtained from Sigma Aldrich, New York, USA.

Sample collection

Cracker waste samples were used for isolating nitrile-degrading microorganisms. These samples were obtained from the cracker manufacturing industries of Sivakasi, Tamilnadu, India and transported to the laboratory in sterile Himedia bags and stored at 4 °C until further use.

Isolation of nitrile-converting new biocatalysts

Conventional methods of isolating microbial colonies and enrichment culture were carried out. The isolated colonies were streaked onto minimal salt media plates supplemented with Isobutyronitrile for selecting nitrile utilizing colonies. In enrichment culture method Mineral salt media components were dissolved in 1 liter of double distilled water and autoclaved. 1.0 g of soil sample added to 1 liter mineral salt medium spiked with an isobutyronitrile (2%, v/v) as an inducer for a period of 15 days at pH 7.0 and the flask was incubated at 37 °C with agitation at 150 rpm. After enrichment, serial dilutions were performed and plated on solid Enriched mineral salt agar medium plates containing isobutyronitrile as substrate. Colonies that appeared after 72 h were picked up and further purified by streaking on enriched agar plates to obtain single isolated pure colonies.

Screening of mandelonitrile converting bacteria

Nitrilase activity was determined qualitatively by pH sensitive calorimetric assay (**Banerjee *et al.*, 2003**). The reaction mixture consist of 201µl phosphate buffer (10 mM pH 7.2), 23.25 µl cell suspension (200 mg/mL), 5.75 µl mandelonitrile (from a 500 mM stock in ethanol) 0.01% bromothymol blue.

Analysis of mandelic acid by Thin layer chromatography (TLC)

The reactions with positive strains were repeated at large scale. Cells were harvested by Centrifugation, washed with phosphate buffer, pH 7 and re suspended in same buffer to give a resting cell concentration of 200 mg/mL. Reaction mixture volume was scaled up 10 ml and the product is extracted and analyzed by TLC (Precoated TLC sheet ALUGRAM). A mobile phase of toluene: dioxane: acetic acid (90:25:4), reference samples mandelic acid and Mandelonitrile (0.1 g) dissolved in 5 ml of methanol were used. Sample solution (20 µl) along with the reference solution (20 µl) was loaded on the TLC plates. Chromatogram was run till the solvent reached 3/4th of the TLC plate. The plates were dried and observed under UV light (**Pathak *et al.*, 2008**)

Estimation of ammonia

Nitrile-hydrolyzing activity was measured by phenol-hypochlorite method containing Reagent A - Phenol plus nitroprusside; 5 grams of phenol with 25 mg of sodium nitroprusside per 500 ml of solution. Store in amber bottle in refrigerator, Reagent B-Alkaline hypochlorite; 2.5 grams of sodium hydroxide, 4.2 ml of sodium hypochlorite to 500 ml of solution. Nitrilase enzyme activity was studied at 37 °C for 30 min. The reactions were carried out based on 3.7 and The Reaction was stopped by adding 0.05 ml 2N HCl and the mixture centrifuged at 10,000 rpm for 10 min at 4 °C. Supernatant was taken for ammonia assay (**Badoei-Dalfard *et al.*, 2016**). One unit of the enzyme activity was defined as the amount of cells that hydrolyzed the nitrile to release one micromole of ammonia per minute under standard assay conditions. The weight of wet cells in milligrams was calculated and used for the assay.

Microbial identification

The positive strain for nitrilase SK1 was identified by conducting various Biochemical tests, SEM analysis and 16S rRNA. The primers used for the analysis were; 27F AGAGTTTGATCCTGGCTCAG 1492R CGGTTACCTTGTTACGACTT.

Optimization of culture condition

Different parameters were studied for the optimum production of enzyme and its activity. Further substrate specificity of the enzyme were evaluated by using different nitriles at optimal condition for the optimum production of enzyme the following parameters like substrate, concentration of substrate pH carbon source nitrogen source biomass time of incubation were investigated.

Substrate specificity

The *Acidovorax* sp. SK1 was grown in minimal salt media, cells were harvested by centrifugation and suspended in phosphate buffer and the reaction was carried out using different nitrile substrates like mandelonitrile, 4-methoxy, 2-nitrobenzonitrile, 3-phenyl propionitrile and isobutyronitrile. Enzyme assay was carried out to determine the substrate specificity.

Substrate concentration

To check the effect of concentration of substrate on the activity of enzyme, assay was carried out with various concentrations (1mM to 4mM) of mandelonitrile.

Effect of pH

Enzyme assay was carried out at different pH (5-8) to study the suitable pH for enhanced nitrilase activity.

Effect of carbon source

The *Acidovorax* sp. SK1 grown on different carbon source such as sucrose, glucose, maltose and fructose of 10 g/L concentration keeping other media constituents and physiological parameters constant and the activity was assayed after 24 h of growth.

Glucose concentration

The concentration of glucose in the minimal salt media was varied from 5-20 g/L. ammonia assay was carried out and enzyme activity was calculated.

Effect of nitrogen source

To check the effect of nitrogen source the *Acidovorax* sp. SK1 grown on different sources like yeast extract, urea, ammonium chloride and peptone of 1 g/L concentration keeping other media constituents and physiological parameters constant.

Yeast extracts concentration

The concentration of yeast extract in the minimal salt media was varied from 0.5-2 g/L. The ammonia assay was carried out and enzyme activity is calculated.

Time of incubation and enzyme activity

In order to achieve the maximum synthesis of nitrilase enzyme, *Acidovorax* sp. SK1 was cultured at optimal conditions. Different Erlenmeyer flasks were inoculated with fresh culture in triplicate and were removed at different time intervals for enzyme assay.

Effect of biomass

To attain the maximum nitrilase enzyme, the biomass production was investigated under optimal conditions. 50 to 200 mg/mL wet cell weight was taken the reaction mixture containing 4 mM mandelonitrile in phosphate buffer (10mM, pH 7.0) with these concentrations of wet cell weight incubated at 37 ºC and the nitrilase activity was assayed.

GC-MS analysis of mandelic acid

The optimized product, mandelic acid were further confirmed by GC-MS. Analysis was performed by using Perkin–Elmer GC Clarus 500 system gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with Elite 1. Electron impact mode of the mass spectrometer was set to 70 eV. The GC oven temperature was initially held at 60 °C and raised with a gradient of 5 °C per minute until it reached 180 °C. It was then raised with a gradient of 10 °C per minute until it reached 260 °C. The flow rate of the mobile phase was set to 1 ml min^{-1}.

Quantification of mandelic acid by HPTLC

After optimization minimal media prepared based on the optimized parameters and *Acidovorax* sp. SK1 cultured in large scale. HPTLC is used to quantify the mandelic acid produced by the bacteria. Standards of mandelic acid (obtained from sigma) were prepared in 1 µg, 10 µg and 100 µg concentration in methanol. Mobile phase comprises of toluene: dioxane: acetic acid (90:25:4) was used. The sample solution (20 µl) along with the reference solution (20 µl) was loaded on the TLC plates using HPTLC machine. Chromatogram was run till the solvent

reached 3/4th of the TLC plate. The plates were dried and observed under 240 nm. Mandelic acid is quantified using winCATS Planar Chromatography Manager Software.

Immobilization

To check suitable matrix for immobilizing *Acidovorax* sp. sodium alginate, agar and agarose were used.

Sodium alginate

Cells suspended in phosphate buffer thoroughly mixed with sodium alginate solution 3% w/v then this mixture added drop wise to a stirred solution of calcium chloride 2% w/v by using syringe. After stirring beads were filtered through muslin cloth and stored in calcium chloride solution

Agarose

In the case of agarose, the harvested cells were washed, suspended in phosphate buffer (100 mM, pH 7), mixed with 3% w/v agarose above gelling temperature, and allowed to cool. The resulting gel was cut in cubes and used as biocatalyst.

Agar

For immobilization, 3% w/v of agar-agar (Hi-media, Mumbai, India) was dissolved in buffer and autoclaved. The cell suspension was mixed aseptically with the agar at 40–50 °C and poured on a flat-bottom petriplate gel was cut in cubes. The agar beads so formed were washed with phosphate buffer and sterile distilled water twice and finally stored at 4 °C till further use (**Nigam *et al.*, 2009**).

RESULTS

Sample collection

Fourty seven different bacterial strains were isolated from cracker waste samples from the cracker manufacturing industries of Sivakasi, Tamilnadu, India and were used for isolation of nitrile-degrading microorganisms.

Screening of mandelonitrile converting bacteria

This method is based on decrease in pH that occurs as the reaction proceed due to the formation of acid by nitrile hydrolysis, which is reflected by color transition of the pH indicator, bromothymol blue from deprotonated form to protonated form over an interval of 4 h (Fig. 1).

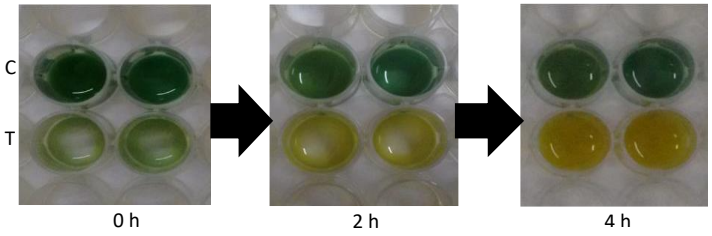

Figure 1 The color transition of bromothymol blue from deprotonated form toprotonated form over an interval of 4 h, depicting the conversion of nitrile compound to respective acids

Analysis of mandelic acid by Thin layer chromatography (TLC)

The TLC data (Rf) of extracted product showed presence of mandelic acid where we used mandelonitrile and mandelic acid as standards (Fig. 2).

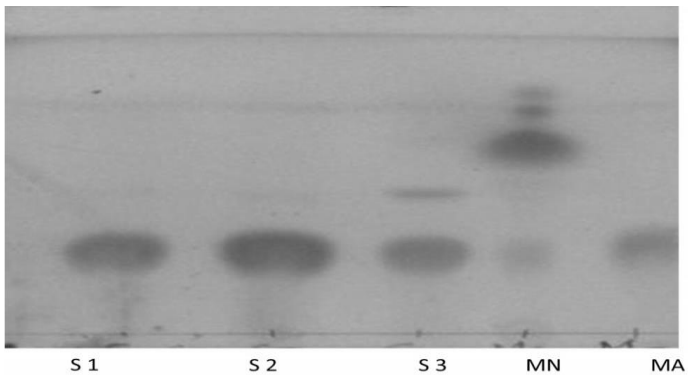

Figure 2 TLC profile showing the presence of mandelic acid

Estimation of ammonia

From the standard plot for ammonia, ammonia released during the reaction was observed to be 1.06 µg/mL. Nitrilase activity was found to be 0.021 I.U. and is calculated in I.U units by using the following equation,
IU/mL = mg/mL x 1000 / Incubation time x volume of enzyme x molecular wt

Microbial identification

The positive strain for nitrilase SK1 was identified by conducting various Biochemical tests, SEM analysis and 16S rRNA. The 16S rRNA sequencing followed by sequence similarity search in NCBI GenBank and comparative phylogenetic analysis revealed the isolated bacteria to be *Acidovorax* sp. SK1 with 82% homology (Fig. 3A). The selected bacterium was further identified using SEM analysis (Fig. 3B).

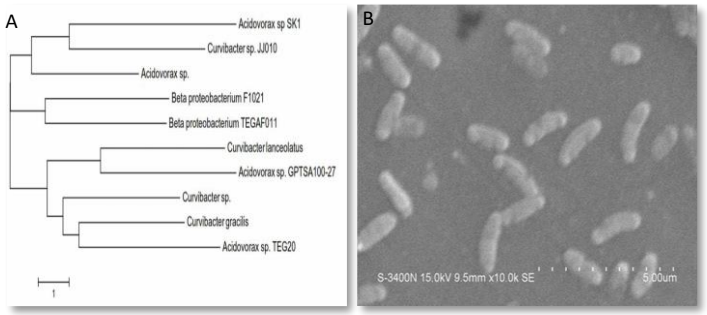

Figure 3 (A) Phylogenetic tree analysis of *Acidovorax* sp. SK1 with other proteobacteria using neighbor joining and maximum likelihood method, (B) Scanning electron micrograph (SEM) of *Acidovorax* sp. SK1

Optimization of culture condition

Substrate specificity

The *Acidovorax* sp. SK1 showed highest specificity towards mandelonitrile for nitrile biocatalytic activity (Fig. 4).

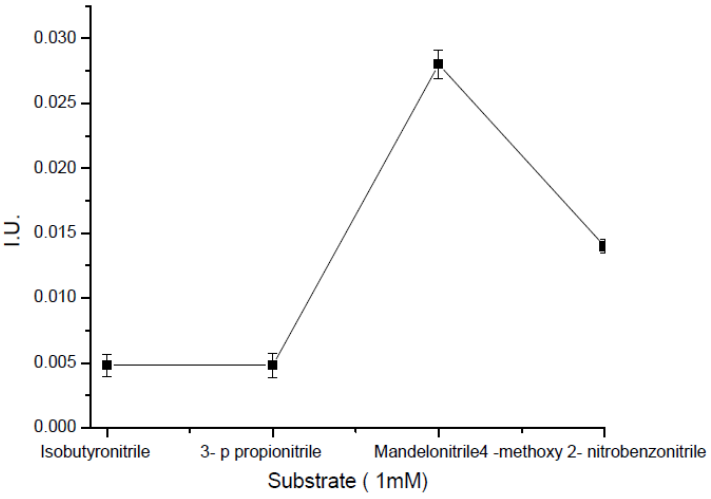

Figure 4 Substrate specificity of *Acidovorax* sp. SK1 for nirilase activity using different nitrile compounds

Substrate concentration

For the different concentrations of mandelonitrile used, the nitrilase activity was increased with concomitant increase in the concentration of mandelonitrile. The highest activity was observed at 4 mM concentration and hence all further investigations were carried out at 4 mM concentration (Fig. 5A).

Effect of pH

From the pH optimization analysis, it was observed that at pH 7.0, the nitrilase activity of *Acidovorax* sp. SK1 was observed to be highest. The nitrilase activity was increased with increase in pH from 5.0 to 7.0, and beyond pH 7.0 there was a significant decrease in the enzyme activity (Fig. 5B).

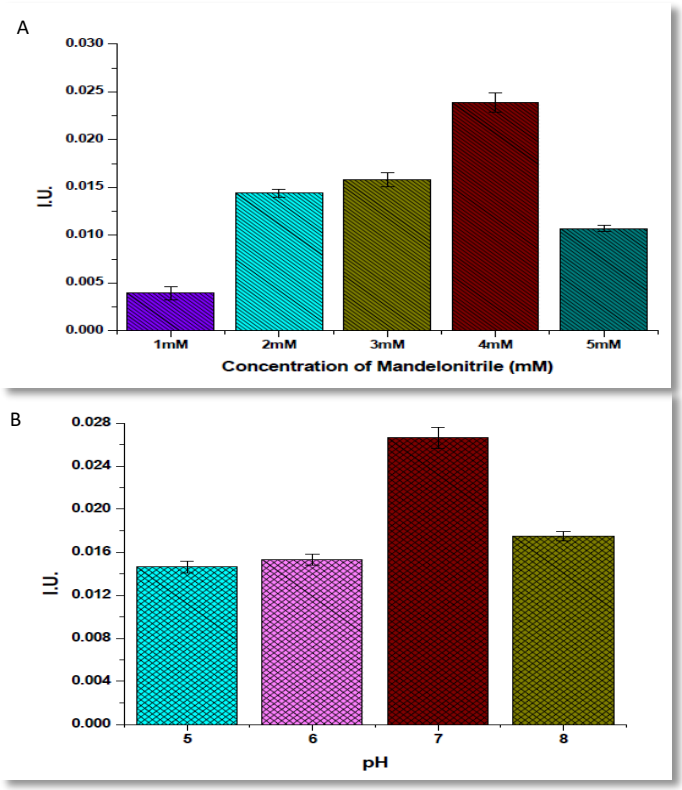

Figure 5 (A) Effect of different concentrations of mandelonitrile on the nitrilase activity of *Acidovorax* sp. SK1; (B) Effect of different pH on the nitrilase activity of *Acidovorax* sp. SK1

Effect of carbon source

From the different carbon sources used, *Acidovorax* sp. SK1 showed highest nitrilase activity when glucose was used as carbon source and 10 g/L concentration of glucose was found to be optimum for enhanced nitrilase activity (Fig. 6A, 6B).

Effect of nitrogen source

From the different carbon sources used, *Acidovorax* sp. SK1 showed highest nitrilase activity when yeast extract was used as sole nitrogen source and 1.0 g/L concentration of yeast extract was found to be optimum for enhanced nitrilase activity (Fig. 6C, 6D).

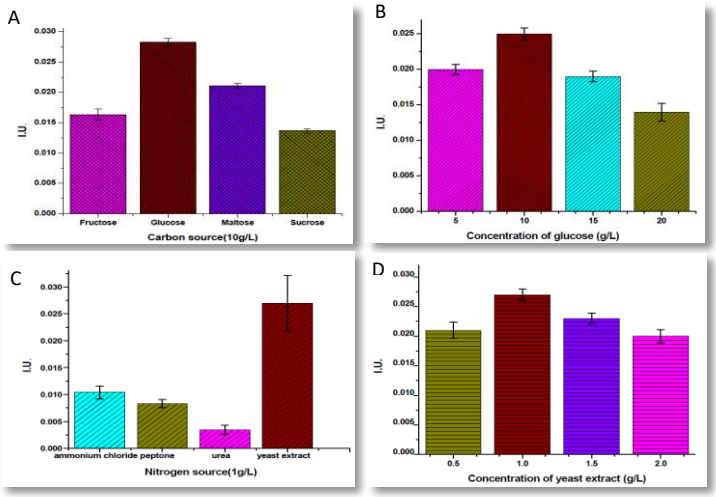

Figure 6 (A) Effect of different carbon sources on the nitrilase activity of *Acidovorax* sp. SK1; (B) Effect of different concentration of glucose on the nitrilase activity of *Acidovorax* sp. SK1; (C) Effect of different nitrogen sources on the nitrilase activity of *Acidovorax* sp. SK1; (D) Effect of different concentration of yeast extract on the nitrilase activity of *Acidovorax* sp. SK1

Time of incubation

The nitrilase activity of *Acidovorax* sp. SK1 was found to be increased with increase in the incubation time upto 18 h, beyond which a marked decrease in activity was observed. Hence, 18 h was optimized for highest enzyme activity (Fig. 7A).

Effect of biomass

The reaction mixture containing different concentrations of wet cell weight (50-200 mg/L) was used and the nitrilase activity was assayed. The highest activity (0.026 IU) was observed when 100 mg of wet cell weight was used (Fig. 7B).

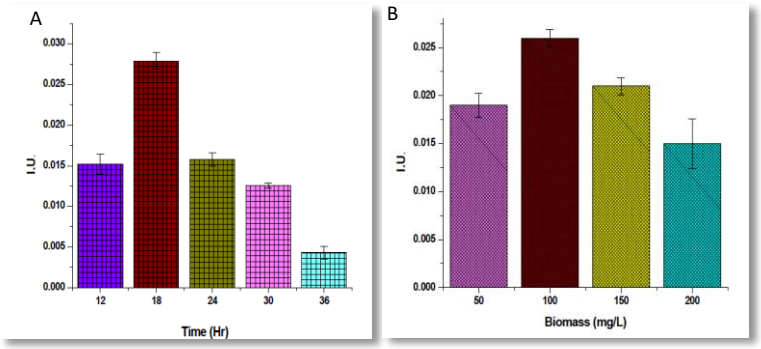

Figure 7 (A) Effect of incubation time on the nitrilase activity of *Acidovorax* sp. SK1; (B) Effect of biomass on the nitrilase activity of *Acidovorax* sp. SK1

GC-MS analysis of mandelic acid

From the GC-MS analysis, the ions at m/z = 77.10 [M-H]- suggested the presence of methyl mandelate, esterified product of mandelic acid at a retention time of 7.5 min. The interpretation on mass spectra was conducted using NIST database. The spectrum of the product was compared with the spectrum of known compounds stored in the NIST library (Fig. 8).

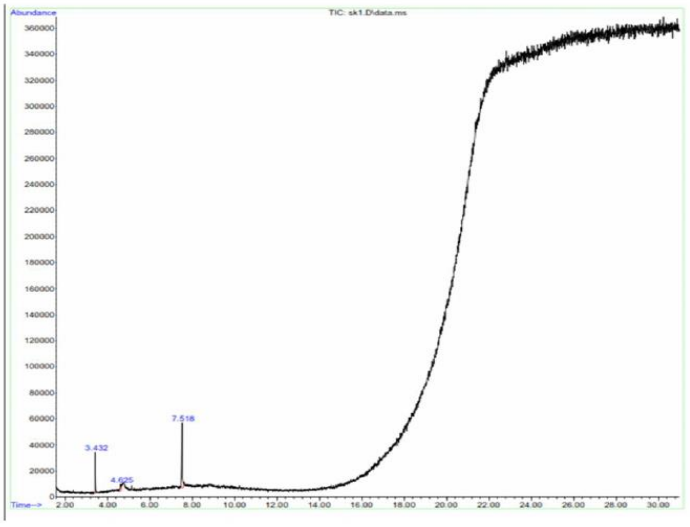

Figure 8 GC-MS analysis of mandelic acid

Quantification of mandelic acid by HPTLC

From the HPTLC analysis, the quantity of mandelic acid present in bacterial sample was quantified to be approximately 751.48 ng obtained from the standard plot (Fig. 9).

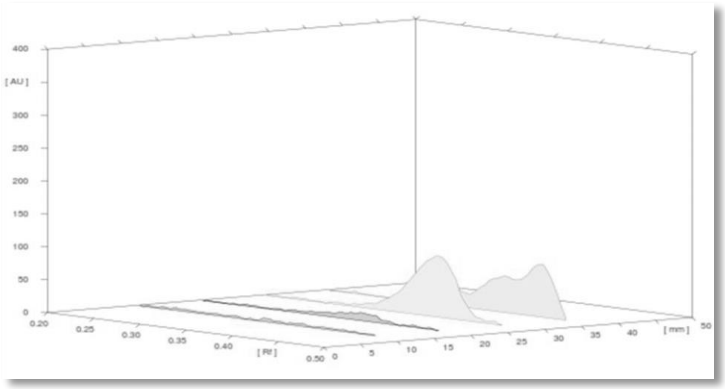

Figure 9 Quantification of mandelic acid with three different concentrations of standards by using HPTLC

Immobilization

From the different materials used for immobilization, agar was found to be more effective showing the highest enzyme activity as compared to sodium alginate and agarose (Fig. 10).

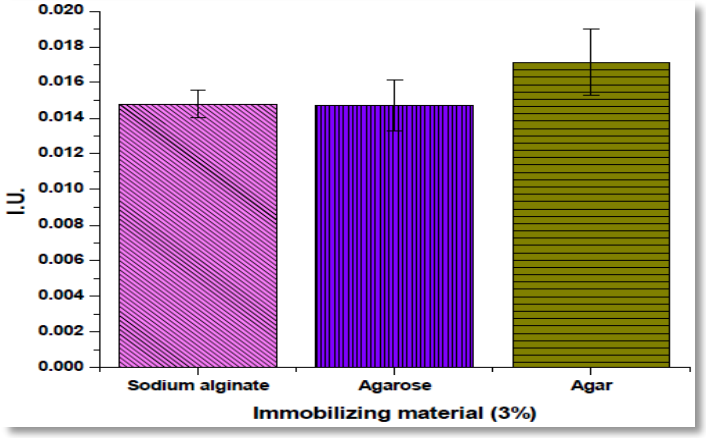

Figure 10 Nitrilase activity of different immobilized cells

DISCUSSION

The biotransformation of nitriles has been studied in great detail because of its widespread biotechnological and industrial potential. The conversion of nitriles by nitrilase enzyme into carboxylic acids is valuable and the scope of possible applications has been broadened recently. Isolation and screening of nitrilase producing organisms require sophisticated methods like high throughput screening rather than conventional methods (**Novikov *et al.*, 2018**). Hence, the importance of continuing the screening for microorganisms of interest always remains. Here we used pH dependent calorimetric assay for screening a large group of bacterial isolates. By using pH responsive method, one can analyze large numbers of microorganisms simultaneously in a short period, thus reducing the number of samples to be analyzed. The assay requires very little substrate (5.75 µl of a 500m, stock), Moreover, this demands special instrumentation (micro plate reader), which can be avoided by visualizing the enzyme catalyzed reaction using a suitable indicator. Our goal is to use the method not for precise quantitation but for the screening of large numbers of microorganisms, allowing quick identification of organisms.

Acidovorax sp. are important microbial species with the ability to convert dinitriles into other valuable products (**Chen *et al.*, 2009**). The *Acidovorax facilis* 72W converts 3-hydroxynitriles to the corresponding 3-hydroxycarboxylic acids in high yield (**Hann *et al.*, 2003**). The substrate specificity of *Acidovorax* sp. SK1 is also following the same trend. Here the *Acidovorax* sp. SK1 has the potential to catalyze mandelonitrile and to form mandelic acid.

To establish a viable industrial process, the growth medium design and reaction parameters are critical and need to be improved for the maximal conversion of nitriles. Therefore, we have investigated the optimization of different environmental conditions that play an important role in the synthesis of nitrilase from *Acidovorax* sp. Most nitrilases are inducible enzymes, and in the literature a number of inducers have been used for the production of nitrilase, but not much attention has been given to the effect of different inducers on substrate specificity and also very little information is available on physicochemical parameters and other constituents of the medium that is used for the production of enzyme The nitrilase-producing cultures are known to synthesize the enzyme mostly at neutral pH, whereas the maximum enzyme activity was observed to be in the pH range 7.0–8.0 (**Zhang *et al.*, 2014**). In our study using *Acidovorax* sp. SK1 the maximum enzyme production was observed in the pH of 7.

A number of microbes have been reported in the literature but the optimization of different parameters for the maximum synthesis of enzymes has been scanty. Therefore we report in this article the optimization of different environmental conditions that play an important role in the synthesis and characterization of nitrilase produced from *Acidovorax* sp. SK1. A thorough literature review was performed and it was noticed that though a number of strains and a number of inducers have been used for the production of nitrilase, not much attention has been paid to the physico-chemical parameters and other constituents of the medium used for biosynthesis of enzyme. The nitrilase synthesizing isolates (*Alcaligenes faecalis* JM3, *Rhodococcus rhodochrous* J1, *R. rhodochrous* K22, *Arthrobacter* sp. J1, *A. facilis* 72W etc.) have been found to synthesize the enzyme mostly at neutral pH, but the maximum activity with respect to the specified substrate was observed to be in the pH range 7.0 to 8.0 (**Liu *et al.*, 2014; Lan *et al.*, 2017; Bhalla *et al.*, 2018**).

Investigation of substrate preferences of nitrilase showed that nitrilase secreted from different organisms prefers different nitriles. For example *R. rhodochrous* K22 hydrolyze aliphatic nitriles but the concentration of nitriles was very low (0.2 mmol/L). Similarly, nitrilase produced from *Bacillus pallidus* showed a broad substrate specificity hydrolyzing aliphatic, aromatic and heterocyclic nitriles (**Dennett and Blamey, 2016**), whereas *Acidovorax* sp. SK1 shows substrate specificity towards mandelonitrile and also to 4-methoxy, 2-nitrobenzonitrile. The whole-cell nitrilase activity of *A. facilis* 72W is very stable at temperatures of up to about 55° C.; a cell suspension in 0.10 M phosphate buffer (pH 7.0) (**Gavagan *et al.*, 1998**). Although *A. facilis* 72W, a mesophilic bacterium, has an optimal temperature for growth of 32 °C., nitrilase enzyme itself has a thermal stability that compares favorably to the nitrilase enzyme of a thermophilic bacterium such as the *B. pallidus* strain DAC521 (**Cramp *et al.*, 1997**) whereas *Acidovorax* sp. SK1 is stable at 37 °C with comparatively high enzyme activity of 0.026 I.U.

GC-MS analysis of mandelic acid shown the presence of methyl mandelate which is an esterified product of mandelic acid and it can be form when mandelic acid reacts with alcohol. Since, mandelonitrile is the cyanohydrin from benzaldehyde; there are chances of having residual benzaldehyde in the sample, accounting for a benzaldehyde peak in HPTLC and also in GC-MS analysis. Moreover, at neutral pH mandelonitrile has a tendency to deteriorate into benzaldehyde, which may get oxidized to benzoic acid and other compounds. The immobilization of whole cells and purified enzymes can make the biocatalytic process more economical. Most industrial processes for nitrile biocatalysis were performed using immobilized biocatalysts. However, little information is reported on the biotransformation of nitriles by free cells in commercial scale. Examples include immobilized *C. testosteroni* 5-MGAM-4D (nitrilase) for hydrolysis of 3- hydroxyalkanenitriles to 3-hydroxyalkanoic acids with >98% yield (**Hann *et al.*, 2003**), and immobilized *A. facilis* 72W (nitrilase) for the regioselective hydrolysis of (E, Z)-2- methyl- -butenenitrile for production of (E)-2-methyl-2-butenoic acid at 100% yield. Despite the sensitivity of nitrilase enzymes to various inactivating mechanisms, the *A. facilis* 72W nitrilase appears quite robust, losing little activity, even after numerous recycle reactions. The suitable matrix for immobilization of *Acidovorax* sp. SK1 were sodium alginate in terms of efficiency whereas agar immobilized cells were found to effective matrix comparing with its enzyme activity. Bacterial nitrilases that hydrolyze aromatic nitriles have been known for many years, but the identification of bacterial nitrilases that convert aliphatic nitriles is relatively recent. As per the recent reports, an aliphatic nitrilase from *A. facilis* 72W that catalyzes the hydrolysis of a wide variety of nitriles, including the highly regioselective hydrolysis of many dinitriles.

CONCLUSION

Enzymes that hydrolyze nitriles have enormous potential as industrial biocatalysts in which they can provide the common functional groups of carboxylic acids subsequent to the facile chemical addition of a carbon to a simpler (and cheaper) substrate. The application web of nitrilases is increasing as the knowledge on structural, biochemical and functional properties of enzymes is elucidated. Hence, scientific community is always on their toes to expedite the isolation of specific microbial strains exhibiting nitrilase activity from natural and exotic samples. In the present investigation, an effort was made to isolate a promising nitrilase producing microbial strain, identify the strain using 16S rRNA amplification and SEM analysis, improve the mandelic acid production by optimizing media and also the different biotransformation strategies, screening of immobilization matrix for reuse of enzyme and to increase the commercial importance immobilizing the enzyme.

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