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Biodegradation of acrylamide by acrylamidase from *Stenotrophomonas acidaminiphila* MSU12 and analysis of degradation products by MALDI-TOF and HPLC

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ABSTRACT

The present study examines an improved detoxification and rapid biological degradation of toxic pollutant acrylamide using a bacterium. The acrylamide degrading bacterium was isolated from the soil followed by its screening to know the acrylamide degrading capability. The minimal medium containing acrylamide (30 mM) served as a sole source of carbon and nitrogen for their active growth. The optimization of three different factors was analyzed by using Response Surface Methodology (RSM). The bacteria actively degraded the acrylamide at a temperature of 32 °C, with a maximum growth at 30 mM substrate (acrylamide) concentration at a pH of 7.2. The acrylamidase activity and degradation of acrylamide was determined by High Performance Liquid Chromatography (HPLC) and Matrix Assisted Laser Desorption and Ionization Time of Flight mass spectrometer (MALDI-TOF). Based on 16S rRNA analysis the selected strain was identified as Gram negative bacilli *Stenotrophomonas acidaminiphila* MSU12. The acrylamidase was isolated from bacterial extract and was purified by HPLC, whose mass spectrum showed a molecular mass of 38 kDa.

1. Introduction

The world's chemical production has elevated 400 times when compared to past century. The modern life can increase more chemical usage, polluting the landscapes around the world (WWF, 2013). Some chemicals severely damage the ecological system by interference with hormones and genes leading to hormonal and genetic disorder etc., in various living organisms and persists environmental accumulation (WWF, 2013). The harmful chemicals contaminate the world ecosystem from land to deep Ocean. Some toxic chemicals can be observed in animal's blood at Arctic zone. Annually millions of hectares of farming lands are lost due to hazardous wastes and toxic chemicals. About, 125 million peoples are at risk due to toxic pollution whereas 1.5 million peoples are at high risk towards the exposure of volatile organic compounds from chemical manufacturing sites (FAO, 1996; WWPP, 2012). Due to the heavy chemical pollution, the world needs innovative solutions to control the chemical pollution. Presently, human beings are taking attempts to reduce the world chemical pollution by waste management and recycling systems. Some industrial toxic chemicals were treated by physical and chemical treatment methods to reduce the harmfulness of chemicals during release. The biological degradation may play a key role in effective removal of chemical pollutant and converting it into economically valuable products.

Acrylamide (C_3H_5NO) is a toxic aliphatic amide which is colourless and odourless flake like crystals with a relatively high melting point (84.5 °C), water solubility (2155 g/l at 30 °C) and low vapour pressure (7×10^{-3} torr at 20 °C) which is usually formed industrially from the hydration of acrylonitrile (Lee and Chang, 1989; Budavari et al., 1989; ACGIH, 1991). While heating, it violently undergoes polymerization reaction (Keith and Walters, 1985). There are many research reports dealing with genotoxicity, carcinogenicity and neurotoxicity of acrylamide in both human beings and animals (US EPA, 1985; Shelby et al., 1987; Summer et al., 1999; Abramsson-Zetterberg, 2003; Hogervorst et al., 2007). Acrylamide has numerous industrial applications like manufacture

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of dyes, paper making, wastewater treatment, ore processing, thickening agent, agricultural sprays, latex dispersions and research laboratories (Mannsville, 1993) etc. Due to the low vapour pressure and high water solubility nature, acrylamide easily enters into the environment. At low concentrations (0.5–3±0.2 mM), acrylamide rapidly degrades but, in elevated levels (>10 mM) the possibility is less and it persists in the environment (Cherry et al., 1956; Brown et al., 1980). Many microorganisms produce amidase enzyme that catalyze the hydrolysis reaction against carboxylic amides and release free ammonia and carboxylic acid (Shukor et al., 2009; Minseok and Glenn, 2010). Particularly, many bacterial species (*Geobacillus thermoglucosidasius*, *Variovorax boronicumulans*, *Bacillus licheniformis*, *Kluyvera georgiana*, *Xanthomonas maltophilia*, *Rhodopseudomonas palustris*, *Ralstonia eutropha*, *Helicobacter pylori*, *Enterobacter aerogenes*, *Pseudomonas* sp, *Moraxella osloensis* etc.,) produce amidase enzyme that are capable of hydrolyzing acrylamide into acrylic acid and free ammonia by utilizing acrylamide as a sole source of carbon and nitrogen (Nawaz et al., 1992; Van et al., 2003; David et al., 2005; Sathesh and Thatheyus, 2007; Minseok and Glenn, 2010; Kanokhathai and Jittima, 2011; Richi et al., 2012; Uthumporn et al., 2012; Minseok and Glenn, 2013; Liu et al., 2013; Emmanuel et al., 2013). With the above perspective in view, a detailed investigation was carried out in isolation and characterization of *Stenotrophomonas acidaminiphila* MSU12 capable of degrading 30 mM of acrylamide.

2. Materials and methods

2.1. Chemicals used

Acrylamide (C_3H_5NO), other chemicals, reagents, biochemical test kits and media components used for this study was purchased from Hi-Media, Mumbai, India. All chemicals were of analytical grade, available commercially and were used without any further purification.

2.2. Isolation of acrylamide degrading bacteria

The soil samples were collected from a depth of 15 cm from the laboratory effluent at Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi and were transferred into an aseptic bag. The Phosphate Buffered Medium (PBM) [K_2HPO_4 – 1.0 g; KH_2PO_4 – 1.0 g; $MgSO_47H_2O$ – 0.2 g; $CaCl_22H_2O$ – 0.02 g per litre of deionized water (pH 7.3 ± 0.2)] was used to isolate the acrylamide degrading bacterial species (White et al., 1988; Van et al., 2003). In addition, 30 mM acrylamide was added prior to autoclaving. After autoclaving, the media was allowed to cool prior to use. Then 1 g of soil sample was inoculated and incubated in a rotary shaker (120 rpm) for 24–48 h at 30 °C. After incubation the cultures were plated into solid medium. The isolated colonies were differentiated based on the colony morphology. The isolated colonies were transferred into fresh 10 ml PBM broth with 30 mM acrylamide. After 48 h, the culture medium was centrifuged at 6000 rpm for 5 min. The supernatant was tested for ammonia concentration, as an indicator of acrylamide degradation (Nawa et al., 1989; Nawaz et al., 1993).

2.3. Identification of bacteria

The acrylamide degrading bacterial genomic DNA was extracted by alkaline lysis method. PCR amplification was performed using Eppendorf Mastercycle Gradient PCR (Eppendorf, India). 16S rRNA-targeted universal bacterial primers; FAM63F 5'-CAGGCCTAACACATGCAAGTC-3' and HEX1389R 5'-ACGGGCGGTGTGTACAAG-3' corresponding to the forward and reverse primers of 16S rRNA respectively were used (Marchesi et al., 1998). PCR was performed under the following conditions: initial denaturation at 94 °C for 3 min; 25 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min; and a final extension at 72 °C for 10 min. Sequence data were initially recorded and edited using CHROMAS Version 1.45. The resultant 976 bases were compared with the GenBank database using BLAST server at NCBI. The phylogenetic analysis was performed using ClustalW-multiple sequence alignment software package at http://align.genome.jp/site. The sequence data has been submitted to the GenBank databases under accession no. KF395361.

Table 1
The coding levels applied in optimizing the medium components (n = 3 experiment).

Sl.No.	Variables	Symbols	Coded levels				
			$-\alpha$	−1	0	+1	$+\alpha$
1.	Acrylamide (g/L)	A	21.59	25	30	35	38.41
2.	pH	B	5.52	6.2	7.2	8.2	8.88
3.	Temperature (°C)	C	28.64	30	32	34	35.36

Table 2
The response surface methodology, successive experimental design and results of central composite.

Run	A	B	C	Ammonia release (mg/L)
1.	−1	−1	−1	7.1
2.	+1	−1	−1	6.7
3.	−1	+1	−1	4.7
4.	+1	+1	−1	4.5
5.	−1	−1	+1	6.4
6.	+1	−1	+1	6.3
7.	−1	+1	+1	4.9
8.	+1	+1	+1	5
9.	$-\alpha$	0	0	6.7
10.	$+\alpha$	0	0	6.5
11.	0	$-\alpha$	0	5.2
12.	0	$+\alpha$	0	2.2
13.	0	0	$-\alpha$	7.4
14.	0	0	$+\alpha$	7.3
15.	0	0	0	8.5
16.	0	0	0	8.5
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18.	0	0	0	8.5
19.	0	0	0	8.5
20.	0	0	0	8.5

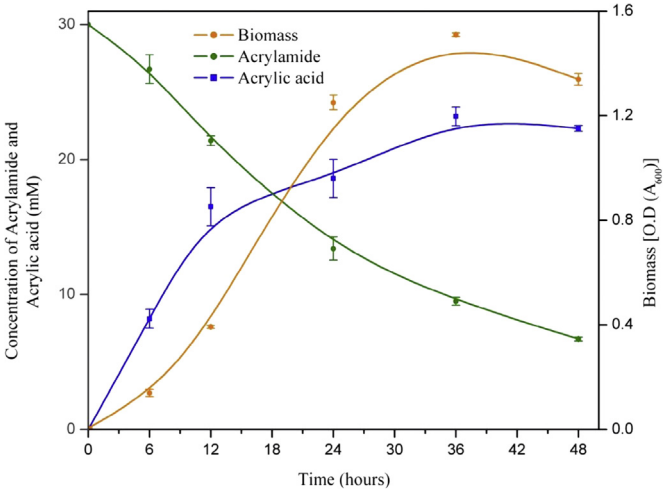

Fig. 1. The bacterial growth and acrylamide degradation on minimal medium containing non immobilized cells of *Stenotrophomonas acidaminiphila* MSU12. (values are mean ± SD, n = 3).