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Biodegradation of acrylamide by a novel isolate, *Cupriavidus oxalaticus* ICTDB921: Identification and characterization of the acrylamidase produced

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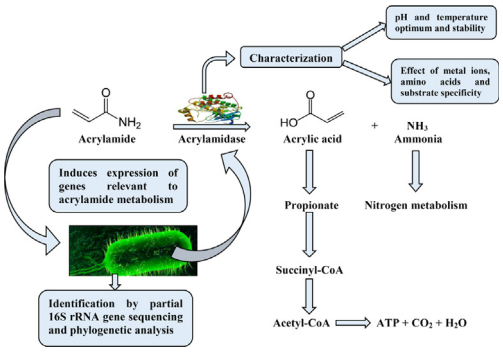
GRAPHICAL ABSTRACT



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ABSTRACT

Acrylamide is neurotoxic, genotoxic, teratogenic and carcinogenic. Its widespread use in various industrial processes leads to environmental contamination. Acrylamidase produced by certain bacteria degrade acrylamide to acrylic acid and ammonia. The present study details the isolation and identification of soil bacterium which could degrade acrylamide. Among the 18 acrylamide-degrading isolates tested, isolate ICTDB921 demonstrated superior acrylamide degradation which was confirmed by HPLC, FTIR and GC-MS. The partial 16S rRNA sequencing confirmed the isolate to be *Cupriavidus oxalaticus* ICTDB921, which showed highest growth at 60 mM acrylamide, neutral pH and 30 °C. The kinetic model predictions were consistent with experimental results. The acrylamidase from this isolate showed potency at pH (6–8) and temperatures (30–60 °C), with reasonable pH (6–8) and thermal stability (upto 60 °C). The enzyme was stable against most metal ions and amino acids, and also degraded other aliphatic amides, demonstrating its potential in remediation of acrylamide from the environment and food systems.

1. Introduction

Soil and water pollution by direct/indirect discharge of untreated industrial effluents containing toxic compounds (Kusnin et al., 2015) is a growing concern in many countries. During last few decades researchers have been focusing on isolation of promising microorganisms from environment for bioremediation, which is most promising strategy to remove acrylamide from polluted environment (Charoenpanich, 2013).

Acrylamide (C_3H_5NO), a crystalline aliphatic amide is most commonly used in the synthesis of various polymers such as plastic and polyacrylamide (Scott et al., 1996). Some acrylamide is used in

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scientific research for selective modification of −SH group in protein, manufacturing of dyes and other monomers (Charoenpanich, 2013; Cha and Chambliss, 2011). Polyacrylamide gels prepared from acrylamide are used for electrophoretic separation of proteins and other compounds. Polyacrylamide contains traces of residual acrylamide which is a known toxicant. Environmental conditions such as heat and UV light depolymerizes polyacrylamide to acrylamide, thereby contaminating soil and water (Smith and Oehme, 1993).

Acrylamide is reported to show neurotoxicity, mutagenicity, carcinogenicity, and reprotoxicity (World Health Organization, 1985; Molak, 1991). There are numerous reports on acrylamide formation in foods such as potato chips, French fries, breads and roasted coffee during high-temperature processing (roasting, frying and baking). Acrylamide is formed in these foods through Maillard reaction of amino acid, asparagine and the naturally present reducing sugars (Tareke et al., 2002). Several strategies to mitigate acrylamide formation by eliminating or removing their precursors in food system are reported in the literature (Hendriksen et al., 2009; Raquel et al., 2012). However, there are no reliable methods available to remove preformed acrylamide from food systems. A possible method of reducing or eliminating acrylamide formed in food systems is to employ the action of acrylamidases produced *in situ* by soil microorganisms.

Polyacrylamide is generally resistant to biodegradation (Caulfield et al., 2002), but reports on bacterial as well as fungal degradation are available (Wakaizumi et al., 2009). Acrylamide reacts with sulfhydryl proteins of microorganism to inhibit their growth (Cavins and Friedman, 1968). The acrylamidase (E.C.3.5.1.4) in these microbes hydrolyzes acrylamide to acrylic acids and free ammonia, which are then utilized as carbon and nitrogen sources *in situ* for growth and metabolic activities. Although some microbial acrylamidases are constitutive (Nawaz et al., 1994), these enzymes are generally inducible, especially when acrylamide is itself present (Potts and Clarke, 1976; Sharma et al., 2009).

It is known that microorganisms belonging to genera *Pseudomonas* (Prabu and Thatheyus, 2007; Chandrashekar et al., 2014), *Bacillus* (Shukor et al., 2009), *Enterobacter* (Buranasilp and Charoenpanich, 2011) and *Rhodococcus* (Nawaz et al., 1998), can degrade acrylamide. Other genera such as *Xanthomonas maltophilia* (Nawaz et al., 1993), *Ralstonia eutropha* (Cha and Chambliss, 2011), *Geobacillus thermoglucosidasius* (Cha and Chambliss, 2013), *Variovorax boronicumulans* (Liu et al., 2013), (Jebasingh et al., 2013), *Stenotrophomonas acidaminiphila* (Lakshmikandan et al., 2014), and most recently, *Arthrobacter* sp. (Bedade and Singhal, 2017) have also been shown to possess significant acrylamidase activity. It is however evident from these publications that only few bacterial strains can degrade higher concentrations of acrylamide. Hence efforts to investigate strains that could efficiently degrade high concentration of acrylamide in shorter time are warranted and timely.

Several physicochemical factors such as temperature, pH, acrylamide concentration, nature of the media and microbial growth time, influence the biodegradation of acrylamide (Chandrashekar et al., 2014; Nawaz et al., 1998). The nature of the bacterial strain strongly determines the tolerance and potency of acrylamide degradation, and its complete degradation may take several days (Guezennec et al., 2014). Although bioremediation is a cost effective and eco-friendly technique, finding microorganisms which could degrade acrylamide has not been an easy task since acrylamide cause toxicity in microorganisms.

There are different kinetic models for evaluation of substrate utilization, biomass production and product formation. Hence it becomes necessary to test the best model fit for microbial processes. Some industrially important microbial strains *viz. Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* are well characterized by kinetic modeling (Almquist et al., 2014).

In an earlier publication, we reported on a culture, identified as *Arthrobacter* sp. that could degrade acrylamide (Bedade and Singhal, 2017). This paper reports on the isolation, screening, identification and characterization of one of the most potent acrylamide degrading soil microorganism. The isolate was also investigated to establish the relationship between acrylamide degradation and microbial growth, temperature, pH. The activity and stability of acrylamidase isolated therefrom were also characterized as a function of pH temperature and other substrates present. In addition, the effect of the presence of activators/inhibitors such as amino acids and metal ions were also investigated. In this context, our study highlighted, for the first time, the promising potential of a newly isolated bacterial strain, *C. oxalaticus* ICTDB921 in degrading acrylamide and other aliphatic amides.

2. Materials and methods

All experimental methods employed here are well known and reported in our earlier paper (Bedade and Singhal, 2017). However, these methods will be briefly discussed here for the sake of completeness.

2.1. Chemicals

Acrylamide (99% purity), acrylic acid (99% purity), and all the other chemicals were of AR grade. These chemicals as well as all the media components were procured from Hi-Media, Mumbai, India. HPLC grade solvents were obtained from Merck, Mumbai, India.

2.2. Media components

Acrylamide degrading bacterial strains was isolated using phosphate buffered medium (PBM) supplemented with 40 mM of acrylamide and trace element solution devoid of nitrogen (pH-7.2) (Bedade and Singhal, 2017). Qualitative screening for acrylamide degradation was performed using indicator agar plate method as per our earlier protocol (Bedade and Singhal, 2017). The cell free crude extract was prepared by growing the cells in R2A medium (Bedade and Singhal, 2017).

2.3. Isolation of acrylamide degrading bacteria

Samples were collected from surface layer of the garden soil from university campus (Institute of Chemical Technology Mumbai, India) and transferred aseptically into a pre-sterilized bag. Isolation of acrylamide degrading bacteria was carried out as per the protocol given in our earlier work (Bedade and Singhal, 2017). Eighteen colonies were isolated on the basis of morphology and growth characteristics. These colonies were individually inoculated aseptically into nutrient broth and further incubated by placing on a rotary shaker incubator (180 rpm, 30 °C and 24 h). This 24 h old inoculum formed the seed culture for further study.

2.4. Screening of acrylamide degrading bacteria

The isolation and screening of acrylamide degrading bacteria was also done following our earlier work (Bedade and Singhal, 2017). In brief, Petri plate (12 cm) containing solidified mineral salt medium (MM1) was divided into three equal sections in which one section was kept un-inoculated (control), and the remaining two sections were inoculated with one loopful of seed culture. These plates were incubated (30 °C, 48 h) and checked for changes in plate color.

2.5. Acrylamide degradation study

The PBM was supplemented with acrylamide (10–100 mM) as only source of carbon and nitrogen. This medium was inoculated with 18 h old bacterial seed culture (2% v/v, OD_{600nm} = 9.40 ± 0.03, cell count of 18.46 ± 0.042 × 10^9 CFU/ml), and incubated by placing on a rotary shaker (30 °C, 180 rpm) for 48 h. Samples were withdrawn at interval of 3 h from each flask for determination of growth at 600 nm