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Characterization of An Acrylamide-degrading Bacterium Isolated from Volcanic Soil

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ABSTRACT

Due to the fact that it breaks down into acrylamide over time, polyacrylamide is one of the most important sources of acrylamide in soil. As a strategy for bioremediation, the breakdown of acrylamide by the action of microbes has seen a gradual but consistent increase in attention all over the world. In this work, a bacterium, tentatively identified as *Pseudomonas* sp. strain Neni-12 that had been isolated from volcanic soil showed the ability to grow on acrylamide. The acrylamide-degrading bacterium grew best in the presence of glucose with acrylamide as the sole nitrogen source. At concentrations of acrylamide ranging from 400 to 600 mg/L, the organisms saw the greatest amount of growth, where ANOVA analysis shows no difference among these temperatures; however, growth was entirely halted at concentrations of 800 mg/L and above. The optimum pH was at 7.0, and growth was maximum between 25 and 35 °C. The bacterium is also capable of growing while using acetamide as the only source of nitrogen. An acrylamide-degrading bacterium that was isolated from volcanic soil is reported for the very first time here.

INTRODUCTION

The Maillard reaction is a cooking process that can result in the formation of acrylamide, a substance that is both carcinogenic and neurotoxic. Acrylamide can be created when meals that are heavy in carbohydrates are cooked at a high temperature. The Maillard reaction can produce acrylamide in some foods, particularly those that are heavy in carbohydrates. The Maillard reaction takes occurs in response to the combination of sugars and amino acids. This is the primary reaction that leads to the formation of acrylamide [1]. On the other hand, acrylamide may be produced from a variety of other carbonyl compounds [2]. Cattle and fish both perished in Sweden and Norway as a direct result of acrylamide contamination in streams in the surrounding area. Acrylamide is most commonly put to use in the manufacturing of polyacrylamide, which has applications not only in the printing, plastics, and adhesives sectors but also in the purification of drinking water. As of the year 2005, commercial polyacrylamides are frequently tainted by the poisonous monomer of acrylamide, a situation that has had a substantial impact on our food supply chain as a direct result of the widespread use of these substances. The Roundup herbicide, which pollutes agricultural land with acrylamides, includes polyacrylamide in a concentration of thirty percent. Acrylamide must be remediated by a biological process in order to address this problem, which must be addressed in order to be resolved [3]. Despite the fact that it has been discovered that acrylamide can cause cancer in experimental animals [4], it is unknown whether this is the case with individuals who are exposed to the toxin because there has been no research done on the subject.

Acrylamide has been demonstrated to bind to DNA and mouse protamine at all phases of the spermatogenic process in mice, leading researchers to conclude that it is responsible for genetic damage [5] throughout this time. Acrylamide exposure in rats has been linked to an increased risk of perinatal mortality, mutagenicity, clastogenicity, endocrine-related cancers, and male reproductive toxicity, according to research conducted on the subject [6]. According to Yang et al. [7], *Salmonella* strains TA100 and TA98 that have been exposed to acrylamide could develop mutations as a result of the chemical. Following administration of the medication, an increased number of chromosomal aberrations were seen in the bone marrow of mice that had received an intraperitoneal injection of acrylamide at a

concentration of 50 mg/kg. The incidence of chromosomal aberrations in lymphocytes from mice that were given intraperitoneal dosages of acrylamide up to 125 mg/kg did not substantially increase when the acrylamide was provided in this manner. This finding was seen when the acrylamide was administered intraperitoneally. [8].

The reproductive systems of male rats are also affected as a result of histological abnormalities in the seminiferous tubules that are induced by acrylamide. These histological abnormalities are caused by the chemical. If acrylamide is breathed or absorbed via the skin, it may cause a burning sensation or a rash to appear. An overactive sweating gland, a sluggish physique, and trembling in the tongue are all signs that something is wrong with the neurological system [4].

Acrylamide, which has a high-water solubility, has the ability to be absorbed via the skin, the lungs, the digestive system, and even the placental barrier. It is possible to assess the amount of acrylamide that the general public is exposed to as a result of their profession by measuring the number of acrylamides adducts that are present in haemoglobin. According to the data, there were a total of 41 workers at an acrylamide production factory that had neurotoxicity scores that were associated with the biomarker haemoglobin adducts. The levels of haemoglobin adducts rose in a Chinese plant that manufactures acrylamide, indicating that the workers had been subjected to extremely high levels of acrylamide exposure [9].

As a result of acrylamide pollution in the water supply of the country, many cases of acute acrylamide poisoning have been documented in Japan. These occurrences have occurred in multiple people. Igisu and his colleagues made the discovery [10] in a well that had been polluted by a grouting operation that was 2.5 meters deep, an acrylamide content that was as high as 400 mg acrylamide/L was found to be present. According to the findings, five people who drank poisoned drinking water experienced symptoms such as truncal ataxia and disorientation. These symptoms are assumed to be the result of acrylamide poisoning, which was produced by drinking the water.

In order to get acrylamide into your body, you must either breathe in contaminated air or consume or drink something that has been tainted in some way. This material may be absorbed by the mucous membranes in the lungs, the digestive system, or the skin, depending on how it comes into contact with the body. On the other hand, it will be eliminated from the body through the urine [11–13]. The facilitation of the acrylamide impact is contributed to by the presence of acrylamide in biological fluids as well as the distribution of acrylamide throughout the body. In spite of the fact that it is swiftly metabolized and eliminated after exposure, acrylamide poses a risk to people and employees due to the high degree of reactivity it exhibits toward proteins. This is the case even though it is not a carcinogen. The facilitation of the acrylamide impact is contributed to by the presence of acrylamide in biological fluids as well as the distribution of acrylamide throughout the body. In spite of the fact that it is swiftly metabolized and eliminated after exposure, acrylamide poses a risk to people and employees due to the high degree of reactivity it exhibits toward proteins. This is the case even though it is not a carcinogen. Volcanic soils are known to harbor several xenobiotics-degrading and biotransforming microorganisms [14–17], mostly hydrocarbon-degrading microorganisms. Microorganisms that have been reported as capable of utilizing acrylamide include the yeast *Rhodotorula* sp .[18], the fungi *Aspergillus oryzae* [19], an Antarctic bacterium [20], *Pseudomonas* sp. [20], *Burkholderia* sp. [21], *Enterobacter aerogenes* [22], *Bacillus cereus* [20], *Pseudomonas chlororaphis* [23] and *Pseudomonas stutzeri* [24]. Here we describe the isolation and characterization of an acrylamide-degrading bacterium from a previously reported acrylamide-degrading consortium strain from volcanic soil.

MATERIALS AND METHODS

All of the chemical reagents were produced in large amounts and employed in the analysis in their unpurified forms. Additionally, the analytical grade was maintained for all of the materials that were utilized in this investigation. Experiments were performed in triplicate in every case unless specified otherwise in the accompanying notes.

Growth and maintenance of acrylamide-degrading bacterium

A bacterium from a previously isolated acrylamide-degrading consortium was utilized in this study [25]. A 0.1 mL for a glycerol suspension of the pure bacterium previously purified by dilution streaking was added into a 45 ml of acrylamide enrichment medium in a 250 ml volumetric flask. Growth was carried out at 25 ℃ on an incubator shaker at 150 rpm (Certomat R, USA) for 48 h. The pH of the medium was adjusted to 7.5. Growth was carried out on a minimal salt medium (MSM). The medium was composed of 0.5 g acrylamide g/L as a nitrogen source, glucose as the carbon source at 10 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $FeSO_4 \cdot H_2O$ 0.005 g/L, KH_2PO_4 6.8 g/L, and $ZnCl_2$ 0.03 g/L, $CoCl_2 \cdot 6H_2O$ 0.003 g/mL, 10 mL of H_3BO_3 0.05 g/mL, 0.002 g of $FeCl_2 \cdot 6H_2O$ and $Cu(CH_3COO)_2 \cdot H_2O$ 0.01g [25].

Morphological, physiological and biochemical characterization of the isolated strain

A variety of standard methods were used to determine the strain's biochemistry and phenotype, including Gram staining, colony shape, the size and colour of the agar colonies, motility, oxidase activity (for 24 hours), ONPG (beta-galactosidase), ornithine decarboxylase (ODC), catalase activity (for 24 hours), lysine decarboxylase, arginine dihydrolase (ADH), [26]. Interpretation of the results was carried out via the ABIS online system [27] as before [28].

Statistical Analysis

Graphpad Prism was utilized in order to do the analysis on all of the data (v 5.1). A p-value of less than 0.05 was taken to indicate statistical significance.

RESULTS

Identification of molybdenum-reducing bacterium

The bacterium was Gram-negative, motile, and had the form of a short rod. The bacterium was recognized by referring to Bergey's Manual of Determinative Bacteriology in conjunction with the results obtained from culture, morphological, and biochemical examinations (**Table 1**) [26] and by utilizing the ABIS online software [27]. The computer offered three other possibilities for the identification of the bacterium, but *Pseudomonas aeruginosa* was the one that had the highest homology (97 percent) and accuracy (85 percent). In the future, in order to identify this species more precisely, molecular identification approaches that are based on the comparison of the 16srRNA gene will be necessary. In honour of the late Dr. Neni Gusmanizar, the bacterium is now provisionally named as *Pseudomonas* sp. strain Neni-12.

Table 1. Biochemical tests for *Pseudomonas* sp. strain Neni-12.

Motility	+	*Utilization of:*	
Hemolysis	+	L-Arabinose	–
Growth at 4 ºC	–	Citrate	+
Growth at 41 ºC	+	Fructose	+
Growth on MacConkey agar	+	Glucose	+
Arginine dihydrolase (ADH)	+	meso-Inositol	–
Alkaline phosphatase (PAL)	–	2-Ketogluconate	+
H_2S production	–	Mannose	–
Indole production	–	Mannitol	+
Nitrates reduction	+	Sorbitol	–
Lecithinase	–	Sucrose	–
Lysine decarboxylase (LDC)	–	Trehalose	–
Ornithine decarboxylase (ODC)	–	Xylose	–
ONPG (beta-galactosidase)	–	Starch hydrolysis	–
Esculin hydrolysis	–		
Gelatin hydrolysis	+		
Starch hydrolysis	–		
Oxidase reaction	+		

Note: + positive result, – negative result, d indeterminate result

Effects of Initial pH and Temperature on Growth

The growth of the bacterium was analyzed at room temperature in a phosphate buffer with a concentration of 0.05 M in order to assess the influence that the initial pH had (pH 5.7 to 8.5). After an incubation period of 48 hours, a measurement of the growth rate was taken. It was discovered that the ideal pH range was 7.0. pH values lower than 6.0 and higher than 8.5 did not support growth (**Fig. 3**). A considerable slowdown in expansion was seen outside of this range. There was no discernible difference, in terms of the growth of bacteria capable of breaking down acrylamide, between the two temperatures that were investigated (**Fig. 4**). Growth was maximum between 25 and 35 °C.

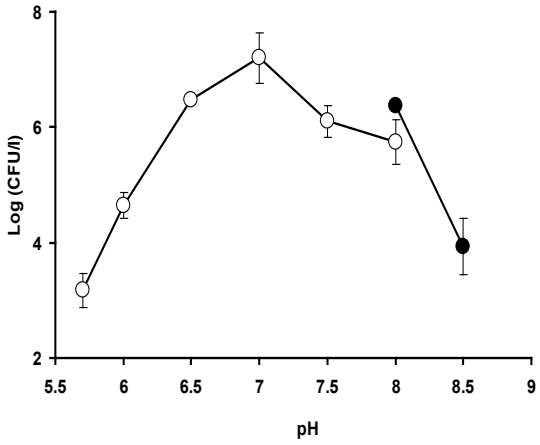

Fig. 3. Growth of the bacterium at various pHs (overlapping buffer system, phosphate and Tris.Cl). Data point represents mean ± standard deviation of triplicates.

Effects of Carbon Sources on Growth

The effects of a starting concentration of 1.0 percent (w/v) of numerous organic carbon sources such as fructose, glucose, lactose, maltose, mannitol, citric acid, and diesel on the breakdown of acrylamide were examined in great detail.

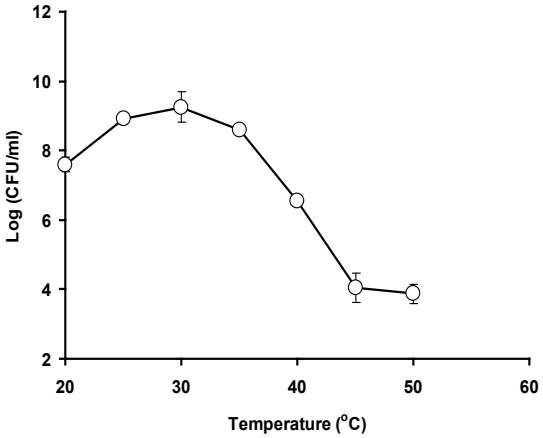

Fig. 4. Growth of the bacterium at various temperature. Data point represents mean ± standard deviation of triplicates.

After 72 hours of incubation, the findings revealed that glucose provided the greatest amount of cellular growth. Fructose and maltose both equally support growth based on ANOVA analysis. Mannitol and citric acid did not support growth. When compared to the control, the results showed that all carbon sources boosted cellular development when compared to control (p0.05) (**Fig. 5**).

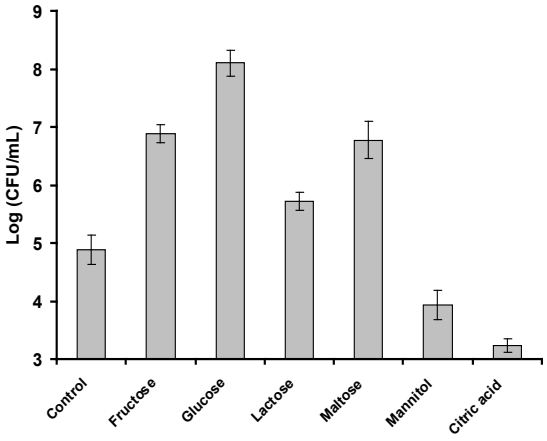

Fig. 5. Growth of the bacterium at various carbon sources. Data point represents mean ± standard deviation of triplicates.

Effect of Acrylamide Concentration on Growth

During the course of this research project, acrylamide concentrations ranging from 200 to 1000 mg/L were utilized in an effort to locate the optimal acrylamide concentration. At concentrations of acrylamide ranging from 400 to 600 mg/L, the organisms saw the greatest amount of growth, where ANOVA analysis shows no difference among these temperatures; however, growth was entirely halted at concentrations of 800 mg/L and above (**Fig. 6**).

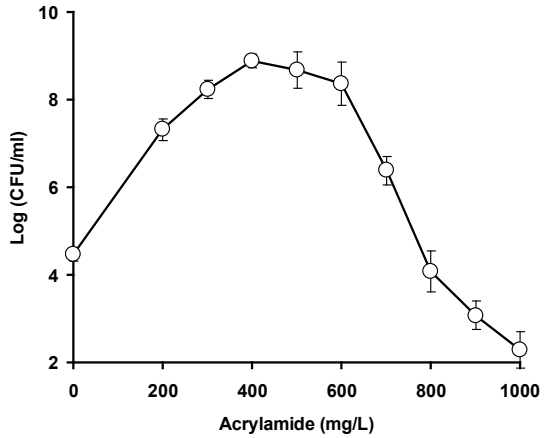

Fig. 6. Growth of the bacterium at various acrylamide concentrations. Data point represents mean ± standard deviation of triplicates.

Effect of Different Amides on Growth

The influence that a number of amides had on the expansion of this bacterium is presented in **Fig. 7**. On the other hand, this bacterium grew on acrylamide and acetamide but not on 2-chloroacetamide, methacrylamide, propionamide, or nicotinamide, which implies that it is resistant to these substances.

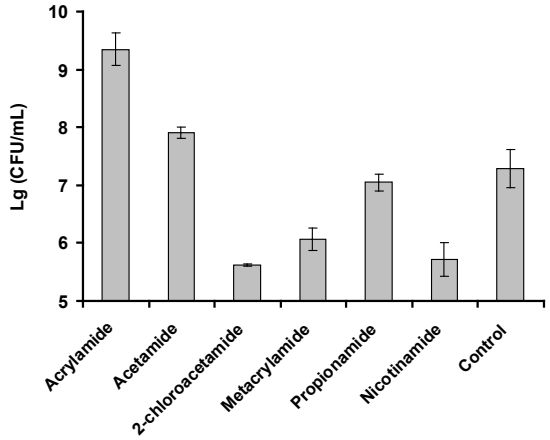

Fig. 7. Growth of the bacterium at various amide sources. Data point represents mean ± standard deviation of triplicates.

DISCUSSION

The optimum conditions supporting the growth of acrylamide for this bacterium are not far different from its parent consortium [25]. The optimum pH for the recently reported acrylamide-degrading consortium was broad at between the pHs of 6.0 and 8.0, perhaps due to the existence of several degraders in the consortium. The optimum temperature for the acrylamide-degrading consortium is at 30 °C whilst glucose was the best carbon source and optimal growth on acrylamide occurred at between 300 and 500 mg/L of acrylamide [25].

The result of this experiment, which is compatible with the findings of a variety of previous publications regarding the impact of beginning pH, was accomplished. The result of this experiment, which is compatible with the findings of a variety of previous publications regarding the impact of beginning pH, was accomplished. Several bacteria have shown that a pH of about 7.0 is their ideal environment such as *Pseudomonas* sp. MCI3434 [29], for *Rhodococcus* sp. [30] and the yeast *Rhodotorula* sp. Rahim *et al*. [18] and *Pseudonocardia thermophilic* [31]. Because of the high levels of metabolic activity in tropical environments, the soils there often have lower pH values. This activity includes the formation of organic acid and carbon dioxide. As a consequence of this, pH-regulating chemicals need to be supplied so that the environment may be brought as close as possible to neutral [32].

Temperature is one of the most important factors that play a role in biodegradation. The growth of the isolated bacterium is excellent, as is the growth of other microorganisms that degrade acrylamide, such as the temperature is one of the most important factors that play a role in biodegradation. The growth of the isolated bacterium is excellent, as is the growth of other microorganisms that degrade acrylamide. The temperature optimum is similar to several bacteria such as *Pseudomonas chlororaphis, Pseudomonas aeruginosa* and *Pseudomonas stutzeri* at 26, 28 and 30 °C, respectively. *Pseudomonas* sp. strain DRYJ7 is the only documented acrylamide-degrading bacterium that degrades acrylamide optimally at 15 °C [33]. *Helicobacter pylori*, found in the human gut, the optimum temperature is 37°C [34]. A temperature of 30 °C was reported as the best temperature for the growth of *Rhodococcus rodochrous* and *Rhodococcus* sp. [23,24,35] [36] and [30] whereas the thermoactive bacteria require a greater temperature to develop well. *Pseudonocardia thermophilic* and *Brevibacillus borstelensis* BCS-1 for instance grow optimally at 50 and 55 °C respectively [31,37].

When given on a low-salt medium that has the barest essential amount of salt, carbon sources are often favorable to the growth of bacteria on acrylamide. This bacterium is not an exception to the norm that glucose is the best possible supply of carbon for many bacterial processes. *Rhodococcus rhodochrous* [38], *Bacillus clausii* and *Burkholderia* sp. [18], *Pseudomonas* sp. [33] and *Bacillus cereus* [3] need glucose from 0.5 to 2.0% (w/v). Other complex carbon sources such as soluble starch have been reported for *Pseudonocardia thermophilic* [31] while salad oil was the carbon source for the growth of *Pseudomonas aeruginosa* acrylamide [39].

The results of this study show that the bacterium capable of digesting acrylamide may survive acrylamide concentrations of up to 600 mg/L, with optimum development occurring at a concentration from 400 to 600 mg/L. This is considered a medium level of acrylamide. Fungal strain *A. oryzae* was successful in degrading acrylamide concentrations of around 100 mg/L using the carbon source sucrose which is a low feature [19]. Medium degraders include *Pseudomonas stutzeri* at 440 mg/L and *Pseudomonas* sp. strain DRYJ7 at 500 mg/L [20,38] while high degrader [40] *Ralstonia eutropha* TDM-3 and *Ralstonia eutropha* AUM-01 can utilize up to 780-1990 mg/L acrylamide as the sole carbon and nitrogen source. Acrylamide poses toxicity to nearly all organisms due to its ability to form interlinking bonds with many molecules.

The results of this study show that the bacterium responsible for the degradation of acrylamide was able to make use of basic aliphatic amides, as was previously documented by other research [28,41–47]. It is also unable of degrading 2-chloroacetamide, an amide molecule that many degraders are unable to use. This is in contrast to the degradation of various short-chain amides [20] and *Bacillus cereus* strain DRY135 [48]. Even though acrylamide and propionamide are both composed of three carbon atoms, the fact that acrylamide has more double bonds than propionamide does not change the fact that acrylamide is a polyunsaturated (less stable) complex that is more

easily attacked than propionamide. This is because acrylamide has more double bonds than propionamide [49,50]. The use of a single bacterium compared to a consortium may be beneficial in certain circumstances especially where the growth of the bacterium before the augmentation process is needed.

CONCLUSION

A novel bacterium capable of degrading acrylamide has been identified from volcanic soil. The bacterium was originally in a consortium and was purified and characterized. The optimal conditions for growth occurred at temperatures between 30 and 35 degrees Celsius and at neutral pH. It was discovered that glucose was the most effective carbon source, and growth was maximal using acrylamide and was inhibited by several other amides. The bacterium was able to tolerate acrylamide at a concentration within the range of other reported degraders. Utilizing the bacterium as a tool for acrylamide bioremediation presents a considerable possibility, particularly in soils used for agricultural purposes.

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