Characterization of An Acrylamide-degrading Bacterium Isolated from Volcanic Soil

Rusnam1* and Neni Gusmanizar2

1Department of Agricultural Engineering, Faculty of Agricultural Technology, Andalas University, Padang, 25163, Indonesia.
2Department of Animal Nutrition, Faculty of Animal Science, Andalas University, Padang, 25163, Indonesia.

*Corresponding author:
Prof Dr. Rusnam,
Department of Agricultural Engineering,
Faculty of Agricultural Technology,
Andalas University,
Padang, 25163,
Indonesia.
Email: rusnam_ms@yahoo.com

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.

KEYWORDS

Acrylamide
Pseudomonas
Acrylamide-degrading
Volcanic soils
Sumatra

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 spermatogenetic 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
Aspergillus oryzae

Microorganisms that have been reported as capable of utilizing Pseudomonas 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 °C 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\(_4\)·7H\(_2\)O 0.5 g/L, FeSO\(_4\)·H\(_2\)O 0.005 g/L, KH\(_2\)PO\(_4\) 6.8 g/L, and ZnCl\(_2\) 0.03 g/L, CoCl\(_2\)·6H\(_2\)O 0.003 g/mL, 10 mL of H\(_3\)BO\(_3\): 0.05 g/mL, 0.002 g of FeCl\(_3\)·6H\(_2\)O and Cu(CH\(_3\)COO)\(_2\)·H\(_2\)O 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.
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Pseudomonas 7.0 is their ideal environment such as accomplished. Several bacteria have shown that a pH of about 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. MC13434 [29], for Rhodococcus sp. [30] and the yeast Rhodotorula sp.

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 rodochrous [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 salal 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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