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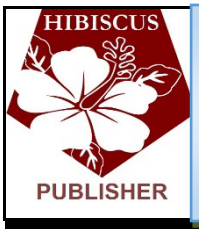

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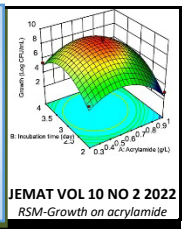

Characterization of the Growth of *Pseudomonas* sp. strain DrY135 on Acrylamide

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ABSTRACT

This study investigated the growth properties of a molybdenum-reducing bacteria previously isolated for its ability to break down amides. The bacterial growth range is 500–1000 mg/L, 6.5–8.0 pH, and 30–35 °C. The presence of hazardous heavy metals such as mercury, silver, and copper impeded this bacterium's development on acrylamide. The protracted lag phase seen when growing on acrylamide demonstrates the compound's severe growth inhibition. This bacterium has the potential to be an effective acrylamide bioremediation agent due to its greater tolerance for acrylamide than other acrylamide-degrading bacteria identified in the scientific literature. The influence of initial pH on bacterial growth at room temperature indicates that the optimal pH range lies between 6.5 and 8.0. The ideal temperature range for plant growth was between 30 and 35 ºC. In a series of experiments utilizing a starting concentration of 1% (w/v) of various organic carbon sources, it was determined that glucose supported the most cellular growth on acrylamide, followed by sucrose, fructose, mannose, and citrate, in descending order of efficiency, whereas mannitol did not support growth. Doses of 300 and 500 mg/L of acrylamide stimulated the most rapid growth expansion, but concentrations of 1500 mg/L and above completely halted development. Copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg) were investigated at a concentration of 2 ppm. Mercury hindered growth by 71 percent, copper by 72 percent, and cadmium by 52 percent, according to our findings. There was a linear association between the acrylamide content and the delay before this bacterium began to develop. A lag time of one to three days was found when the acrylamide content grew from 100 to 1,500 mg/L. As quantities of acrylamide increased, so did the maximal growth rate, indicating an overall pattern of increasing toxicity.

INTRODUCTION

The Maillard reaction can cause the formation of acrylamide, a carcinogenic and neurotoxic chemical, during high-temperature cooking. It's possible that acrylamide will be produced in high-carbohydrate diets during the Maillard reaction. Whenever sugars and amino acids are combined, a chemical reaction known as the Maillard reaction takes place. A considerable amount of acrylamide is generated in this manner [1]. However, many carbonyl compounds can be used to generate acrylamide [2]. In Sweden and Norway, cows and fish died due to acrylamide contamination of local streams. Polyacrylamide (PAM) is made from acrylamide and has several essential uses in the adhesive, plastic, printing, and water treatment sectors, among others. In 2005, the safety of our food supply was significantly impacted by the widespread use of commercial polyacrylamides, which are frequently contaminated by acrylamide's lethal monomer. Roundup herbicide, which has 30% polyacrylamide, is a major contributor to acrylamide pollution. To solve this issue, a bioremediation technique involving acylamide is required [3].

Acrylamide has been demonstrated to bind to DNA and mouse protamine during spermatogenic stages, suggesting it may inflict genetic damage [4] during this time. Rats exposed to acrylamide have a higher rate of prenatal mortality, mutagenicity, clastogenicity, endocrine-related malignancies, and reproductive toxicity [5]. *Salmonella* TA100 and TA98 show that acrylamide is mutagenic [6]. After being injected intraperitoneally at a dose of 50 mg/kg, acrylamide increased the incidence of chromosomal abnormalities in the bone marrow of mice. There was no discernible increase in the number of chromosomal abnormalities in mouse cells after injecting them with acrylamide at doses up to 125 mg/kg [7]. Among acrylamide's many negative impacts on male rat reproductive systems is a histological abnormality in the seminiferous tubules. If acrylamide is breathed in or absorbed via the skin, it can cause a burning feeling or rash. A faulty nervous system manifests itself in symptoms including profuse sweating, fatigue, and unsteady speech [8]. Due to its high solubility in water, acrylamide may be absorbed through the skin, lungs, intestines, and even the placenta.

Concentrations of acrylamide in the blood can be estimated by measuring the amount of acrylamide bound to hemoglobin. For this investigation, haemoglobin adducts were used as a biomarker, and the results showed that 41 workers in an acrylamide plant had significantly increased neurotoxicity. Researchers found that haemoglobin adduct levels increased among workers in a Chinese acrylamide facility, indicating that they were exposed to very high levels of acrylamide [9]. Wells was discovered to contain as much as 400 mg acrylamide per liter (mg/L) due to pollution from a grouting operation at a depth of 2.5 meters, as reported by Igisu et al. [10]. According to the findings, truncal ataxia and confusion were experienced by five of the persons who drank the acrylamide-tainted water.

Air pollution and contaminated food and drink are two common routes of entry for acrylamide into the body. Material can be absorbed through the skin, the respiratory mucosa, and the digestive tract. However, the kidneys and urine will flush it out of the system. The rapid acceleration of the acrylamide effect is due, in part, to acrylamide's availability in biological fluids and its widespread diffusion throughout the body. Although acrylamide is quickly metabolized and eliminated from the body after exposure, it nevertheless poses a concern to workers and consumers [11-13] due to its high protein reactivity. Bacteria are still the most common type of microorganism shown to break down acrylamide [14–23]. Here we describe the isolation and characterization of another *Pseudomonas* acrylamide-degrading strain with metal-reducing capability.

MATERIALS AND METHODS

All of the materials utilized in this investigation were of analytical grade unless otherwise specified. Experiments were conducted in triplicates.

Growth and maintenance of acrylamide-degrading bacterium

The bacterium was previously isolated as a Mo-reducer [24]. From an overnight pure culture of the bacterium grown in 100 mL of nutrient broth, 0.1 mL was added into 45 mL of acrylamide enrichment medium in a 100 mL volumetric flask and the culture was incubated at 150 rpm for 48 h at 25 °C on an incubator shaker (Certomat R, USA). Minimal salt medium (MSM) for growth was supplemented with 0.5 g acrylamide g/L as the sole nitrogen source, glucose 10 g/L as the carbon source, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 6.8 g/L (buffering species and source of phosphorous), $FeSO_4 \cdot H_2O$ 0.005 g/L and 0.1 mL of trace elements [3]. The presence of phosphate in the medium acts as a buffer system, maintaining a pH range that spans from 5.8 to 7.8. Acrylamide was the only source of nitrogen that was employed for the sterilisation process, and PTFE syringe filters with a pore size of 0.45 micron were used. In order to determine the number of bacteria present, samples of one milliliter each were successively diluted in sterile tap water and plated on nutrient agar.

Statistical Analysis

Values are means ± standard deviation (SD) of triplicate experiments. One-way analysis of variance (with post hoc analysis by Tukey's test) or Student's t-test was used to compare between groups. P-value of < 0.05 was considered was significant.

RESULTS

It should come as no surprise that the monomer for polyacrylamide is the hazardous xenobiotic acrylamide (**Fig. 1**). Tunnel and dam stabilization, sewage flocculants, and industrial adhesives are just a few of the many applications for polyacrylamide. Acute acrylamide toxicity caused by pollution has been blamed for the deaths of cows and fish in Sweden [25]. Glyphosate herbicide's polyacrylamide dispersion may be a major source of acrylamide in contaminated soils and waterways [26]. Contamination in the acrylonitrile-acrylamide industry has been linked to acrylamide concentrations as high as 1 g/L [27].

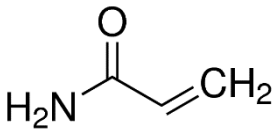

Fig 1. The structure of acrylamide.

Effects of Initial pH and Temperature on Growth

The influence of starting pH on bacterial growth in a 0.05 M phosphate buffer at room temperature was studied (pH 5.7 to 8.5). After 48 hours in incubator conditions, a growth rate was determined. To achieve the best results, the pH should be between 7.0 and 7.5. (**Fig. 2**). Outside this range, cellular development slowed dramatically. Figure 5 shows that acrylamide grows best between 30 and 35 degrees Celsius.

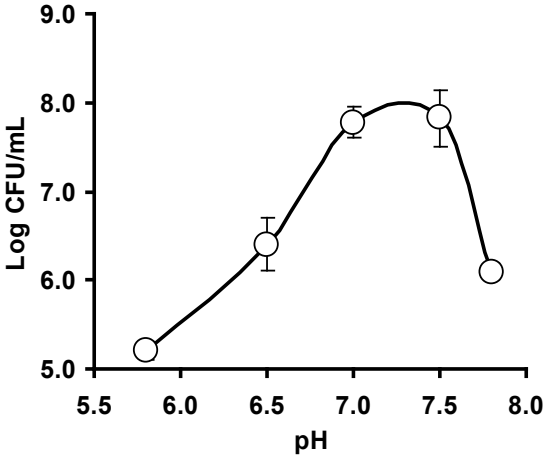

Fig. 2. Growth of the bacterium at various pH. Each data point represents the mean ± SD.

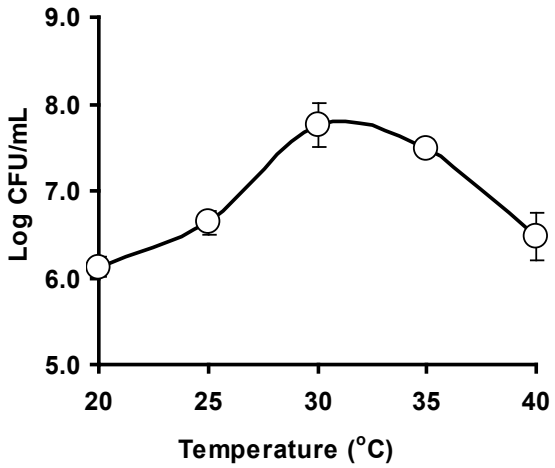

Fig. 3. Growth of the bacterium on various temperature. Each data point represents the mean ± SD.

Effects of Carbon Sources on Growth

Bacterial growth on acrylamide was studied in depth in relation to a number of other organic carbon sources, including fructose, glucose, lactose, maltose, mannitol, citric acid, and diesel, all of which were tested at a starting concentration of 1% (w/v). Compared to other carbon sources and the control, glucose and sucrose supplied the maximum cellular growth after 72 hours of incubation, with growth of 8.792 log CFU/m. In comparison to the control, the results showed that the use of any carbon source promoted cellular growth (**Fig. 4**).

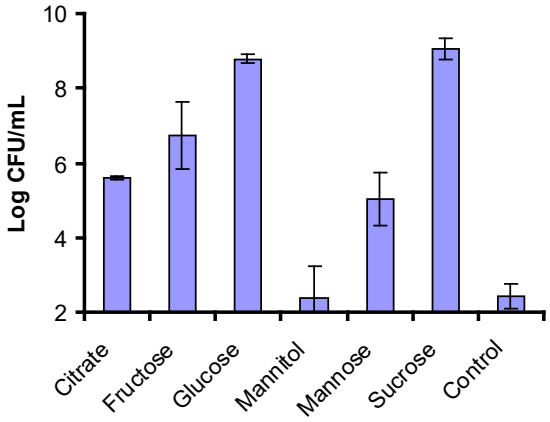

Fig. 4. Growth of the bacterium at various carbon sources and 0.5 g/L acrylamide. The error bars represent the mean ± SD and n=3.

Effect of Acrylamide Concentration on Growth

Acrylamide concentrations up to 2000 mg/L were studied as a sole nitrogen source. The maximum growth occurs between 0.3 and 0.5 g/L or between 300 and 500 mg/L of acrylamide which gave a growth of 8.091 log CFU/mL with no difference between these values as determined via ANOVA analysis, while growth stopped at acrylamide concentration of 1500 mg/L and above (**Fig. 5**).

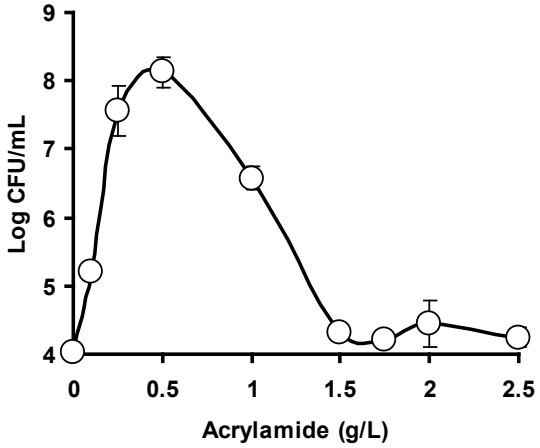

Fig. 5. Growth of the bacterium at various concentrations of acrylamide. Each data point represents the mean ± SD n=3.

Effect of Heavy Metals on the Growth and Degradation of Acrylamide

Heavy metals present at the contaminated site are a major factor that hinders bioremediation. This is because many varieties of bacteria cannot tolerate high levels of heavy metals, and hence lose their ability to degrade their targets. The growth of the bacterium on acrylamide was found to be inhibited to varying degrees by the presence of 2 parts per million of heavy metals (copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg). The three metals that inhibited growth the most were mercury (71%) copper (72%) and cadmium (52%) (**Fig. 6**).

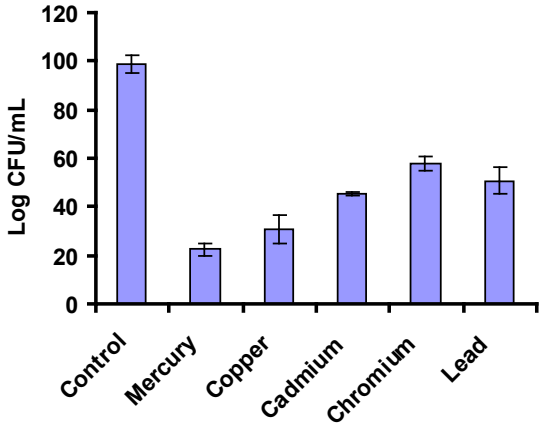

Fig. 6. The effect of heavy metals on acrylamide degradation by acrylamide-degrading bacterium. Each data point represents the mean ± SD.

Growth profile

The growth of this bacterium at various acrylamide concentrations shows an increasing lag period as the concentrations of acrylamide was increased. The lag period ranges from 1 to 3 days as acrylamide was increased from 100 to 1500 mg/L (**Fig. 7**). The maximal growth was also decreased indicating a general toxicity increased trend as the concentrations of acrylamide were increased.

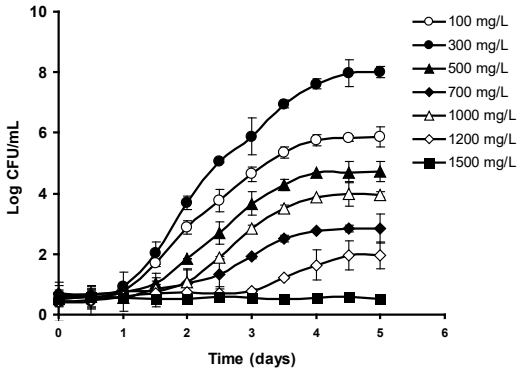

Fig. 7. The growth profile over time of the bacterium on various concentrations of acrylamide. Each data point represents the mean ± SD n=3.

DISCUSSION

Research examining the impact of pH on acrylamide development has yielded consistent results, as seen in the present study. Many microbes that break down acrylamide prefer an environment with a pH of about 7.0 [14-23]. Soils in tropical regions often have a lower pH because of the high levels of metabolic activity that produce organic acid and carbon dioxide. Consequently, chemicals to control the pH level should be supplied to get as close to neutral as possible for efficient cleanup [28].

A key factor in how quickly acrylamide is broken down by bacteria is temperature. Researchers have found that several bacteria capable of digesting acrylamide thrive at temperatures close to 30 degrees Celsius [14–23]. However, thermoactive bacteria, including *Pseudonocardia thermophilic* and *Brevibacillus borstelensis* BCS-1, require higher temperatures for optimum growth, with 50(C and 55(C being required, respectively [29,30]. Most acrylamide-degraders employ acrylamide as the main nitrogen source, therefore readily assimilable carbon sources need to be supplied when growing bacteria on acrylamide in a low-salt medium. In the opinion of this bacterium, glucose is the ideal carbon source. *Bacillus clausii* and *Burkholderia* sp. [31], *Rhodococcus rhodochrous* [32], *Bacillus cereus* [3] and *Pseudomonas* sp. [33] require glucose at concentrations ranging from 0.5 to 2.0% (w/v) for optimal growth. Other than simple carbon sources, complex carbon sources such as starch were used by *Pseudonocardia thermophilic* [30] whilst salad oil was the sole carbon source by *pseudomonas aeruginosa* [34].

According to the findings of this research, the bacterium that degrades acrylamide can withstand acrylamide concentrations of up to 1000 mg/L, with optimal growth occurring in the range of 300–500 mg/L. This behavior is analogous to that of a consortium of bacteria that was isolated from volcanic soil [22,22]. The *A. oryzae* fungus was able to breakdown acrylamide concentrations of roughly 100 mg/L by using nitrate and sucrose as nitrogen and carbon sources, respectively. This amount is regarded to be minimal [35]. [36] reported that *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 while *Pseudomonas stutzeri* and *Pseudomonas* sp. strain DRYJ7 require between 440 and 500 mg/L, respectively, for optimal growth [32,37]. *Cupriavidus oxalaticus* was shown to have the highest tolerance and degrading potential thus far; it can breakdown acrylamide concentrations of up to 60 mM or 4260 mg/L [19]. In most cases, the decomposition of acrylamide results in the formation of acrylic acid, which can be metabolized by a wide variety of bacteria via the Kreb's cycle. For instance, it was shown that the metabolism of acrylate in aerobic bacteria that use acrylate proceeds via hydroxylation to -hydroxypropionate, which is subsequently oxidized to produce carbon dioxide [31]. This was confirmed to be the case in these bacteria.

Based on previous works, heavy metals have a significant impact on the breakdown of acrylamide, with greater inhibition occurring in the presence of mercury, copper and silver than other metals [15–18,20–23].In the published study that is available at this time, there is a dearth of knowledge concerning the effect that heavy metals have on the degradation of acrylamide and even other xenobiotics. This lack of information is due to the fact that the research has not yet been sufficiently conducted. Due to the limited quantity of literature that is currently available on the subject of microbial tolerance to heavy metals, the findings of this study will be of major value for applications of bioremediation that will take place in the future. As acrylamide concentrations grew, the maximum growth rate also slowed, which suggests an overall tendency toward increasing toxicity. Acrylamide prevents the growth of a wide variety of bacteria, and doses of 1000 mg/L or more typically have this effect [15–18,20–23]. The enzyme amidase that is found in certain microorganisms makes it possible for them to grow at these significantly higher concentrations [19,44–50]. According to the findings of this study, the lag period is greatly lengthened whenever growth takes place in the presence of exceptionally high concentrations of acrylamide. It is feasible to extract essential growth features such as the specific growth rate, the maximum growth rate, and the lag time by making use of fundamental growth models such as the modified Gompertz or logistics, or even by making use of other models that are already in existence. [21,51]. The particular growth rate that was determined is a valuable parameter that can be further modeled using secondary models such as Monod, Haldane, Teissier (Tessier), Yano, and Aiba, amongst others [20,21].

Due to the high concentration of heavy metals found in rivers that have been subjected to industrial pollution, research that applies models of metal inhibition is crucial but is underrepresented in the narrative. Extensive research has been conducted into the ability of bacteria to live in extremely dangerous environments and to proliferate there. It was possible to determine the effect of toxic metals on the rates of monoaromatic hydrocarbon degradation caused by *Pseudomonas* species and *Bacillus* species by the use of the Andrews model, which was carried out with great success [38]. Heavy metals probably cause enzyme activity to be inhibited by binding to the sulfhydryl group that is present in many enzyme active sites [39]. This phenomenon can be observed. There are a number of different approaches that can be taken to address the problem of heavy metals preventing biodegradation. Inoculation with metal-resistant bacteria has been shown to reduce the amounts of bioavailable metal, which in turn increases the rate of biodegradation in the presence of a hazardous metal [40]. It is possible to improve the effectiveness of acrylamide degradation by combining a primary bacterial degrader with a bacterium that is resistant to metals. A cadmium-resistant *Pseudomonas* H1, which accumulates cadmium in the cell, and 2,4-D-degrading bacteria were introduced to soil that was contaminated with both cadmium (60 mg total cadmium/kg) and 2,4-D (500 mg/kg), which resulted in a better degradation efficiency of the xenobiotic. This is shown as an example in a soil microcosm experiment study. Treatment additives such as calcium carbonate, manganese oxide, cement, phosphate, and magnesium hydroxide can reduce the bioavailability and mobility of metals, which in turn makes it easier to clean up metal contamination

[41]. One alternate approach is to make use of the minerals found in clay. It has been discovered that clay minerals are useful in lowering the bioavailability of metals as well as the toxicity that follows from the presence of metals in an environment. For example, the toxicity of cadmium was reduced when kaolinite (1-20 percent) or montmorillonite (1-5 percent) was added to agar media containing cadmium so that yeasts, bacteria, and an actinomycete could consume it [42]. Likewise, Kamel (1986) discovered that the toxicity of 150 mg total cadmium/L to Streptomyces bottropensis was decreased by 3 percent bentonite and vermiculite in solution trials. This study was conducted on Streptomyces bottropensis. In spite of the fact that kaolinite was successful in reducing the toxicity of cadmium, it required a larger concentration (six percent as opposed to three percent) and offered less protection all around than the other clays [43].

CONCLUSION

We have found a new kind of bacterium that is capable of degrading acrylamide that is found in volcanic soil. According to the preliminary research, the optimal growing conditions include a pH range of between 7 and 7.5, a temperature range of between 30 and 35 degrees Celsius, acrylamide concentrations of between 0.3 and 0.5 g/L, and glucose as the best carbon source. All of these factors should be present. Acrylamide development was hampered by the presence of toxic heavy metals such as mercury, copper, chromium, and cadmium. Current research involves conducting an experiment with a two-level factorial design in order to identify major growth-supporting characteristics and then apply these parameters in an RSM-based study in order to further increase the growth on acrylamide. The utilization of this bacterium offers a sizable window of opportunity for the process of acrylamide remediation which is known as bioremediation. This potential is particularly prevalent in agricultural soils.

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