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

Mohd Izuan Effendi Halmi[1,2], Mohd Badrin Hanizam Abdul Rahim[2,3], Garba Uba[4] and Hafeez Muhammad Yakasai[5]*

[1]Department of Land Management, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Agribiotechnology Group, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
[3]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
[4]Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic, Dutse, PMB 7040, Nigeria.
[5]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Science, Bayero University Kano, P.M.B. 3011, Kano, Nigeria.

*Corresponding author:
Dr. Hafeez Muhammad Yakasai,
Department of Biochemistry,
Faculty of Basic Medical Sciences,
College of Health Science,
Bayero University Kano,
P.M.B. 3011,
Kano,
Nigeria.
Email: hmyakasai.bch@buk.edu.ng

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ABSTRACT

Acetamide is heavily used as a plasticizer and stabilizer and in the methylamine manufacturing process. There has been a modest but steady rise in worldwide interest in microbe-mediated acetamide decomposition or degradation as a bioremediation method. A previously isolated molybdenum-reducing bacterium with amide-degrading capability is characterized for its growth on acetamide in this study. The bacterium growth optimally in between 500 and 1000 mg/L of acetamide, an optimal pH of between 6.5 and 8.0, and optimal temperatures supporting growth of between 30 and 35 °C. Toxic heavy metals, such as mercury, silver and copper slowed down the growth of this bacterium on acetamide. Growth of acetamide exhibits potent inhibition on growth as evidenced from the considerable lag phase that increases as the concentrations of acetamide was increased. As the tolerance level to acetamide for this bacterium is relatively higher than other acetamide-degrading bacteria in the literature, this bacterium can potentially be an excellent acetamide bioremediation agent.

INTRODUCTION

Acetamide (**Fig. 1**) belongs to the larger group of amides derived from carboxylic acids. In addition, it is a chemical that can be found in nature. In most cases, acetamide is created when old coal is burned. It's fascinating that this chemical was discovered at the galactic core, where the Milky Way is located. Acetylacetone can be converted to acetamide via ammonolysis under the same conditions as are typical in reductive amination. Manufacturing acrylonitrile yields acetonitrile, which can be reacted with water to produce acetamide, or ammonium acetate can be dehydrated to produce acetamide. There is a wide range of observable variations out there, from transparent to colorless to gray. A white smear may be seen on it as well.

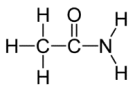

Fig. 1. Acetamide

Depending on how pure it is, it presents as a colorless, odorous, hygroscopic solid. Additionally, it tastes bitter. It is also an acetamide, which is a chemical made by combining ammonia and acetic acid in a sterically closed system. In particular, acetamide is produced through the coordinated electron transfer between carbonyl, methyl, and amine groups. It is largely exploited as a solvent in explosives and for several chemical and inorganic compounds. It's also used as a plasticizer and hygroscopic agent in manufacturing. It also serves as a stabilizer and is used in the methylamine manufacturing process. It can also

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be used to penetrate and put out fires. Due to its low toxicity, the sole negative impact is weight loss, which is triggered by a particularly large oral dose. The eyes, nose, and throat are also gently irrigated. The combustion process also releases toxic gases or fumes. The coagulation system may be damaged. Long-term ingestion has been linked to an increased risk of developing lymphoma and liver cancer. Microorganisms that have been reported as capable of utilizing acetamide as a carbon or nitogen cource ara available in the literature including bacteria [1–11], fungi [12,13] and yeasts [14,15] very few that has been characterized. Here we describe the isolation and characterization of another *Pseudomonas* acetamide-degrading strain with metal reducing capability.

MATERIALS AND METHODS

All chemical reagents were generated in large quantities and utilised in the analysis in their unpurified forms, and all of the materials used in this study were of analytical grade. In all cases, unless otherwise noted, experiments were carried out in triplicate.

Growth and maintenance of acetamide-degrading bacterium
The bacterium; *Pseudomonas* sp. strain DrY135**,** was previously isolated as a Mo-reducer [16] and could use several amides as nitrogen sources such as acrylamide , acetamide and propionamide. From an overnight pure culture of the bacterium in nutrient browth, 0.1 mL was added into 45 mL of acetamide enrichment medium in a 100 mL volumetric flask and the culture was incubated at 25 ℃ on an incubator shaker (Certomat R, USA) at 150 rpm for 48 h.

Minimal salt medium (MSM) was used to isolate the strains with constituents including 0.5 g acetamide g/L, glucose 10 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 6.8 g/L, $FeSO_4 \cdot H_2O$ 0.005 g/L [17]. The phosphate in the medium serves as a buffer system with a pH range between 5.8 and 7.8. For the sterilisation, PTFE syringe filters (0.45 micron) were used and acetamide was used as the sole supply of nitrogen. Samples of one mL each were serially diluted in order to count microorganisms. Acetamide was determined via HPLC on an Agilent 1100 series HPLC on a C18 column (*ZORBAX* StableBond *C18*, *4.6* x 250 mm, *5* µm). The mobile phase was acetonitrile-water-tri-fluoroacetic acid (25:75:0.3, v/v) and the UV detector was set at 205 nm [18].

Statistical Analysis
Values are means ± SD. In triplicate. 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

Effects of Initial pH and Temperature on Growth
The bacterium development was examined at room temperature in a 0.05 M phosphate buffer to determine the impact of the initial pH (pH 5.7 to 8.5). After 48 hours of incubation, the growth rate assessed. pH 6.5 to 7.5 was found to be optimal (**Fig. 2**). Outside of this range, growth was low. Growth was optimum between 25 and 35 °C, whilst temperatures higher than 40 °C were highly inhibitory (**Fig. 3**).

Effects of Carbon Sources on Growth
The effects of a 1.0 percent (w/v) starting concentration of several organic carbon sources such as fructose, glucose, lactose, maltose, mannitol and citric acid on acetamide breakdown were thoroughly investigated.

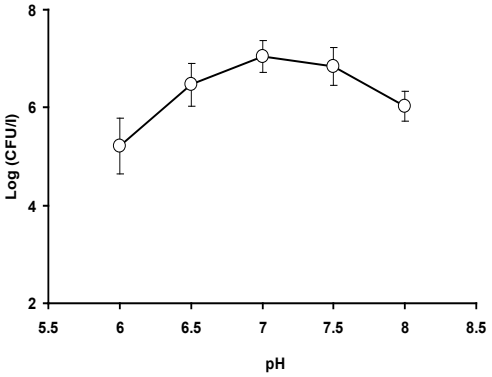

Fig. 2. Effect of pH on acetamide degradation and growth of *Pseudomonas* sp. strain DrY135. Each data point represents the mean ± standard deviation of three replicates.

The results showed that the carbon sources glucose, fructose and maltose (in descending order of efficacy) while other carbon sources were unable to support growth on acetamide as a nitrogen source (**Fig. 4**).

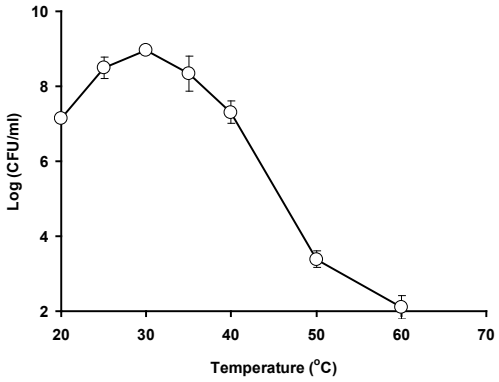

Fig. 3. Effect of temperature on acetamide degradation and growth of *Pseudomonas* sp. strain DrY135. Each data point represents the mean ± standard deviation of three replicates.

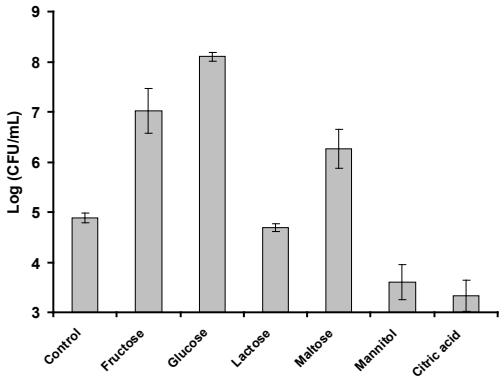

Fig. 4. The effect of carbon sources on degradation of 0.5 g/L acetamide and bacterial growth of *Pseudomonas* sp. strain DrY135. The error bars represent the mean ± standard deviation and n=3.

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Effect of Acetamide Concentration on Growth

Acetamide concentrations ranging from 200 to 1000 mg/L were employed in this study to determine the best acetamide concentration for bacterium growth. The maximum growth was achieved at the concentrations of between 300 and 600 mg/L of acetamide, while growth was mildly inhibited at concentrations of 800 mg/L and above (**Fig. 5**).

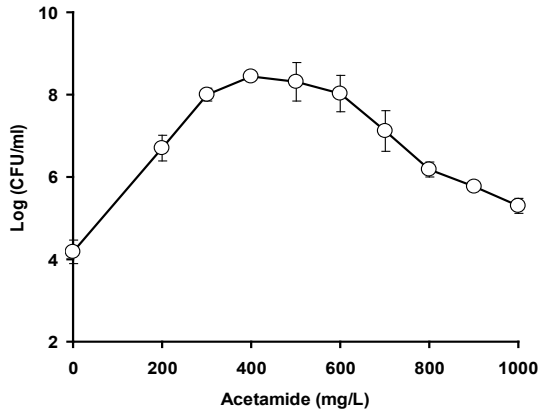

Fig. 5. Effect of different acetamide concentrations on the growth of *Pseudomonas* sp. strain DrY135. Each data point represents the mean ± standard deviation n=3.

Effect of Heavy Metals on the Growth and Degradation of Acetamide

Heavy metals at the pollution site are a major limiting factor for bioremediation. This is because many bacteria cannot withstand high heavy metal concentrations and hence lose their ability to break down target molecules. A concentration of 2 ppm of heavy metals (nickel (Ni), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), silver (Ag) zinc (Zn), and mercury (Hg)) was evaluated. The most severe inhibition was mercury followed by silver and copper causing 87.67, 76.9 and 51.2% inhibition, respectively (**Fig. 6**).

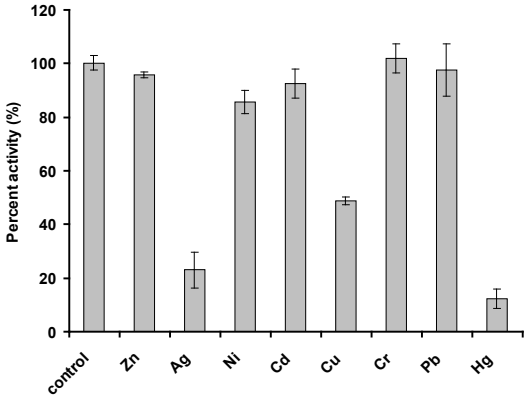

Fig. 6. The effect of heavy metals on acetamide degradation by *Pseudomonas* sp. strain DrY135. Each data point represents the mean ± standard deviation of three replicates.

Growth of bacterium and degradation of acetamide

The growth of this bacterium at 400 mg/L acetamide shows a lag period that ranges from 4 to 8 hr (**Fig. 6**). Acetamide concentration was decreases concomitant to cellular growth. Abiotic degradation of acetamide was minimal as judged by the control.

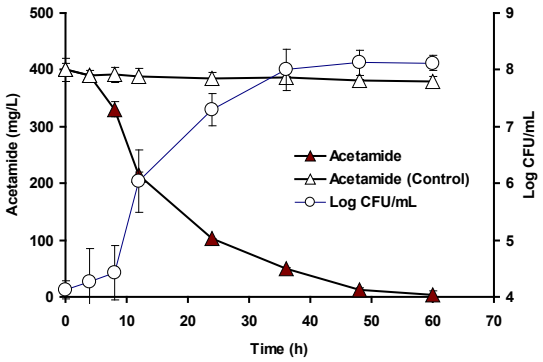

Fig. 6. Growth profile of *Pseudomonas* sp. strain DrY135 on 400 mg/L acetamide. Each data point represents the mean ± standard deviation of three replicates.

DISCUSSION

There is very limited information on acetamide degradation or growth by isolated microorganisms. Characterization of acetamide degradation as sole source of carbon was reported in *Bacillus megaterium* [19], but the characterization of the bacterial growth on acetamide such as the effects of pH, temperature and acetamide as a carbon source is not available.

Many of the publications listing acetamide as a carbon or nitrogen sources originated from acrylamide or nitrile-degrading bacterial activity and mentions acetamide degradation as a secondary activity. Thus, information on its closest relative acrylamide is used for comparison instead. This study's result is in agreement with that of other studies that have looked at the effect of initial pH on acrylamide, a similar compound to acetamide. Several bacteria are reported to exhibit optimal pHs of around 7.0 such as *Pseudomonas* sp. MCI3434 [20], *Rhodococcus* sp. [21] and the yeast *Rhodotorula* sp. Rahim *et al*. [22] and *Pseudonocardia thermophilic* [23]. Strong metabolic activity of microorganism produce organic acids and carbon dioxide, typically results in a lower pH in tropical soils. Therefore, pH-regulating chemicals should be provided to achieve close to neutrality for optimal cleanup [24].

Temperature is a crucial component determining biodegradation. The isolated bacterium grows optimally, at temperatures that spans temperature similar to other acrylamide-degrading microorganisms isolated from soils and waters such as *Pseudomonas chlororaphis, Pseudomonas aeruginosa* and *Pseudomonas stutzeri*, *Rhodococcus rodochrous* and *Rhodococcus* sp. [25–27] [28] and [21] where the optimal temperature ranges from 26 to 30 °C. The gut pathogen *Helicobacter pylori*, which is able to utilize acrylamide, shows an optimum temperature of 37 °C [29]. Very few thermophilic amide-degrading bacteria have been isolated; expectantly, these bacteria require higher temperatures for optimal amide growth. *Pseudonocardia thermophilic* and *Brevibacillus borstelensis* BCS-1 grow optimally at 50 °C and 55 °C respectively [23,30]. *Pseudomonas* sp. strain DRYJ7 is the only documented acrylamide-degrading bacterium that degrades acrylamide optimally at 15 °C (cold-loving) [31].

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Carbon sources promote bacterial growth on amide as the latter is not utilized universally as carbon sources for bacterial growth [6,32–38]. As is generally accepted, this bacterium also finds glucose to be the best carbon source similar to other recently reported amide-degrading bacteria [39,10,40–42]. The concentration of glucose supporting optimal growth on amides typically ranges from 0.5 to 2.0% (w/v), which is exhibited in numerous amide-degrading bacteria such as *Rhodococcus rhodochrous* [43], *Bacillus clausii* and *Burkholderia* sp. [22], *Pseudomonas* sp. [31] and *Bacillus cereus* [17]. Other carbon sources have been reported. For example, salad oil was used by *pseudomonas aeruginosa* [44] while soluble starch was preferred by *Pseudonocardia thermophilic* [23] when amides are utilize as nitrogen sources. *Aspergillus oryzae* KBN1010, on the other hand, requires sucrose at 3% (w/v) for optimal growth on amides [45].

This work demonstrates that the acetamide-degrading bacterium can withstand acetamide concentrations of up to 600 mg/L, similar to the other reported amide-degrading bacteria mentioned above. The fungal strain *A. oryzae* breaks down acrylamide at a modest optimal concentration of 100 mg /L [45]. Compared to acrylamide, which is reported to be toxic to microorganisms, acetamide has not been reported to be toxic to microorganisms and future studies is needed to assess growth at elevated concentrations using inhibitory kinetic models such as Haldane, Teissier, Yano Koga, Aiba, Han-Levenspiel, Luong and Andrews [33,46,47]. Similar range of amide concentrations supporting optimal growth is exhibited in the bacteria *Pseudomonas stutzeri* and *Pseudomonas* sp. strain DRYJ7, at 440 and 500 mg/L, respectively [43,48] while it is reported that *Ralstonia eutropha* TDM-3 and *Ralstonia eutropha* AUM-01 shows a far higher tolerable amide concentrations supporting optimal growth of between 780 and 1990 mg/L acrylamide. The purified acrylamidase acetamide-degrading activity was about 20% less than that of acrylamide [49].

The degradation of acrylamide is significantly affected by heavy metals, with Cd, Cr, and Hg being particularly inhibitive. There is a dearth of data in the current literature about the impact of heavy metals on acrylamide degradation. The results of this study will have significant future applications in bioremediation because of the scarcity of literature on microbial tolerance to heavy metals [50]. Despite the fact that primary models like the modified Gompertz and the modified Logistics may quantitatively identify growth characteristics that can show the presence of inhibition, many investigations on amide degradation failed to use these models. The modified Gompertz model has been proven useful in modeling bacterial growth on acrylamide and has been incorporated into several models of acrylamide biodegradation [39,10,40–42].

CONCLUSION

An acetamide-degrading ability and characterization studies of a previously isolated acrylamide-degrader is reported. Growth was optimum at neutral pH and temperatures of between 25 and 35 °C. Glucose was determined as the best carbon source. Mercury caused 87.67 percentage inhibition, silver caused 76.0 percent inhibition, and copper caused 51.2% inhibition.
The identification of the bacterial consortia, as well as the examination of the metabolites of degradation and the kinetics of growth on acrylamide, are all planned for the future. The bacterium presents a promising prospect for acrylamide bioremediation, especially in agricultural soils.

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