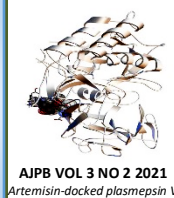


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Characterization of *Morganella* sp. for its Paraquat Degradation Potential

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ABSTRACT

One of the beneficial roles of the microbial population is their ability to convert toxic herbicides to lesser toxic compounds such as water and carbon (IV) oxide. Paraquat which is an acutely toxic herbicide is used on farmlands and has been found to affect human health. This study was aimed at characterizing bacteria with the potential to degrade paraquat. Previously isolated bacteria from culture collection labelled A-F were screened for their potential to degrade and utilized paraquat as the sole carbon source in Bushnell Hass agar media. Of the six isolates, isolate E (*Morganella* sp.) was observed to have the highest growth and tolerance to paraquat after 72 h of incubation at 37 °C. Characterization study revealed that *Morganella* sp. can utilize and grow with optimum conditions of pH 6.5, the temperature of 30 °C and can tolerate up to 400 mg/L paraquat concentration with an increase in growth as inoculum size increases. Thus, these findings showed that *Morganella* sp. can degrade toxic paraquat to a less toxic form and therefore can be a good isolate for the future bioremediation process of the pollutant.

INTRODUCTION

The use of chemical compounds in modern agricultural systems have become common, one group of such chemicals is a herbicide, used to control weed, protect crops and increase agricultural yield [1]. Paraquat is a non-selective rapid-acting herbicide that destroys plants on contact [1]. Paraquat toxicity has been reported by several researchers. In vivo toxicity study of paraquat showed the herbicide can cause lipid peroxidation of cell membranes, therefore, damaging the cells in mice [2]. Study of lungs tissue of rats after a single oral dose of paraquat indicated pulmonary oedema, swelling of the epithelium, an increase in collagen, and an effect on the ribosomes of the membranous pneumocytes [3]. It also produces degenerative lesions in the lung after systemic administration to man and animals [4]. Moreover, the use of the herbicide paraquat (1,1'-dimethyl-4,4-bipyridylum dichloride; PQ) has been strongly challenged due to its critical acute toxicity, putative neurotoxicity after long-term exposure and lack of antidotes to counteract its effects [5]. Paraquat is classified as a Category II oral toxicity and a Category III cutaneous toxicity. ingesting pure paraquat may result in

severe inflammation and even death in humans, as well as in the development of the life-threatening condition known as acute respiratory distress syndrome (ARDS). According to several sources, the death rate ranges from 60 to 90 per cent [6,7]. Ingestion of paraquat is often the cause of accidental death or suicide. Paraquat poisoning, for example, is responsible for more than 5,000 annual fatalities in China [8]. Due to the lack of a particular antidote for paraquat (PQ), suicides in India are a major source of illness and death [9]. Damage to organs like the pulmonary alveolar epithelium results from free radicals generated during the metabolism of phenylephrine (PQ). Acute multiorgan failure and mortality occur during the first two days after PQ consumption of more than 40 mg/kg, but PQ consumption less than 20 mg/kg is associated with moderate symptoms and the majority of patients survive.

Severe mucosal damage and multiorgan failure are the results of paraquat exposure at doses between 20 and 40 mg/kg. Lung fibrosis claims the lives of those who are lucky enough to make it through the first week. It is only the polyamines essential for the cellular activity that is stored in the pulmonary alveolar

cells. Because of its structural resemblance to polyamines, PQ builds up in these cells and causes prolonged harm to the lungs. Despite the lack of a particular antidote, most patients are given steroids/cyclophosphamide/antioxidants to counteract the effects of free radicals [10–12]. The U.S. Environmental Protection Agency (EPA) found no evidence of reproductive/fertility harm from long-term exposure to paraquat, even though it is prone to causing lung and eye injuries. Pumping the stomach to remove as much paraquat as possible is the first step in the traditional therapy for paraquat poisoning. It's possible that using activated charcoal or Fuller's earth can boost results, but it depends on when you use it. Additionally, hemodialysis, hemofiltration, haemoperfusion, and antioxidant treatment may also be recommended. Anti-inflammatory drugs including glucocorticoids and cyclophosphamide have been proposed by some, although the data supporting their use in combination with normal treatment is inconclusive. Even if glucocorticoid is added to normal therapy, it isn't known whether it has undesired side effects including an increased risk of infection. High quantities of oxygen should not be provided until SpO₂ is less than 92 per cent. Death may occur up to 30 days after ingestion [10–12].

PAs are an acute inhalation hazard, paraquat is rated as Toxicity Category I (the most hazardous) by the EPA. The Environmental Protection Agency (EPA) ruled those agricultural operations (400–800 m) are not in the range of particles that may be inhaled. The eye and skin may also be irritated by paraquat. The biggest danger of unintentional paraquat poisoning occurs during the mixing and loading of paraquat for usage. Poisoning has a significant impact on the lungs. In a few days to weeks, the liver, heart, lungs, and kidneys may all fail, which can lead to death within 30 days after intake.

The chances of surviving a substantial exposure are slim. Lung damage, renal failure, heart failure, and oesophageal strictures are all possible outcomes of prolonged exposure. Paraquat's harmful effects on people are still a mystery. When highly reactive oxygen species and nitrite species are generated, oxidative stress occurs, which leads to acute inflammation. Damage to the organs might be caused by inducing cell death, inducing the death of mitochondria, or inducing oxidative stress. For example, it is known that paraquat is concentrated in the alveolar epithelium of the lung. Small amounts, even if taken from the stomach or spat out, may still lead to asphyxiation from fibrous tissue in the lungs, even if they are eliminated from the body [12–15].

Bioremediation techniques involve the use of microorganisms, these strategies can eliminate or reduce xenobiotics in the environment [16–18]. The technique also displayed great potential regarding cost compared to other methods [19,20]. Nevertheless, microbial degradation has been proven to be the safest method of ridding the environment of its pollutants [21–23]. The search for multi-degrader microbe is a continuous process, as no single isolate can tolerate or detoxify all toxic chemicals. This work will thus, screen previously isolated bacteria for their potential to degrade paraquat as the sole carbon source.

MATERIALS AND METHODS

Chemicals and equipment

Paraquat with high purity was purchased from local distributors in the Kano market, Nigeria. Bushnell Hass media containing (g/L⁻¹): CaCl₂, FeCl₃, MgSO₄, KH₂PO₄, K₂HPO₄, NH₄NO₃, was used to study paraquat degradation by bacterial isolates. The nutrient broth was used for the growth of the bacterial culture.

Media and Media Preparation

Nutrient Agar

In a 250 ml conical flask, 3.4 g of nutrient agar was added to 120 ml of distilled water and autoclaved at 121 °C for 45 minutes. The medium was used to determine the viability of isolates

Mineral salt medium

Liquid media was prepared by adding (g/L) KH₂PO₄, K₂HPO₄, MgSO₄, CaCl₂, FeCl₃, NH₄NO₃ to 700 ml of distilled water in a 1000 ml conical flask. A measured volume of the medium was then taken and transferred each into a conical flask, the flask containing the medium was then autoclaved at 121 °C for 45 minutes. 100 mg (unless otherwise stated) concentration of paraquat was used in the different flasks prepared in triplets which were used for degradation study. Agar was added to solidify the media.

Screening for Paraquat-Degrading Isolates

Screening of isolates was carried out in a petri dish. In each plate, 20 ml of prepared solid media was added under a lamina flow and allowed to solidify. To all the Petri dish, bacterial isolates, labelled (A-F) were streaked using a wire loop and incubated for three days. Screening of maximum growth was done macroscopically.

Characterization of Paraquat-Degradation

Effect of Paraquat Concentration

The effect of paraquat concentration media was tested by adding *Morganella* sp. inoculum of 100 µl concentration into the liquid media containing different paraquat concentrations (mg/L); 50, 100, 200, 400, 600 and 800 concentrations were added in triplicate and incubated. Optical densities were measured at 24 h intervals using a spectrophotometer at a wavelength of 600 nm to observe the growth rate of the isolate. Control bottles were prepared without the inoculum and kept under the same condition as the inoculated test bottles.

Effect of pH

The effect of pH of the media was tested by adding *Morganella* sp. inoculum of 100 µl concentration into the liquid media of varying pH (5.5, 6.0, 6.5, 7.0, 7.5, 8.0) and incubated. The pH was adjusted using NaOH and HCl prior to the sterilization. Optical densities were measured at 24 h intervals using a spectrophotometer at a wavelength of 600 nm to observe the growth rate of the isolate. Control bottles were prepared without the inoculum and kept under the same condition as the inoculated test bottles.

Effect of Inoculum Size

The effect of inoculum size was tested by inoculating different volumes (100, 200, 400, 600, 800 and 1000 µl) of *Morganella* sp. into the liquid media and incubated for 120 hrs. Optical densities were measured at 24 h intervals using a spectrophotometer at a wavelength of 600 nm to observe the growth rate of the isolate. Control bottles were prepared without the inoculum and kept under the same condition as the inoculated test bottles.

Effect of Temperature

The effect of temperature was tested by inoculating *Morganella* sp. into 100 ml of the liquid media in 250 ml bottles and incubated for 120 h at different temperatures of 25, 30, 37, and 40 °C respectively. Optical density was measured at 24 h intervals using a spectrophotometer at a wavelength of 600 nm to observe the growth rate of the isolate. Control culture were

prepared without the inoculums and kept under the same conditions as the inoculated test flasks.

Effect of Incubation Time

Prepared liquid media (Bushnell Hass) supplemented with 0.1 g/ of Paraquat was inoculated with 100 μ L of *Morganella* sp. in triplicate. Also controls, without inoculation of *Morganella* sp. were kept under similar conditions. Optical densities (OD) were measured at regular intervals of time 24 h up to 120 h using a spectrophotometer at 600 (nm) to determine the optimum growth temperature for paraquat biodegradation.

RESULTS

Screening of Isolates for Paraquat Biodegradation

A total of six (6) molybdenum-reducing bacteria was previously isolated from agricultural soils in Kano state were screened for their potentials to degrade Paraquat and utilize it as a sole carbon source. The isolate E (*Morganella* sp.) was observed to tolerate and grow best on Bushnell Hass media containing Paraquat as a sole carbon source following 48 h of incubation at 37 °C, thus was chosen for further analysis.

Characterization of paraquat-degradation by *Morganella* sp.

Effect of concentration on paraquat biodegradation

The effect of various paraquat concentrations on its degradation was determined between 50 – 800 mg/L. Paraquat concentration was observed to be optimum at 400 mg/L for this isolate with a decline in growth on increasing the concentration to 800 mg/L (Fig. 1).

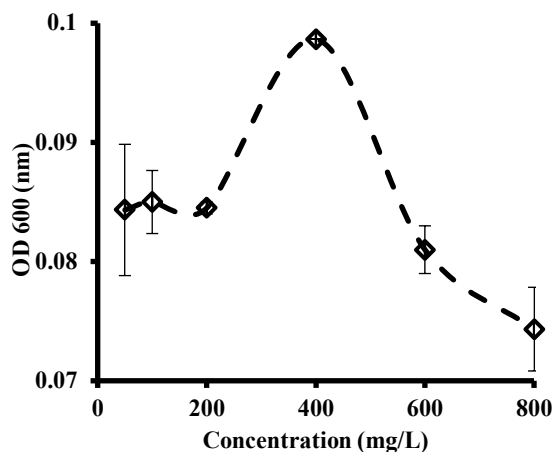


Fig. 1. Effect of concentrations of paraquat (carbon source) on its degradation by *Morganella* sp. after 96 hrs. of incubation at 37 °C. Data represent mean \pm standard deviation of triplicate determination.

Effect of pH on paraquat biodegradations

The effect of initial pH on paraquat degradation was tested at different pH range between 5.5 -8.0. The result obtained showed that the growth of the bacterium was optimum at a pH of 6.5 after 48 hrs of incubation (Fig. 2).

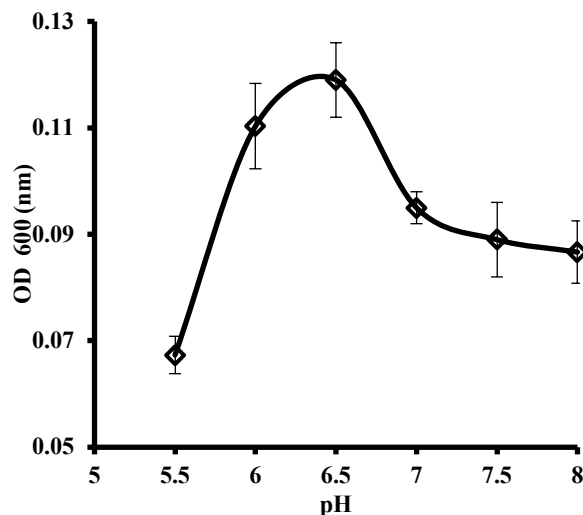


Fig. 2. Effect of initial pH on paraquat degradation by *Morganella* sp. Data represent mean \pm standard deviation of triplicate determination.

Effect of inoculum size on paraquat biodegradation

The inoculum sizes between 100 to 1000 μ L were used to determine the effect of inoculum size. The result obtained shows that the growth of this isolate on paraquat increases with an increase in the inoculum (Fig. 3).

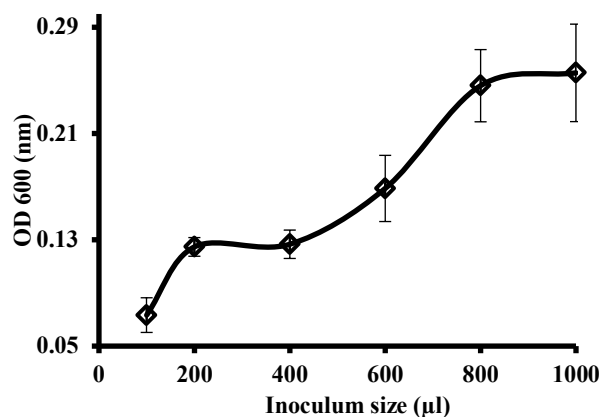


Fig. 3. Effect of various Inoculum sizes on paraquat degradation by *Morganella* sp. after 96 hrs of incubation at 37 °C. Data represents mean \pm standard deviation of triplicate determination.

Effect of temperature on paraquat biodegradation

The effect of temperature on the growth and paraquat-degrading potentials of *Morganella* sp. was tested within a temperature range between 25 – 40 °C. It was observed that 30 °C to be the optimum temperature after 72 h (Fig. 4).

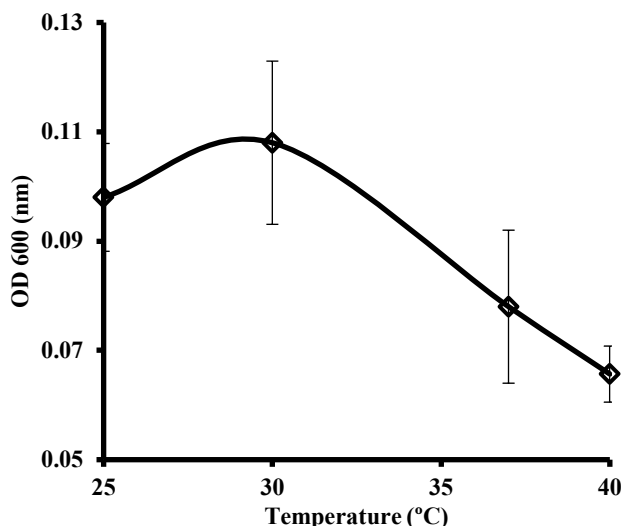


Fig. 4. Effect of temperature on optimum paraquat degradation by *Morganella* sp. after 48 Hrs. Data represent mean \pm standard deviation of triplicate determination.

Effect of incubation time on paraquat degradation

The result of the effect of incubation time shows that *Morganella* sp. was found to grow exponentially from 0 to 96 h at which the maximum growth is observed. Growth declined from that time signifies its death phase.

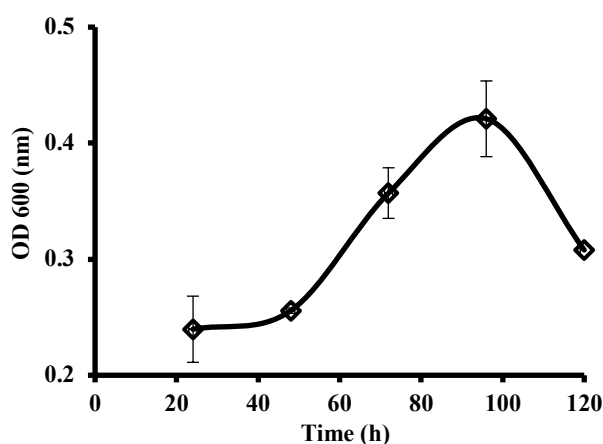


Fig. 5. Effect of Various incubation times on Paraquat degradation by *Morganella* sp. Data represent: Mean \pm Standard Deviation of triplicate determination.

DISCUSSION

Bioremediation exploits the ability of microorganisms to degrade and detoxify organic contaminants. It has been an efficient, economic, versatile and environmentally sound biological treatment method [24]. The herbicide paraquat is comparatively safe for soil microorganisms and plant roots, but its long-term exposure results in harmful biomagnifications in humans and mammals [25]. Extensive paraquat applications lead to widespread residues in the soil surface and aquatic environments that ultimately enter the food chain [26].

Effect of concentration between 50 to 800 mg/L tested in this study showed that the bacterium can grow and tolerate up to 400 mg/L concentration, a concentration that contradicts most if not all of the reported bacteria with paraquat degradation potentials tolerable concentration. On the other hand, a test set to determine the effect of pH on the degradation of paraquat by *Morganella* sp. showed that the bacteria can grow best at pH of 6.5 with an optimum temperature of 30 °C. Similar optimum temperatures and slightly higher pH of 7.0 have been reported on paraquat degradation by *Bacillus aryabhatai* [27].

Finally, the test to determine the effect of inoculum sizes showed an increase in growth as the inoculums size increases, this can be explained by the fact that bacterial growth curve rate increases exponentially with larger healthy inoculum.

CONCLUSION

The potential of previously isolated *Morganella* sp. to tolerate, degrade and utilize paraquat as the sole carbon source was exploited in this study. The bacterium degrades the herbicide best at optimum pH of 6.5, temperature 30 °C, concentration of 400 mg/L and 1000 μ L inoculum after 96 hrs of incubation. This bacterium can be utilized for future biodegradation of this environmental pollutant.

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