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Isolation and Identification of Bacteria from Phytoremediation Plant of *Heliconia psittacorum*

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Abstract. Phytoremediation is a technology that uses plants to degrade, assimilate, metabolize, or decompose organic matter and pollutants. Critical criteria for plants used for phytoremediation include economic benefits, harvest management, and side-use. This cost-effective plant-based recovery utilizes exceptional plant and microorganism capabilities to focus on environmental elements and compounds and metabolize various molecules in their tissues to decompose elements such as heavy metals, toxic substances, and organic pollutants are the main targets. This study aims to analyze the concentration of Gram-positive and Gram-negative bacteria and identify the bacteria using biochemical assays and commercialized API Kit (Biomérieux, France). Three plant samples of *Heliconia psittacorum* were analyzed for their root and soil sample which showed the concentration of Gram-positive bacteria is higher compared to Gram-negative bacteria which ranged from 1.9×10^8 to 3.95×10^9 , while gram-negative bacteria ranged from 2.25×10^7 to 2.95×10^9 . Based on the identification test, seven bacteria were identified. Four gram-negative bacteria are *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Chryseobacterium indologenes* which are generally bacteria that are common to the environment. Meanwhile, three gram-positive bacteria include *Staphylococcus aureus*, *Staphylococcus xylosus*, and *Staphylococcus lentus* have a high impact on metal degradation in the effluent. In conclusion, the concentration of bacteria is relatively higher in the root area of the plant which dominated by gram-positive bacteria and the bacteria that were identified have a prominent effect on the phytoremediation process.

1. Introduction

The growth of the agricultural and industrial sectors in today's challenging economic environment is inevitable. Thereby, it becomes a catalyst for the economic growth of a country. However, human activities have affected the natural environment, especially through industrialization and agriculture.

Soil and sediment are the foundations of the earth's biogeochemical cycle and the microbial community they contain is essential to maintaining the soil-atmosphere-water balance. However, at a critical threshold, land may lose its ability to recover from such disturbances, leading to long-term changes with often unforeseen consequences.

Today, a wide range of treatment technologies are available to restore and maintain the chemical, physical, and biological conditions of wastewaters. Conventional technologies used in the removal of



contaminants from wastewater are limited since they often involve high capital and operational cost and may also be associated with the generation of secondary wastes causing treatment problems. Because of the drawbacks of conventional technologies in the removal of contaminants from wastewater, considerable interest has been expressed in the potential use of a variety of natural biological systems to purify water in a controlled manner during the past 20 years.

Phytoremediation is a technology that utilizes biodiversity to remove pollutions from water, soil, and air which technology is rapidly expanding and commercialized [1]. However, phytoremediation also has its disadvantage which is only a few plants can tolerate the pollutant or contaminated environment [2].

The use of the phytoremediation process in the Constructed Wetlands (CWs) has been used to improve the quality of contaminated waters by acting as a sink for various contaminants discharged from sewage, industrial and agricultural wastewaters, landfill leachate, and stormwater runoff [3]. Phytoremediation technology has been applied to both organic and inorganic pollutants present in soil and water.

Phytoremediation is using the ability of plants to absorb the contaminants from the soil, atmosphere, and wastewater [4] and that there are involvements of the microorganism to facilitate this mechanism [5].

In this study, *Heliconia psittacorum* was used as a model of phytoremediation plant. Genus of these plants was belonged to *Heliconia* under the family *Heliconiaceae* and originated from the neotropical areas of South America [5]. Approximately a total of 182 *Heliconia* species in the whole world, while 37 of the species, natural growth in Brazil. In Malaysia, *Heliconia psittacorum* was known as “*sepi nuri udang*” in Malay and “*false bird-of-paradise*” in English.

The inflorescences part of the plant has distinct colours varied from yellow to orange, red and purple and often contrast with the leaves. On the other side, the leaves are long and broad, with a variety of green colours that characterize them as tropical plants in the garden [6].

As it is a flowering plant, *Heliconia sp.* is usually cultivated as ornamental plants. Additionally, this plant can be propagated by rhizome. With the rhizome characteristics, this plant could be another alternative as a phytoremediation plant in constructed wetlands.

There are a lot of microorganisms in the soil of the plants including bacteria, fungi, actinomycetes, protozoa, and algae. Among all the microbes, bacteria are the most researched and abundant in the soils as soils serve as a beneficial host for the bacteria. The variation in genetic of the microbial population gives a pre-eminent impact on the growth of the plants [7].

Therefore, this study aims to analyze the concentration of Gram-positive and Gram-negative bacteria and identify the bacteria using biochemical assays and commercialized API Kit.

2. Methodology

2.1 Experimental Design

The research was used in the existing experiment plant (reactor) that already been developed in the Universiti Teknologi Petronas (UTP). Two rectangular basin reactor tanks were used for the entire experiment with one of the reactors used as a control with no *Heliconia psittacorum*. The rectangular basin tanks were constructed using a concrete based with size 90cm x 40cm x 25cm (Length x Width x Height) each. The detention times were varied for every 5 days by constructing the tank into four baffled compartments with sampling points for each compartment placed on the side of the tank.

The plant (*Heliconia psittacorum*) we collected from the local area in Tronoh and the plants were transplanted into the system (each baffled had individual plants, to have a significant plant root effect on the wastewater treatment). All microcosms were wrapped with aluminum foil during transportation to avoid sunlight penetration and photodegradation of the compounds.

Every reactor consisted of three layers as shown in Figure 1. The first layer contains 10 cm of gravel (10–20 mm), in the middle layer, 5 cm of gravel (1–5 mm), and the top layer, 10 cm of soil without

the supplementation of nutrient or fertilizer. Healthy *Heliconia psittacorum* with possess similar characteristics such as (same plant size & number of leaves) were then transplanted in each reactor.

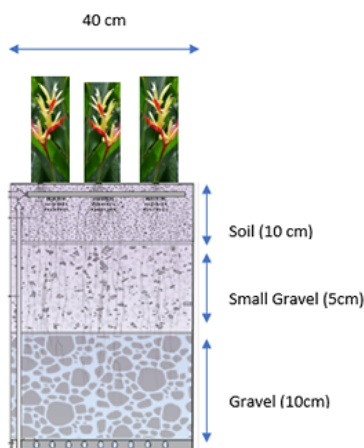


Figure 1. Experimental set up of CW reactor

Effluent from the shrimp pond was pumped using peristaltic pumps (Master Flexx) into the reactor tanks via plastic tubing. Each plastic tube was 6 m in length, and they were sprayed with black spray to prevent algae growth in the channeling tubes. The flow rate of the pump was set at 12 L/d and the water levels were maintained at the lower soil layer to ensure sub-surface flow (SSF) and allowed to infiltrate each system [8]. The systems were designed to operate in batches, with the initial load of water and without any running flow during the tests, having only tap at the base for sample collection.

A two-week acclimatization period was set to stabilize the plant. At the start of the experiment, three young shoots of *Heliconia psittacorum* of the same size were placed in the compartments of each wetland reactor tank. Influent and effluent samples were monitored for COD, ammonia, phosphorus and nitrate, and other Physico-chemical parameters. Four water samples (100 ml) from each reactor tank were collected in a plastic bottle every 2 days within a 60-days study period on each sampling day and stored at 4°C.

2.2 Raw Material

Plant samples that were involved in the phytoremediation process were collected. This including the soil sample that attached to the root and was kept in plastic bags, in an icebox and brought to the laboratory. Samples then kept in a refrigerator at 4°C [9].

2.3 Plant Sample Preparation

Plant parts that were used were the soil and roots. The adhering soils of the root weighed 5 grams and added to 45 ml of 0.85% saline solution. The root then slashed into several small pieces. The pieces weighted and crushed by using pestle and mortar with few drops of saline. An amount of 5 g of the crushed roots was put into 45 ml of sterile saline. Both samples agitated with a rotary shaker at 150 rpm for 30 mins [10].

2.4 Media Preparation (Agar Medium)

Nutrient Agar was prepared by weighing 15 g of agar powder and suspended in 500 ml of distilled water. The mixture stirred until homogenous. Then the solution was autoclaved for 15 minutes at 121 °C and aliquot into sterile petri dishes in laminar flow chambered hood and allowed to solidify. A quality control process of the incubation period of 18 to 24 hours was done to detect any contamination before the agar can be used.

These steps were repeated for Triple Sugar Iron Agar (HiMedia), MacConkey agar (HiMedia) which 50 g suspended in 1000 ml and mannitol salt agar (Oxoid); 111 g in 1000 ml. Triple Sugar Iron allowed drying in a slant position.

2.5 Preparation of Broth Medium

Nutrient Broth was prepared by weighing 13 g of powder and suspended in 1000 ml of distilled water. The solution gently heated and boiled. The solution was distributed into 10 ml universal bottles. The bottles sterilized in autoclave 15 minutes at 121 °C. A quality control process was done by incubating for 18 to 24 hours before the agar can further be used to detect any contamination. These steps repeated for Urea broth. For urea broth, a 40% urea solution will be added.

2.6 Saline Buffer Preparation

An amount of 0.97 g NaCl was suspended in 90 ml of sterile water in a beaker to obtain 0.85% concentration. The saline solution then transferred to Schott bottles. The solution autoclaved for 15 minutes at 121°C.

2.7 Preparation of Bacterial Serial Dilution

Each dilution reduced the concentration of bacteria from the sample by a specific amount. Both samples were serially diluted by pipetting 1 ml of sample into 9 ml saline inside a test tube. This method was repeated for another 7 test tubes which are the dilution up to 10⁻⁸ such as in Figure 2.

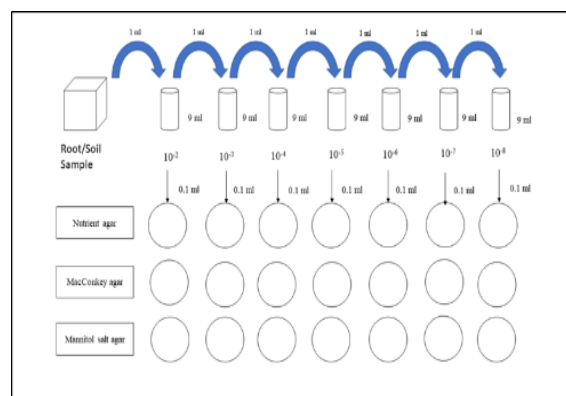


Figure 2. Serial Dilution onto Three Differential Agars

2.8 Pour plating of bacterial serial dilution on Differential Agars

From each test tubes of serial dilution, 0.1 ml were transferred onto nutrient agar, mannitol salt agar, and MacConkey agar and incubated for 24 hours at 37°C. Nutrient agar was used to obtain the overall concentration of bacteria, MacConkey agar to obtain a concentration of gram-negative bacteria and mannitol salt agar was used to obtain gram-positive bacteria concentration.

The colonies was counted, and the concentration of the bacterial culture were determined by using Equation 1. The colony-forming unit should be from a range of 30 to 300 colonies.

$$cfu/ml = (no. \text{ of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

Equation 1: Describes the colony-forming unit per milliliter.

2.9 Isolation of Bacteria

The morphology of the colonies differentiated based on the forms, elevation, and margin [10]. The selected colonies were inoculated into Nutrient Broth and incubated for 18 hours at 37 °C. The bacterial suspension from the broth streaked onto Nutrient Agar to obtain a single colony.

3. Microscopy Analysis

3.1 Gram Staining

The gram staining procedure was done to determine between Gram-positive and Gram-negative bacteria. Bacteria smear was prepared by using the purified single colony. By using the inoculating loop, a loopful amount of single colony inoculated on top a glass slide and mixed with a single drop of water. The smear allowed to air dry and fixed over a gentle flame for a couple of times [11].

The first step was staining the bacterial smear with crystal violet and allowed to stand for 1 minute. This is to fix the dye into the bacterial cell. Then, the slide rinsed gently under slow-moving water. This is a crucial step as the dye should not be washed off. Next, stain the smear with iodine for 1 minute. The slide washed by alcohol for about 3 seconds and rinsed with running water. Lastly, stained the bacteria smear with safranin and allowed to stand for 1 minute before washing it under running water. Observed the bacteria smear under the light microscope until total magnification under oil immersion.

3.2 Biochemical Analysis

3.2.1 Gram-Negative Bacteria

a) Triple Sugar Iron Agar

This test is used to test the ability of the microorganisms to ferment sugars and the production of hydrogen sulphide. In the inoculation procedure, a needle was used to pick up an isolated colony. The needle stabbed at the butt down to the bottom of the slant and streaked onto the surface of the slant. The slant incubated at 37 °C for 18 to 24 hours.

b) Urease Test

A loopful of a bacteria pure culture inoculated in a sterile urea broth with 40% urea solution. The broth then incubated at 37 °C for 24 hours. The positive result indicated by pink colour formation [12].

c) Oxidase Test

A strip of filter paper was soaked with a drop of oxidase reagent. By using a sterile inoculating loop, a small number of colonies from nutrient agar placed onto the soaked filter paper. The positive reaction is indicated by deep purple hue within 10 seconds [13].

3.3 Gram-Positive Bacteria

a) Catalase Test

A microscope slide placed in a petri dish. By using a sterile inoculating loop, a small amount of isolated colony collected and placed onto the microscope slide. A drop of 3% hydrogen peroxide onto the microorganisms on the slide. Then, the petri dish immediately covered with the lid. The immediate bubble presence indicates a positive reaction [14].

3.4 Identification of Bacteria by Using Api Kit (Biomérieux, France)

The kits that were used are API 20 E which is for identification of Enterobacteriaceae and other non-fastidious gram-negative bacteria, API 20 NE to identify Non-Enterobacteriaceae microorganisms and API Staph to identify staphylococcal microorganisms. A well-isolated colony suspended in 5 ml sterile distilled water and emulsify to achieve homogenous suspension.

Sterile Pasteur pipette was used; the homogenous suspension drawn and filled onto the tube and cupule of the tests CIT, VP, and GEL with the bacterial suspension. For the other tests on the strips, the bacterial suspension has only filled the tube. An anaerobic condition will be created for the tests ADH, LDC, ODC, H₂S, and URE by layering with mineral oil.

About 5 ml of distilled water were distributed onto the honeycombed wells of the tray to create a humid atmosphere. The strips then stored in the tray and incubated at 37 °C for 24 hours. After incubation, additional reagents will be added to TDA, IND and VP tests. All the results will be read, and the score recorded. The result compared with the data on the website <https://biomerieux.com>. These steps are repeated with different kits which are API Staph and API 20NE.

However, in API Staph, the bacteria are suspended into an ampoule of API Staph Medium and a microtubule which is PAL. For API 20 NE, the bacteria are suspended in API AUX Medium before inoculated into given microtubules and a microtubule which is TRP Test.

4. Result and Discussion

4.1 Bacteriological Analysis

All the six samples of root and soil were analyzed by serial dilution to determine the colony of bacteria that present in water and soil as shown in table and figure below:

Table 1. Bacteria Concentration of Soil and Root from Plant 1

Sample	Types of Bacteria	Bacteria concentration
Soil	Common Bacteria	3×10^9
	Gram-negative Bacteria	4.7×10^8
	Gram-positive Bacteria	3.95×10^9
Root	Common Bacteria	3.1×10^9
	Gram-negative Bacteria	3.4×10^7
	Gram-positive Bacteria	3.13×10^9

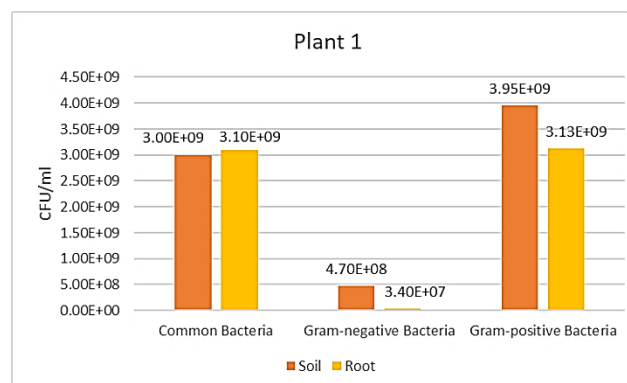


Figure 3. Comparison of Bacteria Concentration of Soil and Root from Plant 1

Table 2. Bacteria Concentration of Soil and Root from Plant 2

Sample	Types of Agar	Bacteria concentration
Soil	Common Bacteria	3.4×10^9
	Gram-negative Bacteria	2.95×10^8
	Gram-positive Bacteria	3.4×10^9
Root	Common Bacteria	2.6×10^9
	Gram-negative Bacteria	2.5×10^7
	Gram-positive Bacteria	1.9×10^8

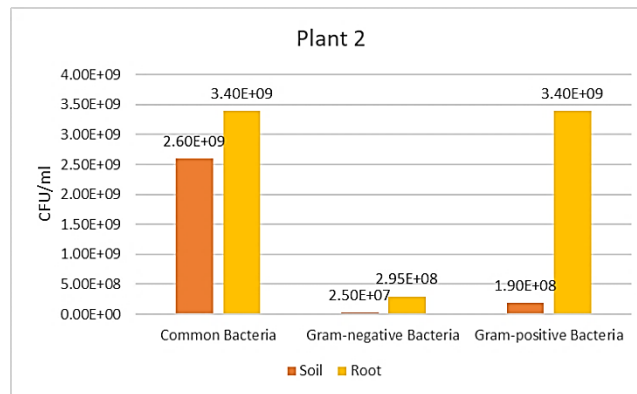


Figure 4. Comparison of Bacteria Concentration of Soil and Root from Plant 2

Table 3. Bacteria Concentration of Soil and Root from Plant 3

Sample	Types of Bacteria	Bacteria concentration
Soil	Common Bacteria	3.1×10^9
	Gram-negative Bacteria	2.7×10^8
	Gram-positive Bacteria	3.1×10^9
Root	Common Bacteria	4.3×10^9
	Gram-negative Bacteria	2.2×10^7
	Gram-positive Bacteria	5.2×10^9

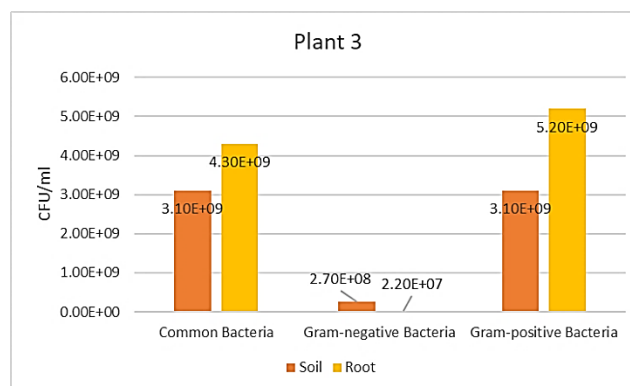


Figure 5. Comparison on Bacteria Concentration of Soil and Root from Plant 3

4.2 Characteristics of Bacteria Colony on Macconkey Agar

MacConkey Agar can grow Gram-negative bacteria when there is a presence of bile salts and crystal violet dye in the agar. The result obtained from the plant samples shows that there are regular and irregular bacterial colonies with the mucoid and nonmucoid surface. The colours of the colony are bright pink, yellow and colourless. Lactose fermenters bacteria grow on the agar as red or pink and surrounded by a zone of acid precipitated bile. The red coloured produced when the production of acid from lactose while non-fermenters are colourless and transparent.

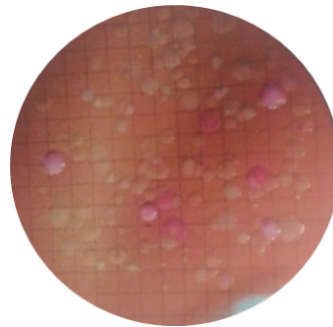


Figure 6. Colony growth on MacConkey Agar

4.3 Characteristics of Bacteria on Mannitol Salt Agar

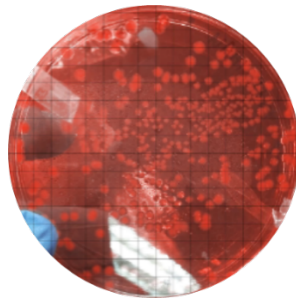


Figure 7. Colony on Mannitol Salt Agar

Mannitol Salt Agar was used in this study as a differential media for Gram-positive bacteria as well as to isolate *Staphylococcus* family bacteria. Based on the resulted bacteria colonies that appeared on this agar, there were different coloured colonies from white to pink.

4.4 Gram Staining and Microscopy Analysis

From the isolated bacteria on MacConkey Agar, the four isolated colonies are all gram-negative based on the red staining. The shapes that were obtained from the four bacteria are all Gram-negative *bacilli*. Gram-negative *bacilli* are very common in causing pathogenic diseases and also a common bacterium in phytoremediation as stated in Table 4. The largest group of Gram-negative *bacilli* are from the family *Enterobacteriaceae* that are found in soil, water and normal enteric flora of humans including animals.

Table 4. List of Bacteria That Commonly Associates with Phytoremediation

Plant growth-promoting bacteria	Mechanisms
<i>Azospirillum</i>	Nitrogen fixation
<i>Azotobacter</i>	Nitrogen fixation
<i>Bacillus</i>	Nitrogen fixation, Auxin synthesis, Cytokinin synthesis, Gibberellin synthesis, Potassium solubilization, Plant stress resistance, Antibiotic production, Siderophore production
<i>Chryseobacterium</i>	Siderophore production
<i>Frankia</i>	Nitrogen fixation
<i>Gluconacetobacter</i>	Nitrogen fixation
<i>Herbaspirillum</i>	Nitrogen fixation
<i>Mycobacterium</i>	Plant stress resistance
<i>Pseudomonas</i>	Chitinase and β -glucanases production, ACC deaminase

Plant growth-promoting bacteria	Mechanisms
<i>Rhizobia</i>	synthesis, Plant stress resistance, Antibiotic production
<i>Rhizobium</i>	Nitrogen fixation, Plant stress resistance, Hydrogen Cyanide production
<i>Streptomyces</i>	Nitrogen fixation, Synthesis of IAA, ACC deaminase synthesis, Siderophore production
	Synthesis of IAA

Meanwhile, from isolated bacteria on mannitol salt agar, the isolated colonies are gram-positive based on the purple staining. The shape that was obtained from the colonies is all coccus in shape. All the isolated bacteria were observed under total magnification using oil immersion which illustrated in the diagram below:

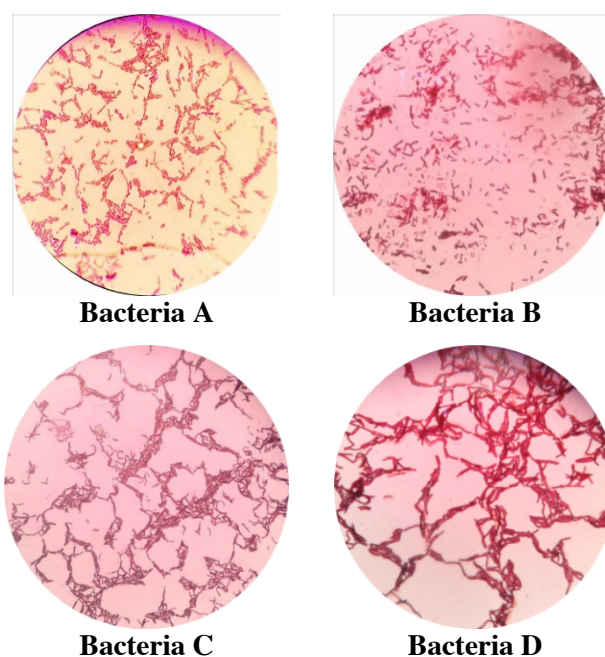


Figure 7. Bacteria A – D from Gram Staining of Gram-Negative Bacteria

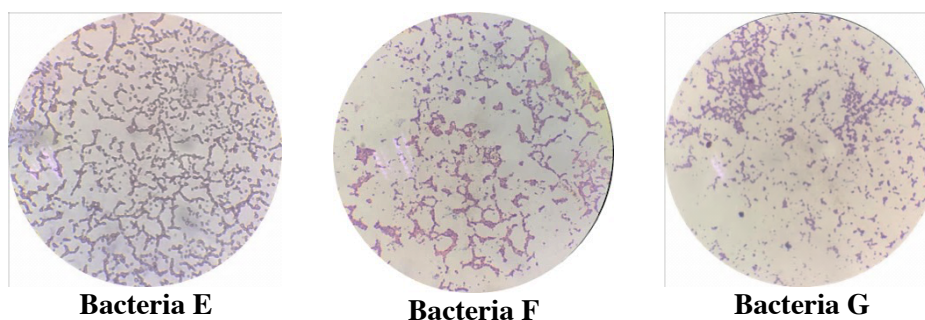


Figure 8. Bacteria E – G from Gram Staining of Gram-positive Bacteria

4.5 Identification of Bacteria Using Biochemical Assays

Biochemical assays were used for microbial identification of the isolated bacteria based on their biological response to the chemical. Biochemical assays that were used were triple sugar iron test, urease test and oxidase test and catalase test for gram-negative while catalase is for Gram-positive. All the results from biochemical tests were recorded after 18 hours of incubation at 37°C. The results were

recorded in Table 5. Meanwhile, the only test that was done on Gram-positive bacteria is a catalase test recorded in Table 6.

Table 5. Results for Biochemical Analysis for Gram-Negative Bacteria Isolation

Identified Bacteria	Biochemical Tests				
	Triple Sugar	Iron/H ₂ S	Oxidase	Urease	Catalase
Bacteria A	A/A/Gas/-		-	-	+
Bacteria B	A/A/Gas/-		-	-	+
Bacteria C	K/K/-/-		+	-	+
Bacteria D	A/A/Gas/-		+	-	+

Notes: (+) sign indicates positive results, (-) sign indicates a negative result. For TSI, A/A indicate acidic fermentation, K for alkaline or no fermentation and Gas represent gas production

Table 6. Results for Biochemical Analysis for Gram-positive Bacteria

Identified Bacteria	Biochemical Test
	Catalase
Bacteria E	+
Bacteria F	+
Bacteria G	+

Notes: (+) sign indicates positive results

4.6 Triple Sugar Iron

The result of triple sugar iron test indicated that if lactose (or sucrose) is fermented, a large amount of acid is produced, which turns yellow for Bacteria A and Bacteria D. Some organisms generate gases, which produces bubbles/cracks on the medium such bacteria are Bacteria A and Bacteria B. If neither lactose or sucrose is fermented, both the butt and slant changed from red to red as showed in Bacteria C.

4.7 Urease Test

Urease Test was used to identify the ability of organisms to digest the urea to become carbon dioxide and water. It is useful to identify *Helicobacter pylori* and several species of *Enterobacteriaceae*. A positive result shows pink colouration after incubation of a minimum of 18 hours at 37°C. Based on Table 5, all the isolated bacteria were urease negative.

4.8 Oxidase Test

Oxidase Test was used to differentiate bacteria that can produce cytochrome c oxidase which is an enzyme in bacteria electron transport chain. This also indicates that all the oxidase-positive bacteria are aerobic. Based on the result in Table 4.8, two bacteria that are oxidase-positive are Bacteria C and Bacteria D. The positive result was indicated by purple colour in a short period amount of time after dropped with oxidase reagent.

4.9 Identification of Bacteria Using API KIT (API STAPH, API 20NE, API 20 E)

The API Kit was used to confirm the bacteria identification from the results from biochemical tests. The API Kit able to identify different bacterial genus and species from *Enterobacteriaceae* family, *non-Enterobacteriaceae* and *Staphylococcus* family. Three bacteria that are *Staphylococcus aureus*, *Staphylococcus lentus* and *Staphylococcus xylosus* were identified by using API Staph.

Meanwhile, *Pseudomonas aeruginosa* and *Chryseobacterium indologenes* were detected by using API 20 NE Kit. Through API 20 E, the kit has confirmed the presence of *Enterobacter aerogenes* and *Escherichia coli* in water and root sample. From Table 4.8 which is result for API Staph, *Staphylococcus lentus* is positive for GLU (glucose), FRU (fructose), MNE (mannose), MAL (maltose), LAC (lactose), TRE (trehalose), MAN (mannitol), MEL (melibiose), NIT (nitrate), VP

(Voges-Proskauer), RAF (raffinose), XYL (xylose), SAC (saccharose), NAG (N-acetyl glucosamine) and supported by positive catalase from the biochemical test.

Meanwhile, *Staphylococcus aureus* is positive for GLU, FRU, MNE, MAL, LAC, TRE, MAN, NIT, PAL (alkaline phosphatase), VP, SAC, NAG, ADH (arginine dihydrolase) and URE (urea) and also supported by positive catalase.

Additionally, *Staphylococcus xylosus* is positive for GLU, FRU, MNE, MAL, LAC, TRE, MAN, NIT, PAL, XYL, MDG (methyl- α D-glucopyranoside) and URE and also supported by catalase-positive.

From Table 8, which is a result for API 20 NE, *Pseudomonas aeruginosa* is positive for ADH (arginine dihydrolase), GEL (gelatinase), GLU (glucose), MAN (mannitol), NAG (N-acetyl glucosamine), GNT (gluconate), CAP (caprate), ADP (adipate), MLT (malate), CIT (citrate). Meanwhile, *Chryseobacterium indologenes* is positive for TRP (tryptophan), ESC (esculin), GEL, PNG (paranitrophenyl- β -D-galactopyranoside), and GLU. Additionally, both of these bacteria are urea negative that similar to the biochemical tests and on the API Kit.

From Table 9, *Escherichia coli* is positive for tests ONPG (ortho-nitrophenyl-galactopyranoside), ADH (arginine dihydrolase), LDC (lysine decarboxylase), ODC (ornithine decarboxylase), CIT (citrate), IND (indole), Glu (glucose), MAN (mannose), SOR (sorbitol), RHA (rhamnose), SAC (saccharose), MEL (melibiose) and ARA (arabinose).

Meanwhile, *Enterobacter aerogenes* is positive for ONPG, LDC, ODC, CIT, VP, GLU, MAN, INO (inositol), SOR, RHA, SAC, MEL, AMY (amygdalase), and ARA. Furthermore, both of these bacteria are urea negative which is similar to the biochemical test and on the API Kit.

Table 7. Result for API Staph

Organism	<i>Staphylococcus lentus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus xylosus</i>
GLU	+	+	+
FRU	+	+	+
MNE	+	+	+
MAL	+	+	+
LAC	+	+	+
TRE	+	+	+
MAN	+	+	+
XLT	-	-	-
MEL	+	-	-
NIT	+	+	+
PAL	-	+	+
VP	+	+	-
RAF	+	-	-
XYL	+	-	+
SAC	+	+	-
MDG	-	-	+
NAG	+	+	+
ADH	-	+	-
URE	-	+	+

Notes: (+) indicates a change in coloration while (-) no colour changes

Table 8. Result for API 20 NE

Organism	<i>Pseudomonas aeruginosa</i>	<i>Chryseobacterium indologenes</i>
NO₃	-	+
TRP	-	+
GLU	-	-
ADH	+	-
URE	-	-
ESC	-	+
GEL	+	+
PNG	-	+
GLU	+	+
ARA	-	-
MNE	-	-
MAN	+	-
NAG	+	-
MAL	-	-
GNT	+	-
CAP	+	-
ADI	+	-
MLT	+	-
CIT	+	-
PAC	-	-

Notes: (+) indicates a change in coloration while (-) no colour changes

Table 9. Result for API 20E

Organism	<i>Escherichia coli</i>	<i>Enterobacter aerogenes</i>
ONPG	+	+
ADH	+	-
LDC	+	+
ODC	+	+
CIT	+	+
H₂S	-	-
URE	-	-
TDA	-	-
IND	+	-
VP	-	+
GEL	-	-
GLU	+	+
MAN	+	+
INO	-	+
SOR	+	+
RHA	+	+
SAC	+	+
MEL	+	+
AMY	-	+
ARA	+	+

Notes: (+) indicates change in coloration while (-) no colour changes

4.10 Discussion

The differential agars that were used are nutrient agar which isolated common or overall bacteria, MacConkey agar to isolate gram-negative bacteria, and mannitol salt agar for gram-positive bacteria. From Figure 3, the concentration of bacteria ranges from 10^7 to 10^9 . The concentration of Gram-positive bacteria is higher compared to Gram-negative bacteria for both soil and root sample of Plant 1 which is 3.95×10^9 and 3.13×10^2 respectively which also means the concentration of bacteria is highest in the soil compared to the root.

Meanwhile, from Figure 4, the concentration of bacteria also ranges from 10^7 to 10^9 . The concentration of Gram-positive bacteria is higher for the root and soil sample from Plant 2 which is 3.4×10^9 and 1.9×10^8 . From Figure 5, the concentration of bacteria is the same for common bacteria and gram-positive bacteria which is 3.1×10^9 that was isolated from the root which also means the concentration of bacteria is higher in root compared to the soil from Plant 3.

The concentration of bacteria in soil is the highest for Gram-positive bacteria which is 5.25×10^8 . However, the concentration of Gram-negative bacteria is relatively low which ranges from 2.25×10^7 to 2.95×10^9 . To put it simply, the concentration of Gram-positive bacteria is higher compared to gram-negative bacteria in all the plant samples.

The composition of bacteria varies in different regions in that is influenced by temperature, moisture, and the presence of other chemicals which the area around the roots rhizosphere is relatively higher than in the rest of the soil due to presence of nutrients such as sugar, amino acid, organic acids, and other small molecules from plant exudates [15]. The concentration of common bacteria, which account for overall bacteria is higher in root compared to soil in all of the plant samples.

Furthermore, the nature of identified Gram-negative bacteria are can be found in soil, water and plants which means that the concentration is low may be due to its characteristics in the environment. Additionally, the identified gram-positive bacteria higher in concentration may due to its ability to aiding phytoremediation in metal tolerance, binding, degradation, and adsorption.

Microorganisms can help in phytoremediation in various ways such as stimulating plant growth, heavy metal tolerance, protection of plants from diseases by the production of antibiotics as well as release chelators which are siderophores and organic acids [16]. Additionally, bacteria can also provide bioremediation which uses microorganisms to promote the degradation of pollutants [17]. *Enterobacter aerogenes* can be found in soil, water, and vegetation [18]. This bacteria can increase plant biomass and metal uptake where this particular bacterium can uptake Ni and Cr. Hence, it can be said that *Enterobacter aerogenes* is beneficial for this plant growth and undergoes the phytoremediation process.

A study on *Enterobacter aerogenes* conducted with *Brassica juncea* as the host plant which shows that this bacterium increases the plant height, root length, wet and dry weight of the plant [19]. *Escherichia coli* is an indicator of fecal bacteria contamination [20]. This bacteria has the characteristics of translocating arsenic from root to shoot which is done by the gene Lsi 2, a homologue in this bacterium.

Then, *Pseudomonas aeruginosa* which can be found in water and soil has the characteristics to increase the plant rooting and biomass of plants. This bacteria species has the effects of plant growth and plant above ground growth with *Brassica juncea* as the host plant [22].

Meanwhile, *Chryseobacterium indologenes* can be found in water, soil, and plants [22]. This bacteria can solubilize phosphate, produce auxin like compounds and had shown a growth-promoting effect on *Lupinus sp*, tomato, pepper, pine, and holm-oak trees. Generally, all the *Staphylococcus sp* has the same benefits in phytoremediation which is metal degradation. The isolated *S. lentus* from the root of the plant were proven the ability to absorb and degrade the arsenic that increases within six hours. Meanwhile, for *Staphylococcus lentus*, a study conducted shows that this bacterium able to degrade or uptake metals which are Chromium, Arsenic, Manganese, and Ferum [23].

Lastly, *Staphylococcus aureus* is also benefits to the phytoremediation through molecular action which is efflux pumps which constitute the main metal tolerance compound [24].

5. Conclusions and Recommendations

In conclusion, the concentration and identification of bacteria from the roots and soil of phytoremediation plant *Heliconia psittacorum* have been experimentally studied. The concentration of Gram-positive and Gram-negative bacteria of this plant was determined by serial dilutions. The bacteriological results of the serial dilutions indicate that there is a high concentration of Gram-positive bacteria compared to Gram-negative bacteria which Gram-positive range from 1.9×10^8 to 3.95×10^9 and Gram-negative from 2.25×10^7 to 2.95×10^9 .

Based on the identification of the bacteria from biochemical assays and API Kit, seven types of bacteria were isolated from biochemical assays and API Kit, there were seven types of bacteria that were isolated from all the samples which are *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Chryseobacterium indologenes*, *Staphylococcus aureus*, *Staphylococcus lentus* and *Staphylococcus xylosus*. Based on previous studies, all of the bacteria found have been studied and have a prominent effect on phytoremediation. For recommendations, the identification method of the bacteria can be tested using the molecular techniques to improve the confirmation of the strain as it can ease the researcher to find out the specific strain of the bacteria.

Additionally, the identified bacteria have been studied to have a prominent effect in phytoremediation and should be used in bacteria assisted phytoremediation so that the process can become more efficient. It is possible to genetically engineer bacteria that can facilitate the absorption of metal or non-metal compounds that strongly adhere to the root surface. Lastly, it is proposed to have support from various jurisdictions that the use of bacteria in the environment is indeed beneficial.

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