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Research Article

Growing of *Ipomoea aquatica* in Seaweed Supplemented Hydroponic System and Identifying Possible Genera of Microbes in the System

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Abstract

Hydroponic system is method of growing plant in nutrient rich solution. The seaweed biomass that is usually disposed as waste once the carrageenan component is extracted from it can be used as a fertilizer. Since nutrients in hydroponic system provide an ideal environment for microorganisms to grow, water-based microorganisms can be easily introduced into the system. This study compared the growth of water spinach (*Ipomoea aquatica*) grown in hydroponic system. The effects on the growth of plants in the hydroponic system when seaweed waste is used as the fertilizer were also studied. Furthermore, the possible genera of the microbes present in the water samples obtained from the hydroponic system were identified. The plants were grown in treated hydroponic tank (with seaweed waste fertilizer) and untreated hydroponic tank (without seaweed waste fertilizer). The mean heights of the plants in each condition were obtained and compared. Water samples were collected from the treated and untreated and biochemical tests were also carried out using the water samples collected to identify the possible genera of the microbes present in the hydroponic system. The plants height of treated tank is significantly taller than untreated tank. The microbe present in the treated tank was either *Chromobacterium* spp./ *Vibrio* spp./ *Plesiomonas* spp./ *Aeromonas* spp., *Acinetobacter* spp., *Bordetella bronchiseptica*/ *Alcaligenes* spp./ *Shewanella* spp./ *Pseudomonas* spp.(alkali producers), *Kurthia* spp. and *Cardiobacterium* spp., and in the untreated tank were *Acinetobacter*, *Bordetella bronchiseptica* / *Alcaligenes* spp./ *Shewanella* spp./ *Pseudomonas* spp. (alkali producers) and *Chromobacterium* spp./ *Vibrio* spp./ *Plesiomonas* spp./ *Aeromonas* spp. Further research need to be carried out to identify the exact bacterial strain of each microbe. By identifying the microbial population of the hydroponic system, further studies can be carried out to identify the beneficial and harmful bacteria. The harmful bacteria can then be eliminated from the hydroponic system to promote healthy growth of plants through hydroponic cultivation.

Introduction

The production of plants is significantly reliant on culture medium and conditions. Vegetable crops are normally grown in soil and the soil acts as a mineral nutrient reservoir. The nutrients are absorbed from the soil, which is dissolved in water, by the plant roots. However, soil is no longer needed for the growth of plants if the essential nutrients are made available to the plants.

Hydroponic system is a method of growing the plants in a nutrient-rich medium solution instead of soil [1]. Growing plants in hydroponic system, fertilizers can be used to enhance the availability of the essential nutrients. Many studies have been carried out on seaweed-derived compounds for their potential use as fertilizers in hydroponic system. Unlike chemical fertilizers, fertilizers derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to human, animals and birds. Fertilizers derived from seaweeds also lead to increased nutrient absorption efficiency of plants, increased rate of growth, resistance to pests and higher yield. Most importantly, sufficient amounts of potassium, nitrogen, growth promoting hormones and micronutrients present in seaweed make it an excellent fertilizer [2].

Carrageenan is a component of red algae seaweed which has many applications. Carrageenan is sulphated linear polysaccharides extracted from seaweeds. This component of seaweed is widely used in the food and beverage industries. Usually once carrageenan is extracted from seaweed, the remaining seaweed content is disposed as waste. However, carrageenan only makes up about less than 5% - 10% of the total seaweed content [3]. In order to utilize the waste or seaweed biomass, seaweed waste can be used as a fertilizer.

The hydroponic system consists of high amounts of nutrient elements. This is simply because the hydroponic system provides an ideal environment for microorganisms to grow. Microorganisms can obtain the nutrients necessary for their growth the hydroponic system [4]. Plus, the pH level and the temperature of the water may be optimum for microbial growth and survival. Although some bacteria, such as *Agrobacterium tumefaciens, Xylella fastidiosa* and *Pseudomonas syringae*, can be

Table 1: Mean height of *I. aquatica* grown in treated tank and untreated tank.

Method of growing	Heights of plants (cm)
Treated tank	24.68 ± 1.03[a]
Untreated tank	15.84 ± 0.63[b]

[a-b]Values in each column that are followed by different letters are significantly different ($p<0.05$). Values are mean ± SD of n = 10 in each group.

pathogenic to the plants, most bacteria in a hydroponic system are beneficial to the plants [5]. Many of these bacteria have a suppressive effect on certain plant pathogenic bacteria. These will help prevent the growth and survival of plant pathogenic bacteria, therefore protecting the plants [6].

This research project was carried out to investigate the *Ipomoea aquatic* cultivation in seaweed supplemented tank and un-supplemented seaweed tank and also to investigate the types of microorganisms that could be present in the system.

Materials and Methods

Seaweed waste preparation using water extraction method

Kappaphycus alvarezii (K. alvarezii) seaweed was purchased from the supplier in Semporna, Sabah. The seaweed was washed with distilled water and dried in a dryer until constant weight was obtained. *K. alvarezii* was soaked in dH_2O with ratio 1:100 and boiled 30 minutes. The carrageenan was extracted and filtered. The extract residues (seaweed biomass) were stored in an air-tight container at 4°C for further usage [7].

Ipomoea aquatica grow in hydroponic system

Ipomoea aquatica (*I. aquatica*) was grown in seaweed biomass (1g/10L) supplemented tank and untreated tank. For untreated tank, the system is neither supplemented with any additive. White light was supplied with 18 hrs per day. After 6 weeks of plantations, the height of *I. aquatic* was measured.

Table 2: Morphology of colonies isolated from treated and untreated tank.

Tank	Isolate Name	Colony Size	Colony Surface	Colony Pigmentation	Colony Shape	Margin of Colony	Elevation of Colony
Treated	T01	Moderate	Smooth	Cream	Irregular	Entire	Flat
Treated	T02	Moderate	Rough	White	Irregular	Undulate	Flat
Treated	T03	Moderate	Rough	Yellow	Irregular	Undulate	Flat
Treated	T04	Small	Smooth	White	Circular	Entire	Convex
Treated	T05	Moderate	Smooth	Cream	Circular	Entire	Flat
Untreated	UT01	Moderate	Rough	White	Irregular	Undulate	Flat
Untreated	UT02	Moderate	Rough	Yellow	Irregular	Undulate	Flat
Untreated	UT03	Moderate	Smooth	Cream	Irregular	Entire	Flat

Table 3: The biochemical test and the possible genera identified of the bacterial isolates.

Tank	Isolate Name	Gram staining / Shape	Oxidase Test	Catalase test	Motility test	Spores	Acid-fast staining	Growth in air	Anaerobic growth	Acid from glucose test	Oxidation-Fermentation test	Bacterial isolate possible genera
Treated	T01	- / Rod	+	+	+	NA	NA	+	+	+	Fermentative	*Chromobacterium / Vibrio / Plesiomonas / Aeromonas*
Treated	T02	- / Cocci	-	+	-	NA	NA	+	-	+	-	*Acinetobacter*
Treated	T03	- / Rod	+	+	+	NA	NA	+	-	-	-	*Bordetella bronchiseptica / Alcaligenes / Shewanella / Pseudomonas* (alkali producers)
Treated	T04	+ / Rod	-	+	+	-	-	+	-	-	-	*Kurthia*
Treated	T05	- / Rod	+	-	-	NA	NA	+	+	+	Fermentative	*Cardiobacterium*
Untreated	UT01	- / Cocci	-	+	-	NA	NA	+	-	+	-	*Acinetobacter*
Untreated	UT02	- / Rod	+	+	+	NA	NA	+	-	-	-	*Bordetella bronchiseptica / Alcaligenes / Shewanella / Pseudomonas* (alkali producers)
Untreated	UT03	- / Rod	+	+	+	NA	NA	+	+	+	Fermentative	*Chromobacterium / Vibrio / Plesiomonas / Aeromonas*

Growth of microbe in Trypticase Soy Agar (TSA) agar

Water samples collected from treated and untreated were used for microbe identification. These water samples were placed on three TSA plates each, using the streak-plate technique and incubated aerobically in an aerobic incubator at 37°C for 24 hours [8].

Biochemical tests

The culture was isolated and proceeds with the biochemical test. The biochemical tests are aerobic and anaerobic growth condition, gram staining test [9], oxidase test, catalase test, acid fast test, spore identification test [8], motility test and oxidation fermentation testand acid from glucose test [10].

Data analysis

The mean values of different data set of hydroponic groups were compared using one way Analysis of Variance (ANOVA). Treated and untreated group were analysed, where p-values of 0.05 (95% confidence) or less were considered significant.

Results

Growth of *I. aquatica* in hydroponic system

The mean height for treated group is 24.68 ± 1.03cm and untreated group is 15.84 ± 0.63cm. The mean height for treated group is significantly taller than untreated group. The mean height of treated and untreated tank is shown in (Table 1).

Growth of Trypticase Soy Agar (TSA) agar

A total of 8 bacterial isolates were obtained, in which 1 bacterial isolate from the water samples, 5 bacterial isolates from the water sample of the treated tank and 3 bacterial isolates from the water sample of the untreated tank. The morphology of the colonies is shown in (Table 2).

Identification of the possible genera of the bacterial isolates

Biochemical tests were used to identify the possible genera of the obtained bacterial isolates. The possible genera identified are shown in (Table 3).

Discussion

The growth of the *I. aquatica* is significant taller in treated tank than untreated tank. The seaweed waste is known for its richness in nutrients that are essential for the growth of plants [11]. Nitrogen source is found in the seaweed waste in the form of ammonia. This nutrient is essential for the development of leaves in the plants. Phosphorus is also found in seaweed waste, which is important for good root system development. Another important nutrient found in seaweed waste would be potassium. When this nutrient is present, it assists the plants to grow more vigorously [12].

Anaerobic bacteria can be divided into 3 types. Obligate anaerobes are anaerobic bacteria that cannot grow in the presence of oxygen and as they can only ferment or respire anaerobically. Oxygen becomes harmful or toxic to these types of anaerobes. Facultative anaerobes are anaerobic bacteria that are able to grow both in the presence and absence of oxygen, as these bacteria can respire both aerobically and anaerobically. Aero-tolerant anaerobes are anaerobic bacteria that do not require the presence of oxygen and also cannot use oxygen for growth. However, they are not harmed by the presence of oxygen [13].

The possible genera of the bacterial isolate identified in the water samples obtained from the untreated culture tank of the hydroponic system were also found in the water samples obtained from the treated culture tank. *Acinetobacter* spp. is isolated from both the treated and untreated culture tanks. Bacteria of this genus are normally found inhabiting soil and waste environments. They have also been isolated from plants and living organisms [14]. *Acinetobacter* spp. has been shown to stimulate plant growth. These bacteria are also said to have the ability to act as bio-control agents against plant diseases that are caused by fungal pathogens. *Acinetobacter* spp. is identified as heterotrophic nitrifying bacteria [15]. These bacteria use organic carbon as their source of food and decompose solid plant and fish wastes. Not only that, they also have the ability to perform nitrification [16].

Another bacterial isolate that was found to be similar in both the treated and untreated culture tanks was the bacteria that belonged to either *Bordetella bronchiseptica*, *Alcaligenes*, *Shewanella* or *Pseudomonas* genera. There are no studies that indicate *Bordetella bronchiseptica* to be pathogenic to plants and also aquatic organisms [17]. *Alcaligenes* spp. can be found in soil and aquatic environments [18]. One strain of bacteria of this genus, that is *Alcaligenes denitrificans*, has been found to impede the growth of algae [19]. Therefore, if the isolates are of this genus, they may have pathogenic effects on the plants. *Shewanella* spp. can be isolated from coastal and ocean seawater. It can also be found associated to marine plants and animals. *Shewanella* spp. has been identified as a Specific Spoilage Organism (SSO), which means it may be responsible for fish spoilage [20]. *Pseudomonas* spp. can be found in soil, faeces, sewage, and water environments [14]. Bacteria of this genus have been found to be pathogenic to plants [21].

Furthermore, bacterial isolate that was found to be similar in both the treated and untreated culture tanks was the bacteria that belonged to *Chromobacterium*, *Vibrio*, *Plesiomonas* or *Aeromonas*genera. *Chromobacterium* spp. can be found in soil and water environments. Some strains of the *Chromobacterium* spp. such as *Chromobacterium lividum*, have been found in food products [22]. *Chromobacterium subtsugae* was isolated from soil and presented high insecticidal properties. *Chromobacterium* spp. hence, has chances of serving as great natural pesticides for plants. Bacteria of this genus can therefore, be beneficial to plants [23]. Some strains of the *Vibrio* spp. is broadly distributed in water environments [14]. They have been known to have toxic effects on aquatic organisms and some strains of *Vibrio* spp. are also finding to be pathogenic to plants. *Plesiomonas* spp. can be found in aquatic environments. Bacteria of this genus have been found to be harmful to fish or seafood, which in turn would harm the consumers when consumed [24,25]. *Aeromonas* spp. can normally be found inhabiting soil and water environments, and also food products such as milk and meat. One bacterial strain of *Aeromonas* spp., which is *Aeromonas salmonicida*, is known as a fish pathogen.

Two other bacterial isolate were found in the treated culture tank of the hydroponic system that were not isolated from the untreated culture tank. These were bacteria of *Kurthia* spp. and *Cardiobacterium*

spp. Bacteria of these genera was only isolated from the treated tank possibly due to the presence of seaweed waste in the treated tank. *Kurthia* spp. can normally be found in faeces of animals, soil, water and food products such as milk and meat. So far, there are no studies conducted that shows *Kurthia* spp. may be pathogenic to plants [26]. There are no studies that indicate *Cardiobacterium* spp. to be pathogenic to plants and also aquatic organisms [27].

Conclusion

I. aquatica in seaweed supplemented tank is significant taller than non supplemented tank. The microbe present in the tank was either *Chromobacterium* spp./ *Vibrio* spp./ *Plesiomonas* spp./ *Aeromonas* spp., *Acinetobacter* spp., *Bordetella bronchiseptica*/ *Alcaligenes* spp./ *Shewanella* spp./ *Pseudomonas* spp. (alkali producers), *Kurthia* spp. and *Cardiobacterium* spp. and in the untreated tank were *Acinetobacter, Bordetella bronchiseptica* / *Alcaligenes* spp./ *Shewanella* spp./ *Pseudomonas* spp.(alkali producers) and *Chromobacterium* spp./ *Vibrio* spp./ *Plesiomonas* spp./ *Aeromonas* spp.

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