Soil and aquaponic phosphorus solubilising organism diversity - Improving aquaponic farming of Swiss chard through microbial ecological methods

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16th October 2019

ABSTRACT

Aquaponic farming is an eco-friendly means of farming. It involves a closed circuit with three main constituents, namely fish, plants and microorganisms. The plants' nutrient requirement is solely provided for by fish effluent, but there is often a need for aquaponic farmers to utilize nutrient supplementation of essential plant growth elements during the growth of their crops. These elements include phosphorus, iron, and calcium. This specific supplementation is usually not required in such abundance in standard soil farm practices. This report focuses on the soluble phosphorus levels available in the water system of aquaponically grown Swiss chard in a system with Koi and Kob fish. The aim is to determine whether solubilised phosphorus (SP) levels could be improved upon through the inoculation of potentially better phosphorus solubilizing microorganisms (PSOs) isolated from the soil rhizosphere. The isolated organisms were taken from the rhizosphere of Swiss chard grown in aquaponic and soil environments and grown on Pikovskaya (PVK) agar plates, which contain calcium triphosphate which is known to produce a clear halo around PSOs. Analysis of the best pure PSO colonies resulted in further testing of one aquaponic bacteria (Pseudomonas sp. strain F4D), one soil bacteria (*Burkholderia pseudomallei)* and one soil fungus (*Aspergillus niger)*. The test included the controlled growth of each PSO in the water environment of the aquaponic system to determine their solubilising ability. The identification of more efficient PSO's and their ability to perform in an aqueous environment could pave the way to a novel means in advancing aquaponic farming abilities.

INTRODUCTION

In an aquaponic closed environment, the plants' nutrient requirement is solely provided for by fish effluent to supplement the plants' growth requirements. In most aquaponic farms this form of synergism between the plants and fish isn't fully sufficient and it is often necessary to supplement farm water with certain nutrients and essential growth elements for the plants. These elements being phosphorous, calcium and iron. (Atkin et al., 2004).

Aquaponics is a water-based agricultural system in which the plant absorbs nutrients provided by the fish effluent and the associated interactions with microorganisms that solubilise minerals (Sharma et al, 2011). This system is also responsible for decreasing dissolved solids and ionic concentrations. This can also include toxic metabolites such as ammonia and nitrite which can be absorbed by the plants, thus improving the overall water which assists optimal fish growth and development (Nozzi et al., 2018).

Mineral phosphate solubilization in aquaponics vs soil environments

Phosphate is the second most important limiting nutrient for the growth of plants after nitrogen, with less than 1% of total phosphorus in the soil bioavailable to plants (Vessey and Heisinger, 2001). Studies show PSOs have potential use as biofertilizers for the increase in the concentration of soluble phosphorus (Collavino et al. 2010; Ghosh et al. 2012) A considerably higher concentration of PSOs can be found in a soil environment in comparison with an aquaponic environment (Raghu et al, 1997). It has been hypothesized that different pH levels can increase the growth and competition of microbes. However, altering pH is not a viable option as the aquaponics system has specific requirements. Any direct or indirect changes could impact the health and sustainability of the three main components in the system (Fitzsimmons et al,. 2016).

Phosphorus solubilizing organisms:

To increase the uptake of phosphorus, the plant (Swiss chard) can be inoculated with Pseudomonas (Parani & Saha, 2012), Rhizobium or Bacillus (Fitzsimmons et al., 2016) bacteria or Mycorrhizae fungi, which have a high phosphorus solubilization ability. These microorganisms can be isolated from the rhizosphere from plants grown in soil and grown in vitro (Sharma et al., 2011). If the microorganisms are absent, the limited phosphorus bioavailability will hinder the growth of the plant.

Interactions with Swiss chard

Plants grown in standard soil conditions emit signals to bacteria and fungi that form a symbiosis with plant roots to allow for increased nutrient uptake (Ahemad et al., 2013). Phosphorus is highly available in the soil but it is predominantly available in its non-soluble form — inorganic phosphate — which cannot be taken up by the plant. Phosphate anions are highly reactive and may be precipitated with cations such as calcium, magnesium, iron and aluminum cations (Nozzi et al., 2018). In aquaculture systems, the availability of microbes that form symbiosis with Swiss chard to solubilise phosphate is limited and insufficient for the plant to be able to obtain the required nutrient levels (Fitzsimmons et al., 2016).

The economic and environmental cost of supplementation:

Nitrogen, calcium, iron, phosphorus, and potassium are just a few nutrients that often need to be supplemented by an external source in agriculture (Ru et al., 2017). Continuous supplementation can become very costly to the aquaponics farmer (Nozzi et al., 2018). To combat the need for continuous nutrient supplementation and minimize costs, plants grown in an aquaponics system can be inoculated with certain nutrient solubilizing bacteria and fungi (Bartelme et al., 2018). Much research has been done on the use of PSOs in soil agriculture (Collavino et al, 2010), however little is known about the use of PSOs in aquaculture.

In this study, two PSOs from the soil and one PSO from the aquaponic environment were isolated and cultured in water samples acquired from the aquaponic farm to determine which PSO was better at solubilising phosphorus. This study aims to determine whether a PSO isolated from a soil environment can survive in the aquaponic environment and adequately solubilize enough inorganic phosphorus to sustain plant growth without the need to supplement the system with phosphorus. It is hypothesised that soil PSOs are more efficient at SP than aquaponic PSOs and that the inoculation of a soil PSO into an aqueous environment would result in an increase of total phosphorus solubilised.

METHODS AND MATERIALS

Sample collection and storage:
Samples were taken aseptically from the rhizosphere of aquaponic and soil-grown Swiss chard from greenhouses at the Welgevallen experimental farms in Stellenbosch, South Africa. Samples were taken from roots at a depth of 15cm and the plants were of similar maturity and growth level. This was done in triplicate from three random locations for each site following a randomised block design. The samples were stored in a 9ml saline (0.85% NaCl) solution.

Conditions of sample sites:

Soil pH will be measured by weighing 15g of soil into two 150ml beakers. 30ml deionized water will be added to each beaker and swirled gently to form a slurry. The solutions will be left to sit for 30 minutes and the pH meter will be standardised using pH 7 and 4. While taking the measurement, the beaker should be swirled gently. The pH is recorded to the nearest 0.01 (Mclean, 1982). The pH of the aquaponic water sample will be measured similarly, without the addition of soil or deionised water. The temperature of each sample site was taken at a 15cm depth using a mercury thermometer.

Preparation of media

PVK agar media (Pikovskaya, 1948) is used as specific isolation for PSO. Ingredients of PVK in grams/L: Yeast Extract 0.500, Dextrose 10.000, Calcium phosphate 5.000, Ammonium sulphate 0.500, Potassium chloride 0.200, Magnesium sulphate 0.100, Manganese sulphate 0.0001, Ferrous sulphate 0.0001, Agar 15.000. Suspend 31.3 grams of powder media in 1000mL distilled water. Heat to boiling point to dissolve media completely and sterilize by autoclaving at 121°C for 15 mins. Mix and pour into sterile Petri plates (Nautiyal & Mehta, 2001).

Isolation of PSOs

PSM were isolated from each sample by serial dilution and spread plate method. One gram (1g) of a sample was dispersed in 9 ml of autoclaved saline water (0.85% NaCl) and vortexed. 1 ml of the above solution was again transferred to 9ml of autoclaved saline water (0.85% NaCl) to form 10^{-2} dilution. Similarly 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} serials were made for each sample. 0.1ml of each dilution was spread on PVK agar and incubated at 26 - 30^{0}C for 7 days. Colonies showing halo zones were picked and purified by 3 times subculture method on PVK agar medium for studying colony morphology (Goenadi et al. 2000). The negative control received no inoculum and the positive control received an undiluted inoculum solution.

Morphological Characterisation

Morphological characteristics of isolates included shape, size, surface form and surface texture, colour were observed for their characterization (Lal, 2002). Light microscopy was used to examine each microorganism's general structure and size in relation to each other. 1000x magnification was used with an oil additive.

Identifying microorganisms

The best solubilising organisms from the chemical analysis had their DNA extracted using a Quick-DNA miniprep DNA extraction kit (Zymo) and amplified using PCR with the following conditions: 5min@94°C, (45s@95°C, 50s@56°C, 70s@72°C)x34 and 7min@72°C. The extracted DNA was checked using gel electrophoresis and submitted to Central Analytical Facilities (CAF), Stellenbosch for sequencing and identification. Phylogenetic trees were produced using https://www.phylogeny.fr/

DNA extraction protocols followed for a bacterial strain from an aquaponic and a soil environment respectively. One fungal strain from the soil environment was analysed. DNA extraction was done twice to ensure accurate results. The following conditions were met for the extraction: A master mix of 16.4 µl of milliQ, 20µl Taq ReadyMix (Kapa biosystems), 0.8 µl forward primer, 0.8 µl reverse primer constituted a 38 µl master mix. 0.5µl of DNA was used respectively from each sample. This was then followed by gel

electrophoresis run for each DNA sample. Primers ITS1/ITS4 (White et al, 2002) were used for the fungi DNA in the gel electrophoresis which produced a 700bp product size. 16s rRNA primers RevB-ForB (Inqaba biosystems) were used as bacterial primers, producing a 700bp product size.

Gel electrophoresis

Agarose gel was prepared by weighing off 0.3g agarose and adding it to 30ml TAE buffer in an Erlenmeyer flask. A lid was placed on the flask and the flask was microwaved until all the agarose has melted (approximately 30-60 seconds). The mixture was swirled to mix. The flask was left to cool down slightly and 1 µl ethidium bromide (Sigma-Aldrich) was added to the gel mixture. The mix was swirled and poured into the prepared tray. The tray was left to set (approximately 20 mins) and the combs removed. The gel was removed from the casting tray and submerged into the electrophoresis tank with enough buffer to cover the gel. Small droplets of blue loading dye were mixed with 2 µl of DNA using the pipette and added to the wells of the gel. 2 µl of KAPA lambda ladder was used in the first well. Parameters for the gel were set to 80V for 40 mins. To visualize the gel it was carefully picked up and an image was taken with a UV doc.

Preparation of water samples for inoculation

50ml water samples were prepared in 100ml Schotte bottles that consisted of 50ml of either distilled water, aquaponic water sourced from the experimental farm or autoclaved aquaponic water. These water samples were set to mimic the aquaponic environment with no competition, and no nutrients, nutrients and competition and nutrients with no competition respectively. 100mg of insoluble tricalcium phosphate was added to each water sample. This was done to test the PS ability of each organism in the aquaponic environment.

Culturing organisms in aquaponic water sample

The three organisms were cultured to a certain OD and then 1ml was inoculated into 50ml of either distilled water, aquaponic water sourced from the experimental farm or autoclaved aquaponic water. The two bacteria strains were cultured in 9ml of Luria Bertani (LB) broth to an OD of 0.6 before inoculation. The fungi strain was cultured in 9ml of Malt Extract Broth (MEB) to an OD of 0.3 before inoculation, due to slower growth and time. The organisms were incubated at 26 - 30^{0}C for 4 days until the required OD was met before inoculation. Spectrophotometry readings were done using a fluorescence spectrophotometer at 600nm. 50ml of autoclaved aquaponic water served as the negative control that wasn't inoculated. The water samples were incubated at 26 - 30^{0}C for 4 days before being put on a shaker for a further 7 days at 26 - 30^{0}C. Before the addition of tricalcium phosphate and after inoculation and incubation the water

samples were sent to Sporatec, Stellenbosch (https://www.sporatec.co.za) to be analysed for the total amount of solubilized phosphorus available.

RESULTS

Sampling:

Sampling was conducted at three different sites from the rhizosphere of both soil and aquaponically grown Swiss chard. The temperature and pH of each sampling site was determined. The temperature from the aquaponics site was 21.5°C at all the sampling sites. The temperature from the soil sites ranged from 16°C - 17°C. The pH of the aquaponics samples was as follows: 7.12, 6.96, 7.05. The pH of the soil samples was as follows: 5.93, 5.95, and 5.92.

PVK microbial plate count:

If the microorganisms produce a visible halo on the PVK agar media they were identified as a PSO. Table 2 represents the initial CFU count of the dilution series on PVK medium. PSOs are highlighted. Colonies were identified using traditional morphology techniques.

Table 1: Morphological microbial plate count for CFUs from dilution series for each system.

		Dilutions					
System	Sample	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Aquaponics	1	Too many to count (TMTC), mat of green hyphae. No halos were formed on any CFUs.	11 white cream, 11 yellow and 100 cream CFUs	10 white 21 yellow 100 cream CFUs	5 yellow 5 cream 5 yellow combined, 6 creams, 18 cream 18 Beige CFUs	8 yellow, 6 cream 2 cream CFUs	1 large cream halo forming colony.
	2	TMTC, mat of green hyphae and no halos.	TMTC, green hyphae and no halos.	4 dark/orange, 2 light yellow 1 dark orange CFUs.	TMTC yellow 1 large light orange 1 small cream CFUs.	2 dark orange 2 light yellow CFUS.	2 orange 2 white CFUs
	3	TMTC. Mat of green hyphae and green	16 black 90 yellow 90 cream	1 Large purple filamentous	9 cream 30 orange 20 pink CFUs	24 cream 1 orange. 7 white	66 orange 5 cream 7 beige CFUs

		fuzzy fungi.	CFUs	24 cream 42 orange CFUS		CFUs	
Soil	1	TMTC. No halos.	Yellow, fuzzy, TMTC cream TMTC small green TMTC yellow colonies.	1 large white 2 orange 1 cream 4 red CFUs	2 small cream 1 big white 1 orange CFUs.	No growth	No growth
	2	TMTC. No halos.	TMTC. No halos.	2 yellow 3 cream TMTC white CFUs	1 cream/white1 fuzzy green middle White mat w/ spores CFUs	1 yellow 1 light yellow 1 green and 1 cream CFUs	12 yellow 11 cream 2 white CFUs
	3	TMTC. No halos.	3 fuzzy 7 orange 2 large orange 7 green 1 small CFUs	1 yellow 12 small yellow 1 fuzzy white 12 orange CFUs	TMTC white, 4 yellow 1 small brown CFUs	1 yellow 4 white 1 cream CFUs	TMTC white 5 brown 31 yellow 1 brown CFUs

From **table 1** it is evident that there was a greater diversity of halo forming colonies in the soil samples than in the aquaponics samples. From the aquaponics samples the most common colony was morphologically identified as cream irregular. From the soil samples the most common colony was identified as white/cream. There were fewer halo forming colonies from aquaponics samples and a greater number of PS colonies from soil samples. Three of the best halo forming colonies were selected to form pure cultures and to be analyzed further.

Analysis of halo forming pure cultures:

Two halo forming colonies were selected from our soil samples. The best halo forming colonies from the soil samples were both taken from S2 (Soil, sample 2) at a 10^{-4} dilution. The first halo forming colony (*B. pseudomallei*) from the soil samples was morphologically described as white/cream in colour and flat with smooth edges. From pure cultures, it could be determined that it had the second-largest formed halo diameters that ranged from 1mm - 3mm in diameter. The second halo forming colony (*A. niger*) from the soil sample was morphologically described as a mass of hyphae that had a fluffy texture with a white

undergarment. It had small black spheres that were raised. This PSO had the largest halo out of all isolated PSOs at 12mm in diameter.

Only one halo forming colony was selected from our aquaponics samples as the best halo forming colony. (*Pseudomonas sp. strain F4D*) from the aquaponics sample was taken from aquaponics sample A3 (Aquaponics, sample 3) at 10^{-6} dilution. When isolated in a pure culture morphological characteristics were determined as small yellow colonies that formed slightly dark orange colonies. This phosphorus solubilizing microorganism produced the smallest halos out of all the isolated PSOs at 0.5mm in diameter.

These colonies were selected for further analysis and were sent for sequencing as they were the best PSOs.

Microscopy:

The three chosen PSO colonies were then identified by light microscopy and the following was observed:
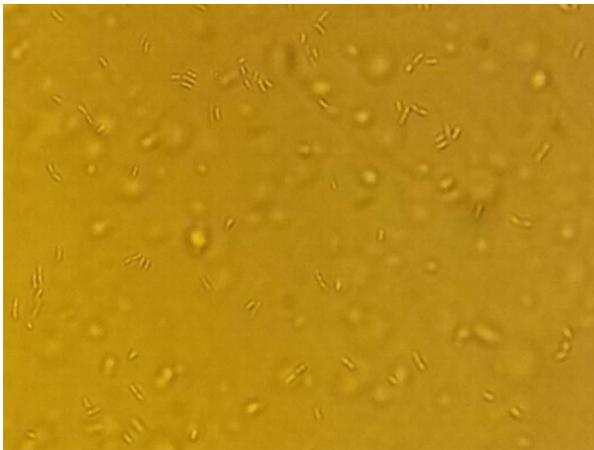
Soil samples:

Figure 1. Microscopy observations were of rod-shaped, non-motile, and small cells. The rod cells of *Burkholderia pseudomallei* mostly were viewed as single cells but occasionally a few cells were clumped together.

Figure 1: SBYLC 1.1 (Soil Bacteria yellow long cream) or Burkholderia pseudomallei. Picture was taken at 1000x magnification.
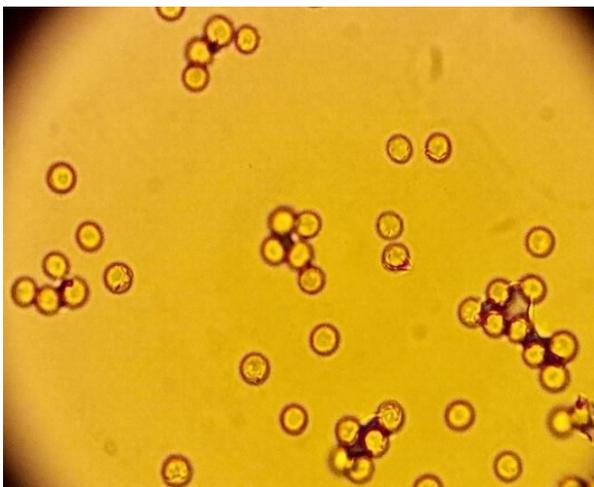

Figure 2. Microscopy observations were of large spherical spores. The *Aspergillus niger* spores are apparent with faint hyphae. The spore cells are much larger than the bacterial cells examined.

Figure 2: SF 1.1 (Soil fungus) or Aspergillus niger. Picture was taken at 1000x magnification
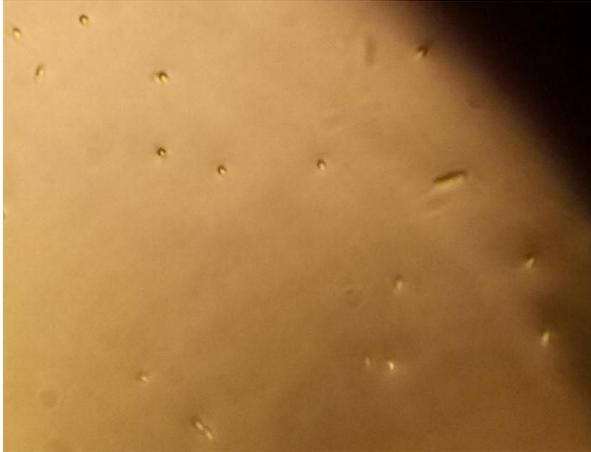

Figure 3. is the same organism as figure 2. Small spherical cells can be faintly seen under a light microscope.

Figure 3: SF 1.1 (Soil fungus) or Aspergillus niger. Picture was taken at 400x magnification.
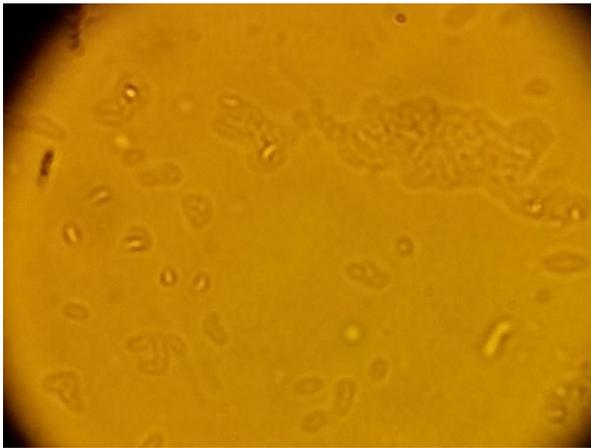
Aquaponics sample:

Figure 4. Microscopy observations were of rod-shaped, non-motile, and small cells. *Pseudomonas sp.* strain F4D form figure 4 is still larger than *Burkholderia pseudomallei* bacteria from figure 2.

Figure 4: AOD 1.1 (Aquaponics orange dark) or Pseudomonas sp. strain F4D. Picture was taken at 1000x magnification.

3

PCR and gel electrophoresis:

The extracted DNA from each of the three isolate samples was amplified and visualised using gel electrophoresis. For *A. niger*, the PCR and amplification were successful and can be seen in figure 5. For *B. pseudomallei* and *Pseudomonas* sp. strain F4D microorganisms, the DNA extraction and PCR reactions were successful and can be seen in figure 6.

DNA bands for figure 5 can be seen at 114bp against the 1Mbp ladder. The two bacteria DNA strands can be seen in figure 6 at 700bp against the 1Mbp ladder.
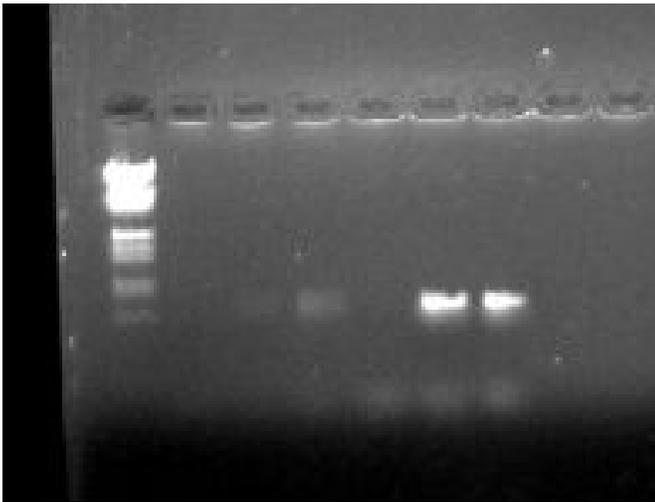

Figure 5: Gel electrophoresis of PCR reaction for Aspergillus niger DNA (key: 2 wells starting from the 5th positon of the ladder were loaded with Aspergillus niger DNA. A negative control was used in the well 4 positions from the ladder)
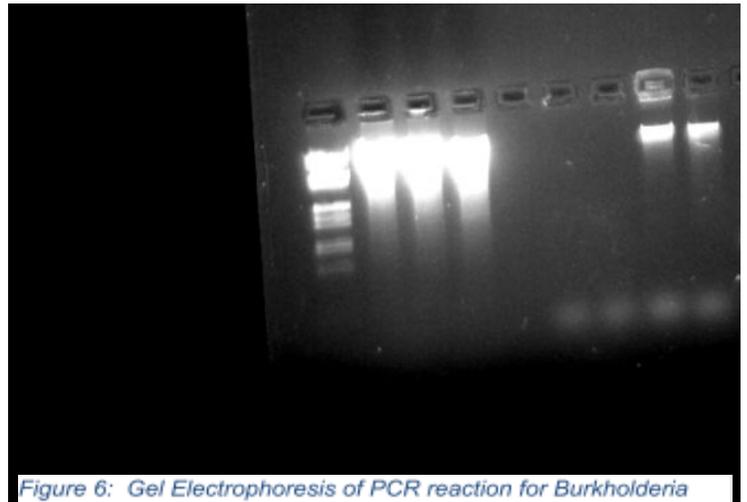
Figure 6: Gel Electrophoresis of PCR reaction for Burkholderia pseudomallei and Pseudomonas sp. strain F4D DNA (key: last two wells on the right hand side of the gel are the DNA samples. A negative control was loaded into the well 6 positons away from the ladder)

Sequencing:

The three PSOss that were isolated in pure culture were sent for sequencing. From the soil samples the colony that was morphologically identified as SF 1.1, visualized in figure 3, returned with a 100% identity match to *Aspergillus niger*. The soil sample that was morphologically identified as SBYLC 1.1, visualized in figure 1, returned from Blastn with a 100% identity match to *Burkholderia pseudomallei.* Both PSOs have an exact identity match and sequence similarities.

From the aquaponics samples, only a single organism was isolated in pure culture. This halo forming colony was morphologically identified as AOD, visualized in figure 4 and returned from Blastn with a 93% identity match to *Pseudomonas* sp. strain F4D.

Phosphorus solubilizing ability in an aquaponics environment:

To determine its PS ability in an aquaponics environment, each organism was inoculated into three water samples. Water sample 1 contained untreated aquaponic water. Water sample 2 contained autoclaved aquaponic water. Water sample 3 consisted of deionised water. A negative control consisted of autoclaved aquaponic water. 100mg of insoluble tricalcium phosphate was added to each water sample. The three best performing PSOs were then inoculated each water sample respectively. After an incubation period the changes in bioavailable phosphorus concentrations were determined.

Table 2: Initial bioavailable phosphorus concentrations of untreated aquaponic water sample before tricalcium phosphate inoculation and concentrations of the control (concentration of phosphorus after inoculation of tricalcium phosphate)

Sample	Bioavailable Phosphorus (ug/ml)
Untreated aquaponic water sample	2.07
Negative control (tricalcium phosphate added)	196.44

Table 2 illustrates the initial concentration of bioavailable phosphorus before the addition of tricalcium phosphate. This water was sourced from the farm and tested immediately after being autoclaved for total phosphorus solubalised. The negative control water sample was used to assess the overall change in bioavailable phosphorus concentration of the different PSOs when inoculated into the different water samples.

Table 3: The overall change of bioavailable phosphorus concentrations for each water sample inoculated with A. niger

Sample	Bioavailable Phosphorus (ug/ml)
Aspergillus niger - untreated	-5.83
Aspergillus niger - autoclaved	-25.83
Aspergillus niger - distilled	-52.14

Table 4: The overall change of bioavailable phosphorus concentrations for each sample inoculated with B. pseudomallei

Sample	Bioavailable Phosphorus (ug/ml)
B. pseudomallei - untreated	74.05
B. pseudomallei - autoclaved	-53.93
B. pseudomallei - distilled	5.24

Table 5: The overall change of bioavailable phosphorus concentrations for each sample inoculated with Pseudomonas spp.

Sample	Bioavailable Phosphorus (ug/ml)
Pseudomonas - untreated	-46.55
Pseudomonas - autoclaved	38.22
Pseudomonas - distilled	2.26

All the changes in bioavailable phosphorus concentrations are based on the negative control.

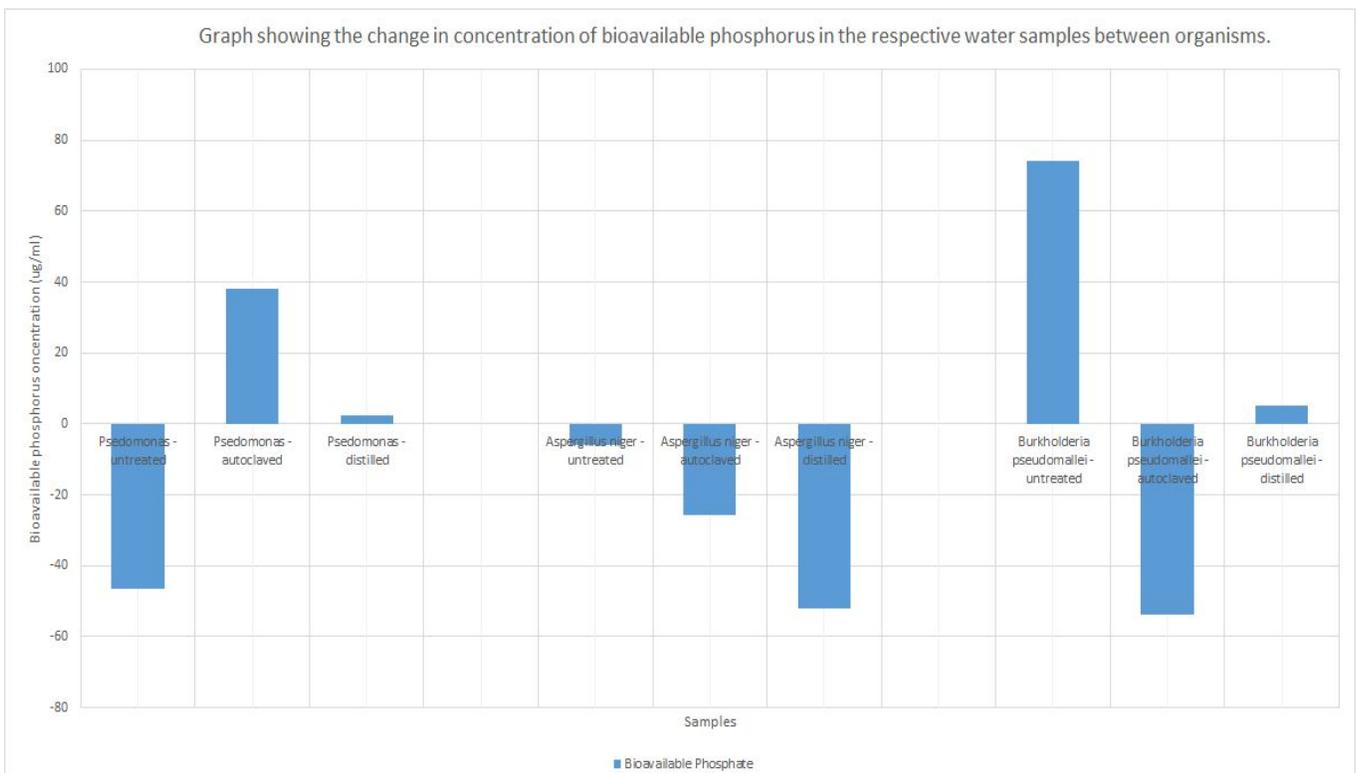**The overall change of phosphorus concentrations between organisms.**

Figure 7: Graph showing the overall change in bioavailable phosphorus concentrations in the respective water samples inoculated with the respective organisms. Figure 7 combines data from tables 4, 5 and 6 and is produced on overall averages.

B. pseudomallei produced the greatest positive change in bioavailable phosphorus in the untreated aquaponic water sample and had a small positive change in bioavailable phosphorus concentrations when inoculated into the deionised water sample. *B. pseudomallei* had a negative overall change in bioavailable phosphorus in the autoclaved water samples. *Pseudomonas* had a high positive change in bioavailable of phosphorus when inoculated into an autoclaved water sample and a small positive change when inoculated into the distilled water samples but a large negative change in the untreated water samples. *A. niger* had negative changes across all three water sample treatments with a noticeable high negative change when inoculated into the distilled water sample. *A. niger* had the second largest negative change in autoclaved water samples and a small change in untreated water samples.

The overall change of phosphorus concentrations between water samples.
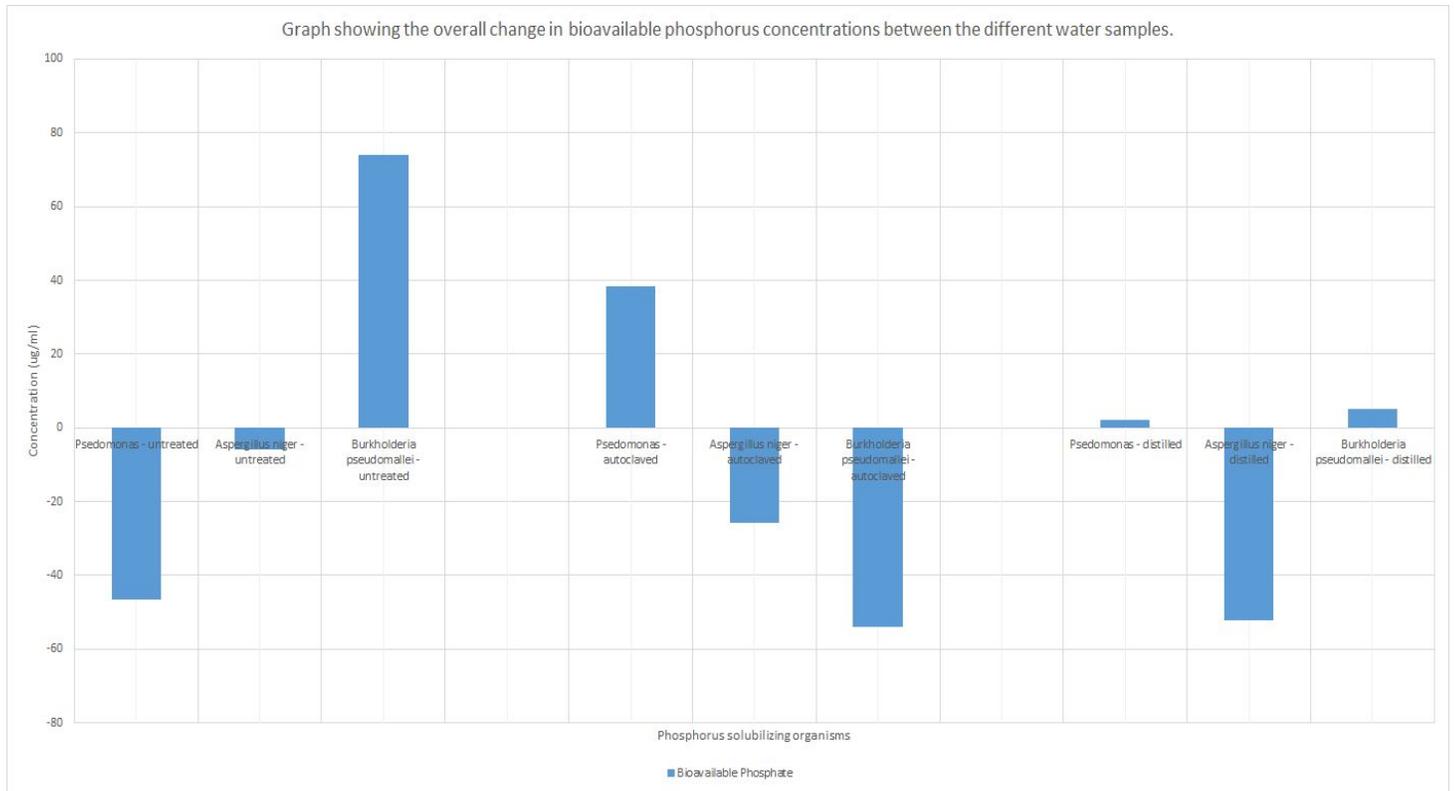

Figure 8: Graph showing the overall change in bioavailable phosphorus concentrations in the respective water samples between organisms. Figure 8 combines data from tables 4, 5 and 6 and is produced on overall averages.

From figure 8 it can be deduced that *B. pseudomallei* had the highest positive concentration change of bioavailable phosphorus between the organisms of the untreated water samples. *Pseudomonas* had the highest negative change in bioavailable phosphorus when the organisms were inoculated into the untreated water samples. *A. niger* had an overall small negative change in bioavailable phosphorus when inoculated into the untreated water samples. *Pseudomonas* had the highest positive concentration change of bioavailable phosphorus between the organisms when the water sample was autoclaved. *B. pseudomallei* had the highest negative change between the organisms when the organisms were inoculated into autoclaved water samples. *A. niger* had a high negative change in bioavailable phosphorus when inoculated into autoclaved water but was still less than *B. pseudomallei*. *A. niger* had the highest negative change in concentration of bioavailable phosphorus when the organisms were inoculated into the distilled water samples. Both *Pseudomonas* and *B. pseudomallei* had a small positive change in bioavailable phosphorus concentrations when inoculated into distilled water samples but *B. pseudomallei* had a slightly greater change.

DISCUSSION

The recorded soil temperatures were measured on the same day and around the same areas. The shallow soil temperature is influenced by the amount of sunlight, moisture, and surrounding air temperature (Pillai and Samual, 1990). This is especially true for close-to-surface rhizosphere of the Swiss chard plant as it has a very shallow root structure (Loomis and Rapoport, 1985). The Aquaponics system, being a closed system, reflected an average temperature of 21.5 degrees Celsius which was higher than the average soil temperature of 16.33 degrees Celsius. This change in temperatures could be due to the reduced exposure to outside air temperature air fluctuations in the aquaponic greenhouse.

In order to get a more comprehensive and diverse data series and to further the accuracy of this experiment, future testing should include a wider range of soil types. The soil samples should be taken at different times of the year to increase the possibility of isolating PSOs. This experiment took samples from the same agricultural crop, and from the same type of environments respective to where they were sampled. Two different sampling environments were used in this experiment which can be used to compare the PSO diversity and ability between environments. This experiment is not an accurate representation of all soil types.

The pH reading represents how alkaline or acidic the environment is based on a scale of 0-14. The soil samples showed an average pH that was slightly acidic of 5.93. Whereas, the aquaponics environment samples showed on average a near neutral pH environment of 7.12. pH and temperature readings were conducted on the same day of sampling. Temperature and pH of the soil influences the microbial ecology

of the soil (Andersson, et al., 2001). pH levels influence factors such as the production and leaching of organic carbon. This affects the growth of microorganisms and thus will influence its solubilizing ability.

The samples were used in a dilution series and were plated onto PVK medium. An understanding of the culturable microbial diversity could initially be established using traditional morphological techniques. PVK media is recommended for detection of PSOs (Pikovskaya, 1948), however a range of media could be explored to increase the possibility of culturing a PSO. The samples were plated at different dilutions to produce pure cultures. Replating of selected colonies allowed for single colony-forming units to proliferate and produce pure colonies.

From table 1, it can be deduced that there was a greater diversity of microbial colonies in the soil samples compared to the aquaponics samples. It can also be deduced from table 1, that there was a larger diversity of PSOs found in the soil samples compared to what was observed in the aquaponics samples. This was identified by identifying halo forming colonies (Sagervanshi, et al., 2012), however, these results cannot be used as a true representation of the microbial diversity due to the great plate count anomaly (Harwani, 2012).

The great plate count anomaly states that not all microorganisms can be cultured in the lab. This could be due to their growth state being altered to a high nutrient growth media and sub-optimal growth conditions. This experiment was limited to three organisms that were chosen based on their halo formation capabilities on the PVK plates. This technique, however, is not a completely accurate representation for choosing the most effective PSOs based on their solubilising capabilities. Many organisms do not form halo zones but do solubilise insoluble inorganic phosphates in a liquid medium, (Nautiyal et al, 1999).

A study by Braz, et al., (2012) showed successful growth on PVK plates illustrates solubilizing phosphorus abilities positively correlated to halo size. From the PVK dilution plate morphological identification, three of the best halo forming colonies were selected and the diameters of the halos were measured. The first soil colony was identified as *Burkholderia pseudomallei* which produced the second-largest halo forming colonies when grown on a solid medium. The diameter of the halos suggests that from the three selected organisms it is the second-best phosphorus solubilizing microorganism that was plated. *Pseudomonas sp. strain F4D* was isolated and grown in pure culture PVK medium from the aquaponic samples. *Pseudomonas sp. strain F4D* produced the smallest halo forming colonies with the smallest diameters, which suggests that *Pseudomonas sp. strain F4D* shows the lowest phosphorus solubilizing ability. *Aspergillus niger* was suggested to be the best phosphorus solubilizing microorganism when plated on solid medium as it produced the largest halo-forming colonies with the largest halo diameters. When grown on PVK medium *Aspergillus niger's* growth was substantially larger than the two bacterial cultures indicating that it is better adapted to this artificial agarose environment.

Figure 5 and figure 6 show the results of the PCR gel electrophoresis. The purpose of the gel electrophoresis procedures were to confirm the presence of DNA, following DNA extractions and PCR reactions of the two bacterial samples and the one fungal sample. Figure 6 confirms the presence of DNA extracted from the soil fungus sample, which was 100% confirmed to be *Aspergillus niger* through sequencing techniques that were outsourced to CAF at The University of Stellenbosch. Two wells showed positive results for the presence of DNA and were visualized in figure 5 to ensure successful results. Figure 6 showed positive results for presence of DNA for both bacterial strains.

The soil bacterial strain was sequenced at CAF and showed a 100% match identification result for *Burkholderia pseudomallei,* whereas the aquaponics bacterial strain showed a 93% match identification result for *Pseudomonas* sp. strain F4D. The percentage similarity results were obtained through bioinformatics techniques made available by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). *Aspergillus niger* is a ubiquitous microorganism and is commonly found in soil, seeds, plant litter, plant rhizospheres, dried fruit and nuts (Sarkari, et al., 2017). *B. pseudomallei* is a soil-dwelling bacterium that has plant growth promotion properties (Vandamme & Coenye, 2003). *Pseudomonas* species tend to live in water, soil and damp areas (Dimitrova, 2016). The identity score of 93% is not a high enough accuracy score to classify this genus as a specific species of *Pseudomonas*, but the possibility of it being the species strain F4D is high due to the nature of the environments sampled and the *Pseudomonas* tend to inhabit.

Figure 7 illustrates groupings of each microorganism separately and illustrates their solubilizing phosphorus abilities within each water sample. Tables 4,5, and 6 represents the amount of bioavailable phosphorus that was solubilised by the growth and metabolic activities of the selected PSO's compared to a negative control. The negative controls concentration level of bioavailable phosphorus represented negligible change over time due no inoculation of phosphorus solubilizing organisms in the water sample. The three water samples tested were: untreated aquaponic water, autoclaved aquaponic water, and deionised water.

The *Pseudomonas* genus is a Gammaproteobacteria, classified as gram-negative. The strain isolated by this study, *Pseudomonas* sp. strain F4D, has close genomic characteristics to the *Pseudomonas fluorescens* species which has been found to improve plant growth in a study done on maize crops (Alori et al, 2017). However, the *Pseudomonas* species which was isolated from the aquaponics rhizosphere, showed an overall negative change for bioavailable phosphorus concentration. This negative result suggests the *Pseudomonas* species, through the process of growth, consumed organic phosphorus at a higher rate than it was able to solubilise inorganic phosphorus. This bacteria has been proven to grow well in aqueous environments with multiple isolations found from analysis of drinking water (Wong et al ,2011*).* This correlates to our data in which the highest change or production of bioavailable phosphorus

was found in autoclaved water which had all remaining microorganisms destroyed through heat, removing any competition. This suggests *Pseudomonas* performs optimally, in terms of providing bioavailable phosphorus, in a non-competitive environment. Similar can be said for the *A. niger* species for bioavailable phosphorus production in aquaponics water samples as it represented a negative result (Chuang et al., 2006).

A. niger, an endophytic fungus, is able to form a mutualistic relationship with the host plant. The fungus is effective in promoting root–shoot length by providing the plant with bioactive products such as phytohormones (Gryndler et al, 2000). This fungus obtains sugars from the host plant and lives out the majority of their life cycle within the root structure of their host plants (Gianinazzi et al ,1996).

Studies have shown a synergistic relationship between *Bukalderia spp*. and *A.niger* concluding that co-cultures produce greater levels of bioavailable phosphorus due to an increased organic acid production, compared to being cultured individually. It is suggested, for future research, to determine the possibilities of performing this co-cultivation method in an aquaponics system to test performance and relevant advantages made to aquaponic system.

B. pseudomallei was the most consistent microorganism in the aquaponics untreated water samples. *B. pseudomallei* is a Gram-negative pathogen and has the ability to cause melioidosis in humans (Wiersinga et al ,2006). A similar study done with two Burkholderia species, *Burkholderia tropica and Burkholderia unamae,* which have been isolated as effective, non-pathogenic, PSOs and should be considered for future studies (Ghosh et al, 2019).

B. pseudomallei grows in an aqueous environment and has been found to form biofilms which have been shown to aid in survival in dynamic environments (Ghosh et al, 2019). Although no biofilms were studied in this project, it could be seen as advantageous in an aquaponics environment due to the environmental disturbances common to the aquaponics systems i.e. constant water flow, up-rooting and replanting of plants into the loose LECA clay balls as well as changes in nutrient availability.

No negative results were found for the change in bioavailable phosphorus for *B pseudomallei* - they did however show a negative result in the autoclaved water samples and a very low level in the distilled water samples. This could suggest they perform better in a symbiosis with other microorganisms which could still have been present in the aquaponic water. Through research of different papers, it is inconclusive as to why *B. pseudomallei* results behaved in this manner.

Figure 8. shows a different interpretation of the data in figure 7, whilst grouping the data in water type group instead of microorganism type groups found in figure 7.

To improve upon this study, suggestions into performing community fingerprinting to gather more data on the community structure, this must be done in triplicate. Comparisons of the microbial community diversity of soil and aquaponic rhizospheres could give insight into the community structure and stability. This study was limited to only culturable phosphorus solubilizing organisms on PVK media and so future studies could use alternate methods like DNA isolation and metagenomics to obtain a further scope of all organisms in the sampled environments that may improve phosphorus solubilisation.

Further studies can also take organisms that performed well in the solubilisation tests and inoculate them into actual aquaponic systems to see their effects on plant growth with and without soluble phosphorus supplementation. This can be suggested to lead to the development of biofertilizers.

This research team was initially interested to see whether the possible microbial structural differences between aquaponic and soil rhizospheres contributed to the differences in taste intensity of the fruit of vegetables grown through each method. This research team hypothesised that this is due to microorganisms in the soil environment, that were unable to survive in an aquaponic aqueous environment, perished when introduced into the aquaponic environment. This is a possible future experiment to conduct ARISA profiles of both aquaponic and soil rhizospheres to compare the microbial diversity and assess whether differences found could be contributing to this phenomenon.

After finding that the soil microorganisms were the most efficient at phosphorus solubilisation, the next step in this study would be to test these microorganisms' ability to solubalise phosphorus in a complete aquaponic trial. This research team used the individual nutrient broths - LB for bacterial species and MEB for fungi species, as the carbon sources. To make this study more accurate, a more optimised aquaponic environment with added carbon sources should be incorporated. Aquaponic systems have not only reported deficiencies in phosphorus, but so too in the elements such as calcium and iron. Further research into microorganisms that have aid in the provision of these elements would also be a useful field of research to aquaponic farmers to work in association with the soluble phosphorus providing microorganisms that have been suggested in this research paper.

CONCLUSION

There is a larger diversity of PSOs in the tested soil rhizosphere compared to an aquaponic rhizosphere growing the same plant. Soil microorganisms showed the greatest ability to produce solubilised bioavailable phosphorus compared to the aquaponic microorganisms. The soil bacteria *Burkholderia pseudomallei* performed the best overall in the aquaponic untreated water and the soil *Aspergillus niger* performed the best overall when grown on PVK plates, however, performed the least effectively when grown in an aqueous environment. This fungi is therefore not an option for aquaponic inoculation.

According to the results of this study, attention in increasing species levels should be given to aquaponic Pseudomonas sp. strain F4D and soil *Burkholderia pseudomallei,* when intending to increase levels of bioavailable phosphorus in an aquaponics environment to assist in plant development.

Acknowledgments

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