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Analytical profile index in smart-vertical farming technique for greenhouse vegetable crops production

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Abstract

Analytical profile indexing was conducted in (CHAT), CLSU Hydroponics, Aquaponics Technologies Demonstration Farm and Experimentation Station, Central Luzon State University, to identify bacteria present in hydroponic production water system using a recirculating prototype module. Water samples were collected from different sampling points, in three collection periods with an interval of 20 days. Environmental factors such as temperature and relative humidity, water quality monitoring parameters like pH, total dissolved solids, water temperature, electrical conductivity and dissolved oxygen were monitored to attain a desirable ecosystem for optimal growth of living organism in the systems. Water samples taken from system of greenhouse hydroponic production were analyzed for total viable bacteria (CFU/ml) in the water. Microbial identification and comparison of bacterial isolates were done using biochemical identification of bacteria based on the methods of API Staph and API Coryne strip. A total of twenty-five (25) bacteria were isolated out of which fifteen (15) strains of microbes have relatively high growth in the medium were identified and described. Eleven (11) bacterial isolates belongs to genus Staphylococcus, namely *S. capitis*, *S. lugdunensis, S. cohnii, S. sciuri, S. auricularis, S. hominis, S. lentus, S. haemolyticus, S. hyicus, S. warneri* and, *S. caprae* one under each genus Corynebacterium, Cellulomonas Leifsonia, and Kocuria were identified and characterized to determine the structure, arrangements, habitat, activity, and pathogenicity of bacteria present in the hydroponic production. The results reveal great diversity of organisms isolated including the presence of pathogenic microorganism, endophytic bacteria (rhizobacter) and commensally occurring bacteria.

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Introduction

The Philippines, due to its geographical location, is no stranger to natural disaster and vulnerable to adverse effects of environment, rendering the optimum capacity greatly affect agricultural food production difficult. Climate change poses a significant hazard to Filipinos due to the frequency and severity of climatic disasters such as typhoons and lengthy droughts (Sace and Fitzsimmons, 2013). Recent studies have also showed that the country's water reservoirs are under jeopardy, which might lead to severe water shortages or, in the worst-case scenario, pollution (Cayabyab, 2012). Water of poor quality has the potential to be a direct source of contamination as well as a vehicle for spreading contamination, potentially leading to outbreaks of foodborne disease. Early research on free-living microorganisms in soil and water suggests that particular strains may help crops by boosting plant development or may be sources of foodborne infection (Falivene, *et al.,* 2005).

The challenge of producing a sustainable and safe food for human consumption with fast-faced growing population with limited resources is still under-developed. Addressing these issues, the potential of modern agriculture using modern and climate-smart farming method a solution to the problem of food shortage (Sace, *et al.,* 2015). Using less space and limited arable land using climate- smart farming, one of its objectives is to promote soilless farming in order to increase vegetable productivity of high-value crops using earth's limited resources even in unproductive space (Sace and Natividad, 2015).

However, the essential of soilless culture farming through its use of mineral based solution, surprisingly few bacteria raid the nutrient stores of living plant cells, apparently because the metabolic intimacy involved in parasitism requires the work of specialists (Alfano *et al.,* 1996). Thus, hydroponic systems offer a unique environment for control of pathogens since various parameters can be managed to favour beneficial microorganisms over pathogenic bacteria and fungi (Paulitz *et al.,* 2001).

This study focused on bacterial evaluation utilizing a domestic hydroponic system, water quality monitoring measures, and the characterization and identification of bacteria present in hydroponic production.

Material and methods

Prototype Module Set-up

The system is enclosed in a tropical greenhouse measuring 2.5 m high x 3.2 m wide x 3.6 m long and is fabricated from locally available materials. The greenhouse is made up of galvanized iron pipes bended and welded together to form a Quonset-type structure as shown in Fig. 1. Prototype Module is a vertical frame measuring 1.6 m high x 0.6 m wide x 0.8 m long consists of five layers of growing tube made from 2" diameter PVC pipes interconnected by rubber hose. The growing tubes contain cut-outs each to hold 360 planting cups. The cups are securely seated on each cut-out of the frame and contain mixture of coconut peat, rice hull and carbonized rice hull and fine sand as growing media. Each cup has holes on the side and bottom surfaces to permit capillary action of the nutrient solution into the media as well as to allow the plant roots to extend into the duct and contact the nutrient solution (Sace and Natividad, 2015).

Preparation of the Nutrient Solution

Solution was prepared and managed using the following preparation and management acquired from the protocol of Sace and Natividad (2015). Tank was filled with clean water and the system run for about an hour to check for leaks and whether the float switch is functioning properly. The water was removed from the tank and the system started with fresh and clean solution. The tank was filled again with 49 liters of water. 0.5 liter of Solution A and 0.5 liter of solution B were mixed for every 49 liters of water to make a 50-liter solution in the tank. Water from the tap is normally chlorinated and allowed to stand for a night to volatilize. Rainwater, when properly harvested, is a better option. Then, the quality of nutrient solution was monitored. The electric conductivity (EC), pH, temperature and dissolved oxygen (DO) should be maintained at optimal level.

EC ranges from 1.0 to 1.3mS/cm, pH from 5.8 to 6.8, and DO of greater than 5 ppm. When pH is high, suitable amount of sulfuric acid (H_2SO_4) is added to bring it down while potash is added to bring the pH up.

Collection of Water Sample and Preparation of Microbial Isolates

The water samples were collected from three setup commercial greenhouse each with initial reservoir tank to final filtered reservoir tank using a sterilized pipette and aspirator. Three samples per each tank were collected from 0, 20th and 40th day. The water samples were placed on sterilized bottles for serial dilution in the laboratory.

Serial Dilution

Eight test tubes were labelled from 1-8. Nine ml of distilled water was placed in each tube using a pipette and was plug with cotton covered with aluminum foil prior to sterilization in an autoclave for 20 minutes in 15 psi.

One ml of the sample was poured into tube #1, this was serve as the initial dilution factor or 10^{-1}. The tube was shaken by grasping it between the palms of both hands and rotating quickly to create vortex. It helped to distribute the microbes and break up any clamp. Using aseptic technique, second dilution (10^{-2}) was made by transferring 1ml from tube #1 to tube #2 the shake. Immediately after shaking, 1ml from the previous dilution was aseptically transferred to the next dilution until the 10^{-8} dilution factor is attained.

Separate sterile pipettes were used in each dilution factor. In computing the dilution factor, the following formula was used:

$$\text{Dilution factor} = \frac{\text{Amount of sample}}{\text{Amount of sample} + \text{Amount in tube}}$$

Pour Plating

Eight sterilized petri plates were labelled from 1-8 and dilution factors with 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} from the previous serial dilution were used. Using sterilized pipette, 1 ml of each dilution factor was aseptically transferred from each petri plate in duplicate. Fifteen to twenty ml of liquefied sterilized nutrient agar (NA) was pour over each plate.

The plates were swirled to mix the medium and diluted sample. The plates were then be incubated in inverted position at 35°C for 24-48 hours.

At the end of the incubation period, plates with more than 250 and 300 colonies cannot be counted were designated as too many to count (TMTC). Plates with fewer than 25 and 30 colonies were designated as too few to count (TFTC).

Pure Culture Preparation

After incubation, transferring of bacterial growth was done by touching the growth from each petri plates using a sterile loop and streaked into the test tube with nutrient agar in slanted position.

Biochemical Test

The API Staph strip consists of 20 microtubes containing dehydrated substrates. These microtubes were inoculated with a bacterial suspension, prepared in API Staph Medium, that reconstitutes the tests. During incubation, metabolism produces color changes that were either spontaneous or revealed by the addition of reagents. The reactions were read according to the Reading Table and the identification was obtained by referring to the Analytical Profile Index or using the identification software.

Results and discussion

The study was conducted at (CHAT) Demonstration Farm and Experimental Station using a recirculating greenhouse backyard model for leafy vegetables. Lettuce (*Lactuca sativa*), Carlo Rossa traditional types of lettuce and cucumber (*Cucumis sativus)* Amata, Green beauty and Bangkok cucumber varieties were planted on the cups securely seated on the cut-outs of each layer. Each cup has holes on the side and bottom surfaces to permit capillary action of nutrient solution in the media.

Environmental Factors

Environmental factors are factor any factor that influences living organisms. Factors include ambient temperature, amount of sunlight, and pH of the water soil in which an organism lives.

Environmental factors such as temperature, humidity and water quality in hydroponic systems are one of the major limiting factors to crop production and directly affect crop productivity of leafy vegetable (Sace and Estigoy, 2015).

In tropical countries like the Philippines, due to its geographical location, normally associated with high temperature, changing and controlling quality parameters are necessary for optimal plant growth and crop production, (Carruthers, 2002 Salve and Hiware, 2008). However, Growth of bacteria also has optimum ranges in which it can carry out its biochemical process, which includes temperatures and a range of pHs (Scattini and Maj, 2017). Microbes affected by ambient environmental conditions, in particular temperature and humidity (Walsberg and Schmidt 1992).

Temperature

In tropical countries, like Philippines, lettuce grows at its best and is productive during rainy season (June to October) and windy season (November to February) with temperatures ranging from 25 to 35°C. During dry season (March to May), lettuce becomes less productive as temperature is even higher ranging from 29-38°C (Sace and Estigoy, 2015). Temperature was at the optimum requirement of crop throughout the growing seasons.

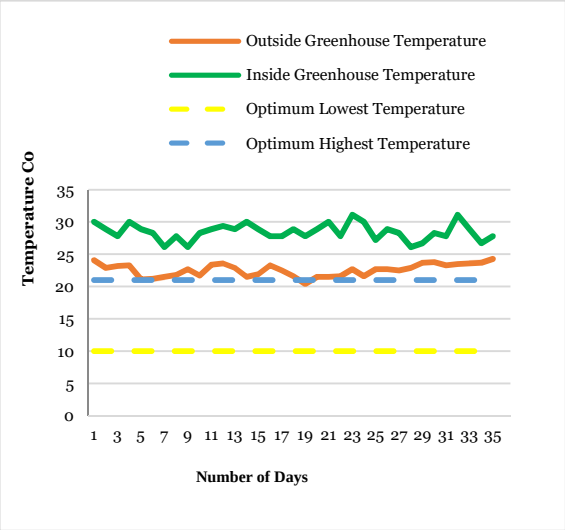

Fig. 2. Temperatures recorded inside and outside greenhouse production.

Result showed that temperature inside the greenhouse ranged from less than 26 to about 32°C while temperature outside the greenhouse ranged from 20 to 25°C. The change in temperature causes the solution to spike. Temperature inside the greenhouse was higher than that of the temperature outside (Fig. 2). Optimum growth of lettuce and cucumber ranged from less than 10 to 20°C.

Nonetheless, the shading net and outer layer roof cover reduce the amount of solar radiation entering the greenhouse. The silver-gray net is made of material proven to reduce solar radiation by as much as 30-40% thereby preventing accumulation of heat. At the same time bacteria experience optimal growth at temperatures of 25-40°C/77-104°F. Thermophilic, or heat-loving, microorganisms experience optimal growth at temperatures greater than 45°C/113°F and up to 100°C/212°F (Bruce and Drysdale 1994).

Alternately, when temperatures reach below 18°C/64°F, microorganism growth decreases and nearly ceases when temperatures reach the freezing point of water. Studies have indicated that the growth of microorganisms, such as bacteria, completely cease when temperatures reach < -18°C/0°F (Bruce and Drysdale 1994).

Relative Humidity

Relative humidity (RH), defined as the amount of water vapor in the air to the greatest amount possible at the same temperature, expressed as percentage of the ratio of the actual water vapor pressure to the saturation vapor pressure.

RH effect on plants plays a crucial role in photosynthetic capability; plant can only absorb a reduced amount of humidity and hence has less water evaporation than most plants. If the plant loses too much water, the stomata will close with the result that photosynthesis stops. If this happens, no further CO_2 can be absorbed, and CO_2 is required to keep the photosynthesis going (Arthur *et al.*, 2011).

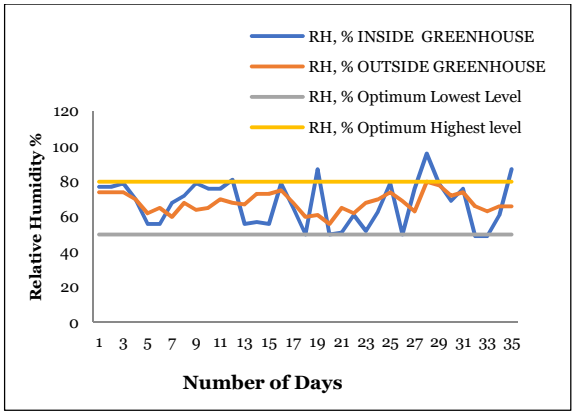

Fig. 3. Relative humidity recorded inside and outside greenhouse production.

Results revealed that greenhouse relatively met suitable RH during the study ranging from 50 to 88 % (Fig. 3). The optimal humidity range for vegetable crops is 50-88% RH with 50% being the best. If it gets too humid, plants are unable to transpire (breathe) properly. At the same time different types bacteria require different amounts of water (in vapor form) to reproduce and grow. The majority require relative humidity (RH) of 60 percent or more, though some can survive and multiply in >20 percent RH. Thus, decreasing temperature and moisture (relative humidity), creates a less hospitable environment for microorganisms to grow (Scattini and Maj, 2017).

Cook *et al.* (2005) reported on the effect of temperature and humidity on bacterial growth and infection plants, the greater bacterial growth in the groups with high humidity under high temperature since those conditions seem optimal for microbial proliferation by the presence of liquid water surface. Overall and against our predictions, we showed that high temperature and low humidity were most favorable to growth of bacteria,

Microbial Identification and Comparison of Bacterial Isolates

The analytical profile index API is a classification of bacteria developed for quick identification of clinically and industrially relevant bacteria. API test system introduced a standardized biochemical identification of bacteria based on the methods.

API Staph is a standardized system for identification of genera under *Staphylococcus*, *Micrococcus* and *Kocuria* while API Coryne is a standardized system for identification of bacteria under genera *Coryne*. The API test consists of strip with microtubes containing dehydrated substrates. These microtubes were inoculated with a bacterial suspension, prepared in API Staph and Coryne medium (Fig. 4). The reactions were read according to the Reading

Table and the identification was obtained by referring to the Analytical Profile Index or using the identification software. Fig. 6 shows biochemical identification of bacteria using API 20 Staph and Coryne.

Identification was obtained with the numerical profile based on the result sheet, the tests are separated into groups of 3 and a value of 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained.

After three subculture and three times of colony purification on agar-solidified medium, a total of twenty five 25 bacteria were isolates and a total of fifteen (15) strains of microbes with relatively high growth in the medium were isolated and identified. Biochemical analysis indicated good identification from significant taxa. Total of bacterial isolates and identified found in the hydroponic greenhouse production were presented in table 1.

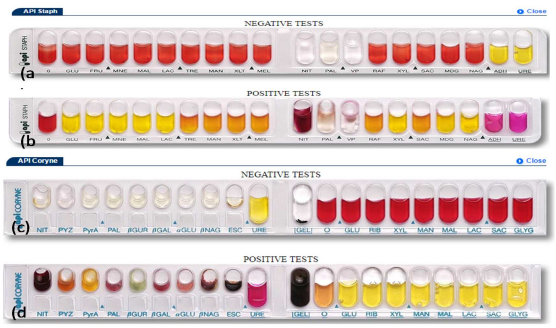

Fig. 4. Biochemical identification of bacteria using API STAPH (a) before inoculation and negative test, (b) after inoculation and positive test, API CORYNE (c) before inoculation and negative test and (d) after inoculation and positive test.

Morphological Characterization of Bacteria

Morphological characterizations of bacteria are noted to determine the structure, arrangements habitat, activity and pathogenicity of bacteria present in the hydroponic production. Active bacterial action in the hydroponic solutions offers a unique environment also hydroponics systems provide the right conditions allowing not only plants to grow also microbial communities.

Table 1. shows morphological characterization of bacteria, habitat and pathogenicity of species present in hydroponic production. The results reveal great diversity of organisms isolated including the presence of pathogenic microorganism, endophytic bacteria (rhizobacter) and commensal occurring bacteria. The presence of these organisms *S. sciuri,* and *S. caprae* associated with domestic animal animals skin such as goat and pig . *S. hyicus*, a type of bacteria originally isolated from skin of pigs, pathogenic only in pig cause exudative epidermitis (Nithya and Babu, 2017).

This led to analyze the surrounding areas from presence of animals such as goat and pig in the immediate areas close to the greenhouse set up, which suggests a possible source of contamination.

The presence of endophytic bacteria also known as plant growth-promoting rhizobacteria (PGPR), bacteria that lives within a plant and around plant environment for at least part of its life cycle without causing apparent disease. Some bacteria may have more than one mechanism for accomplishing plant growth (Ahmad *et al.,* 2008). Included in such mechanisms are antagonism to pathogenic fungi, siderophore production, nitrogen fixation, phosphate solubilization, the production of organic acids.

Table 1. Morphological characterization of bacteria. Habitat and pathogenicity of species present in hydroponic production.

Organism	Pathogenic (+/-)	Morphology	Habitat and Activity
Staphylococcus capitis	–	Circular, raise, smooth, white, opaque, spherical single in form	Potential plant growth promoting characteristics of cultivable strains, normal flora of the skin and from the environment.
Staphylococcus lugdunensis	–	Regular, entire, convex, moist, white, cream, spherical cluster in form	Natural flora of skin harmless and natural on environment,
Staphylococcus cohnii	–	Irregular, entire, dry, white, opaque spherical cluster in form	Associated with foods of plant or animal origin naturally found with high content of Urea, Plant growth-promoting effect through Urease action,
Staphylococcus sciuri	+	Irregular, undulate, mucous, white, opaque, cocci cluster in form	Most often presented as commensal in plant and bioremediation in soil. Pathogenic to animals
Staphylococcus auricularis	–	Circular, entire, convex, moist, clear coccus pair in form	This species was originally isolated from the exterior of a human ear and skin
Staphylococcus hominis	–	Irregular, undulate, moist, clear, cocci clusters in form	Commensal on animal skin and plant, known for producing thioalcohol compounds that contribute to foul odour,
Staphylococcus lentus	–	Irregular, lobate, convex, dry, white, cocci clusters in form	Plant endophytic bacteria associated with high chromium mineral.
Leifsonia aquatica	–	Circular, convex, moist, white, opaque, coryneform rod shape	Aquatic bacterium that is typically found in water habitats. Commensal in plant
Cellumonas spp	–	Circular, convex, moist, white, opaque, coryneform rod shape	Strains are not pathogenic, typically growing in decaying plant-rich soil, microorganism that utilizes cellulose
Staphylococcus haemolyticus	+	Circular,entire, convex, moist, clear, opaque, cocci, single in form	Bacterial endophytes isolated from bioenergy crops sugarcane natural found from environment and domestic animal
Staphylococcus hyicus	+	Irregular,entire, dry, white, opaque, Consisting of clustered cocci	Originally isolated from skin of pigs. Pathogenic only in pig, cause exudative epidermitis

Organism	Pathogenic (+/-)	Morphology	Habitat and Activity
Corynebacterium striatum	–	Irregular, entire, convex, dry, white, opaque coryneform rod shape	Rarely been reported to be a pathogen need co-pathogen. Natural found from environment, initially identified in soil bacteria and in plant
Staphylococcus warneri	_	Regular, lobate, moist, clear, cluster in form	Plant endophytic bacteria, beneficial plant associated microbes. Common commensal in animal
Staphylococcus caprae	–	Circular, entire, convex, moist, clear single in form	Non coagulase usually associated with animal skin, such as goat.
Kocuria kristinae	_	Regular, entire, dry, white, opaque cocci, single in form	Plant growth promoting rhizobacteria (PGPR) is able to inhabit the area close to plant roots and exert beneficial effects

Adapted from *Bergey's Manual of Systematic Bacteriology*, 2nd ed (2005) and (Nithya and Babu 2017)

The agricultural importance of free-living bacteria indicates the diversity of microflora and their mechanism in the production (Ahmad *et al.,* 2008). Results suggest that the primary beneficial effects from the living microbes in the system was created while the water was recirculated and was retained. Bacteria like *S. capitis* was reported isolated and was to characterize the culturable endophytic bacteria of common bean (Phaseolus vulgaris), and leaves from three different cultivars (Costa *et al.,* 2012). Similarly, *S. haemolyticus* was reported bacterial endophytes isolated from bioenergy crops sugarcane (Magnani *et al.,* 2010). Study conducted by Kaul *et al.* (2016) on genomes of rice endophytic bacteria a total of 21 bacterial endophytes isolated from rice seeds, isolates were identified and genomes of endophytic bacteria encodes results show presence of *S. cohnii,* and *S. homini* among these were the study conducted showed that the PGPR abilities. Certain bacteria were common to all sources, while others tended to be found predominantly in a particular site. The primary organisms generally associated with the hydroponic system were *S. capitis* and *S. haemolyticus*. *S. cohnii and S. hominis* was a common isolate but appeared to have a better survival rate in the water and was not frequently transmitted to the lettuce. Certain bacteria were common to all sources, while others tended to be found predominantly in a particular site.

Unusual occurrence of *S. warneri* and *S. lentus* as endophyte in the system are reported already as endophytes in plants and plant-derived produce. Among these were the study conducted by Song and Yen (2002). However, the occurrence of *S. warneri* is unusual. The observation emphasizes the need to further our understanding of the changing ecology of bacteria (Phukon *et al.,* 2013). Moreover, some endophytic bacteria like *K. kristinae* had a property of beneficial microbial allelopathy a study conducted using endoroot bacteria from *Tagetes* spp. can play a role in nematode suppression through the attenuation of nematode proliferation (Stursz and Kimpinski, 2004).

Some bacteria distinguished from other coryneforms by their ability to break down cellulose. *Cellulomonas spp* ability to break down cellulose is due to the expression of exoproteins such as endoglucanase and exoglucanase (Christopherson *et al.,* 2013). Due to their cellulytic nature, *Cellulomonas* species are widely found in soil and decaying plant matter, although they have also been isolated from rumen and activated sludge (Abt *et al., 2010).* Some isolates like *S. lugdenensis* and *S. auricularis* predominant species of the normal flora of humans skin and natural found in environment (Abt *et al., 2010).* Rarely an opportunistic pathogen for humans and primates. These organisms are considered opportunistic human pathogens encountered infrequently in human disease; they are common inhabitants of soil, water, and the surface of plants. The plants like lettuce and cucumber that was grown hydroponically was therefore normally colonized by relatively harmless bacteria, endophytic bacteria and the more threatening opportunist pathogens which were occasionally isolated did not appear to be able to persist on this substrate. The results from this study indicate that bacteria of health

significance in the food industry such as 5. *aureus, C. botulinum, E. coli,* and *Salmonella* spp. did not normally occur in the hydroponic system. The total numbers of microorganisms were not a problem and the presence of coliforms and gram-positive rods were not of concern.

Conclusion

Environmental factors like temperature, humidity and water quality monitoring parameters like pH, total dissolved solids (TDS), water temperature, electrical conductivity (EC) and dissolved oxygen (DO) are targeted and attained a desirable ecosystem for optimal growth of living organism in the systems.

Total coliform counts in three type of collection point, bacterial colonies showed an increase in number for the three collection period. Last day of collection period showed the highest number of grown colonies and least in first day of collection.

Microbial identification and comparison of bacterial isolates using biochemical identification of bacteria, the results reveal great diversity of organisms isolated including the presence of pathogenic microorganism, endophytic bacteria (rhizobacter) and commensal occurring bacteria.

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References

Abajo I, Floh EI, Ferrara F, Barbosa H. 2010. Diazotrophyc Rhizobacteria Isolated from Sugarcane Can Release Amino Acids In A Synthetic Culture Medium. Biol. Fertil. Soils. 2011 **47,** 957-962.

Ahmad F, Ahmad I, Khan MS. 2008. Screening of free-living rhizospheric bacteria fortheir multiple plant growth promoting activities. Microbiol Res **163(2),** 173-181.

Alfano JR, Collmer A. 1996. Bacterial Pathogens in Plants: Life up against the wall. The Plant Cell **8,** 1683-1698.

Arthur MI, Fierer N, Jackson RB. 2011. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. USA **103,** 626-632.

Bruce J, Drysdale EM. 1994. Trans-shell transmission. In: Microbiology of the Avian Egg (eds R.G. Board and R. Fuller) pp. 63-91. Chapman and Hall, London.

Carruthers EA(ND). 2002. Principles of Plant Nutrition- 5th Edition. Fromhttps:// Books. Google. Com. Au/ Books?

Christopherson E, Reis Vm, Baldani JI, Döbereiner JT. 2013. Occurrence of the Endophytic Diazotrophs *Herbaspirillum* spp. In Roots, Stems, and Leaves, Predominantly of Gramineae. Biol. Fertil. Soils. 1996 **21,** 197-200.

Cook MI, Beissinger SR, Toranzos GA, Rodriguez RA, Arendt WJ. 2005. Microbial Infection Affects Egg Viability and Incubation Behavior In A Tropical Passerine. Behav. Ecol **16,** 30-36.

Costa LE, Queiroz MV, Borges AC. 2012. Isolation And Characterization Of Endophytic Bacteria Isolated From The Leaves Of The Common Bean (*Phaseolus vulgaris*) Braz J Microbiol. 2012 Oct-Dec **43(4),** 1562-1575.

Kaul S, Sharma T, Dhar MK. 2016. "Omics" Tools For Better Understanding The Plant Endophyte Interactions. Front. Plant Sci **7,** 955. DOI: 10.3389/Fpls.2016. 00955

Magnani Gs, Didonet Cm, Cruz Lm, Picheth Cf, Pedrosa Fo, Souza Em. 2010. Diversity of Endophytic Bacteria In Brazilian Sugarcane. Genet. Mol. Res **9,** 250-258.

Nithya A, Babu S. 2017. Prevalence Of Plant Beneficial And Human Pathogenic Bacteria Isolated From Salad Vegetables In India. Bmc Microbiol **17,** 2017.

Paulitz TC, Belanger RR. 2001. Biological Control In Greenhouse Systems. Annu. Rev. Phytophatol **39,** 33-103.

Phukon M, Sahu P, Angamuthu Nithya R. 2013. Unusual Occurrence of *Staphylococcus warneri* as Endophyte in Fresh Fruits Along with Usual *Bacillus* spp.

Sace CF, Estigoy PJr Natividad. 2015. Economic Analysis of an Urban Vertical Garden For Hydroponic Production of Lettuce (*Lactuta Sativa).* International Journal of Contemporary Applied Sciences **2(7),** 42-56

Sace CF, Fitzsimmons KM. 2013. Vegetable Production in Recirculating Aquaponic Syatem Using Nile Tilapia (*Oreochromis niloticus*) with and without Freswater Prawn (*Macrobranchium rosenbergii*). Academia Journal of Agricultural Research **1(12),** 236-250

Sace CF, Estigoy JH. 2015. Lettuce Production in A Recirculating Hydroponic System. American Journal of Agricultural Science **2(5),** 196-202.

Salve VB, Hiware CJ. 2008. Study on Water Quality Wanparakalpa Reservoir Nagpur, Near Paili Vaijnath, District Beed. Merathwada Region. J. Aqua. Biol **21(2),** 113-117.

Scattini NA, Maj SP. 2017. Aquaponic Integration And Automation- A Critical Evaluation. Published By Canadian Center of Science and Education ISSN 1913-1844 E-ISSN 1913-1852

Song J, Sen A. 2002. Endophytes: A Rich Source Of Functional Metabolites. Nat Prod Rep **18,** 448-459. The Internacional Potash Institute 324-341, Florence, Italy

Sturz J, Kimpinski AV. 2014. Endoroot Bacteria Derived From Marigolds (*Tagetes* Spp.) Can Decrease Soil Population Densities of Root-Lesion Nematodes In The Potato Root Zone. Education ISSN 1913-1844 E-ISSN 1913-1852

Walsberg GE, Schmidt CA. 1992. Effects of Variable Humidity on Embryonic Development and Hatching Success of Mourning Doves. Auk **109,** 309-314.