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Short communication

Use of *Bacillus* spp. to enhance phosphorus availability and serve as a plant growth promoter in aquaponics systems

Brunno da Silva Cerozi*, Kevin Fitzsimmons

Department of Soil Water and Environmental Science, The University of Arizona, P.O. Box 210038, Tucson, AZ 85721-0038, United States

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ABSTRACT

Plant growth promoters (PGP) are microorganisms essential for sustainable food production systems by improving the productivity of crops and mitigating environmental impacts. Microorganisms enhance the P availability to plants by mineralizing organic P and solubilizing precipitated phosphates. This work is focused on the effect of inoculation of a commercial product containing a mixture of *Bacillus* spp. on hydroponically grown lettuce (*Lactuca sativa*) integrated with tilapia (*Orechromis niloticus*) aquaculture in a closed-loop system, in comparison with an untreated control. We determined plant growth and crop quality parameters to assess the efficacy of the beneficial microorganisms. A nutrient dynamics analysis was conducted to evaluate the effect of *Bacillus* inoculation on the changes of nutrient concentration in aquaponics solutions, as well as the phosphorus accumulation in several components (fish, plants, water and solids). We performed a plate-count assay to quantify the number microorganisms present in systems inoculated or not with the commercial *Bacillus* mixture. In general, nutrient dynamics was affected by the inclusion of the *Bacillus* mixture in the water. Systems that received the product showed faster decreases in ammonia concentration and faster increase in nitrite and nitrate concentrations than the control. The untreated aquaponics systems showed lower accumulation of phosphorus in the water than systems receiving the *Bacillus* mixture, which resulted in poor plant growth, low phosphorus accumulation in the leaves and low chlorophyll content. However, the mass balance analysis showed that an external source of phosphorus possibly contributed to the overall P budget in systems receiving the *Bacillus* mixture. The microbial plate count assay demonstrated an active microbiota in aquaponics systems receiving the treatment while untreated systems showed zero microbial counts. The *Bacillus* mixture used in the present study appears to have PGP properties and to affect P dynamics in aquaponics systems. However, since the product contained traces of phosphorus in its composition, further analysis will be necessary to distinguish whether the advantageous effects promoted by the *Bacillus* occurred as a result of a beneficial microbial activity or a fertilizing effect.

1. Introduction

Integrated aquaculture with soilless vegetable production in a closed-loop system, commonly referred to as aquaponics, has received considerable attention due to the system's capability to raise fish at high density, sustain adequate water quality, minimize water exchange and produce an additional marketable vegetable crop (Danaher et al., 2013). In aquaponics, water from the aquaculture sub-system loaded with fish waste is fed to a biological filtration sub-system mainly responsible for nitrification and solids removal, to then be delivered to a hydroponics subsystem and ultimately utilized by plants as nutrients.

There has been a worldwide trend towards the use of plant-based ingredients in the manufacturing of aquafeeds (Gatlin et al., 2007). Plant-based ingredients (e.g. soybeans, corn and wheat) contain high amounts of phytic acid (or phytate), the main storage form of phosphorus in many plant tissues. However, phytate cannot be utilized by fish as a direct source of phosphorus (Cao et al., 2007; Cho and Bureau, 2001). The addition of the enzyme phytase to liberate free phosphorus from phytate is a necessary step when significant portions of plant-based ingredients are used in aquafeed formulations (Hien et al., 2015). Although a number of plant-produced phytases have been characterized, it has been suggested that the activity of these enzymes in roots is insufficient for effective utilization of phytate by plants (Mudge et al., 2003).

* Corresponding author.
E-mail address: bcerozi@email.arizona.edu (B.d.S. Cerozi).

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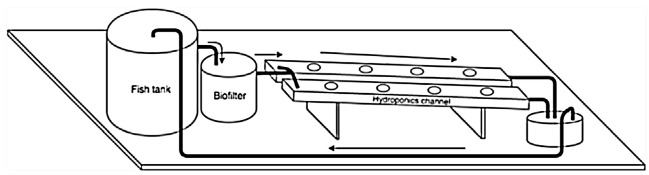

Fig. 1. Diagram of an experimental aquaponics unit used in the study.

Microorganisms known as plant growth promoters (PGP) play an important role in sustainable food production systems by improving the productivity of agricultural crops and mitigating environmental impacts caused by the indiscriminate use of chemical inputs in agriculture (Mangmang et al., 2014). Microorganisms enhance the P availability to plants by mineralizing organic P and solubilizing precipitated phosphates (Khan et al., 2009; Ruzzi and Aroca, 2015). Evidence of naturally occurring rhizospheric phosphorus solubilizing microorganisms dates back to 1903, in which *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* are referred to as the most important species (Khan et al., 2009). *Bacillus* is one of the most studied plant growths promoting rhizobacteria (Ahmad et al., 2008), but its use in aquaponics systems has not been described.

Lettuce is the most frequently cultivated plants in commercial aquaponics operations, as reported by 68% of respondents to an international survey (Love et al., 2015), while the most commonly raised aquatic species is tilapia (Love et al., 2015, 2014). This work focused on the effect of inoculation of a commercial product (Sanolife® PRO-W) containing a mixture of *Bacillus subtilis* and *Bacillus licheniformis* on hydroponically grown lettuce (*Lactuca sativa*) integrated with tilapia aquaculture (*Orechromis niloticus*) in a closed-loop system, in comparison with an untreated control. We determined plant growth and crop quality parameters to assess the efficacy of the beneficial microorganisms. A nutrient dynamics analysis was conducted to assess the effect of *Bacillus* inoculation on the changes of nutrient concentration in aquaponics solutions, as well as the phosphorus accumulation in several components (fish, plants, water and solids). The study also assessed the microbial activity in the aquaponics systems.

2. Material and methods

2.1. Aquaponics system design and operation

The experiment was carried out in a free standing steel A-frame greenhouse with a double wall polycarbonate glazing at the Controlled Environment Agriculture Center (CEAC), Tucson, Arizona, USA. The greenhouse is oriented in a north-south configuration. Greenhouse environmental control consisted of evaporative cooling and natural gas heating to maintain temperatures throughout the growing seasons. Environmental parameters (air and water temperature, humidity, and photosynthetic active radiation) were monitored and controlled for optimal plant production. During the experimental period, plants were grown under natural photoperiod conditions and air temperature set point of 25 °C. Six replicated experimental aquaponics units, each consisting of a 100-L fish tank, 20-L sump partially filled with 10 L of biofilter medium (Biospheres, Amiracle) and two hydroponics channels were used to conduct the study (Fig. 1). Each individual aquaponics system was treated as an experimental unit and three units per treatment (control and *Bacillus*) comprised the experimental design (n = 3).

Potassium was supplemented as potassium sulfate to all aquaponics systems when necessary to maintain a K_2O:N ratio close to 1:1. All systems received the same amount of potassium therefore not all systems were kept under an exact 1:1 K_2O:N ratio. We realized that supplementing different amounts of potassium to each system could have caused an interference in plant growth results, since lettuce responds well to potassium fertilization. Magnesium was supplemented by foliar spraying twice a week using a 1.5% solution of magnesium sulfate salt. Micronutrients (Fe, Cu, Zn, Bo, Mn, and Mo) were also supplemented by foliar spraying with a 0.15 g L^{-1} solution of a micronutrient blends product (S.T.E.M, Peters Professional) every two days during the first two weeks and twice a week during the last two weeks after transplanting.

Air temperature, relative humidity, and solar radiation were monitored using a data logger (Campbell Scientific CR23X). Dissolved oxygen in fish tanks was checked on a daily basis using a YSI Pro20 handheld meter and remained constant above 5 mg L^{-1} in all units. The pH of aquaponics nutrient solutions was monitored on a weekly basis using a handheld pH meter (HACH Hq40d multi) and remained constant at 8.1 ± 0.1 throughout the experimental period.

Table 1
Formulation and chemical composition of basal diet.

Ingredients	Contents
	g kg^{-1}
Soybean meal	538.0
Wheat gluten	80.0
Corn	277.0
Soybean oil	40.0
Calcium carbonate	20.0
DL-methionine	5.0
Mineral and vitamin mix[a]	40.0
Proximate composition (dry matter basis)	
Crude protein (g kg^{-1})	363.9
Crude lipid (g kg^{-1})	79.0
Ash (g kg^{-1})	20.0
Crude fiber (g kg^{-1})	588.2
Energy (Mcal kg^{-1})	3.77

[a] Mineral and vitamin mix contents per kg of product: Fe 40 mg; Cu 4 mg; Zn 50 mg; Iodine 40 mg; Mn 60 mg; Se 0.4 mg; Co 0.5 mg; vitamin A 2 325 000 USP; vitamin D3 65 000 USP; vitamin E 32 500 IU; vitamin K 793.65 mg; vitamin C 87 100 mg; vitamin B1 2600 mg; vitamin B2 3250 mg; vitamin B6 2600 mg; vitamin B12 10 000 μg; pantothenic acid 15 600 mg; biotin 40 mg; folic acid 780 mg; niacin 19 500 mg.

2.2. Diet formulation and manufacturing

A basal diet containing no supplemental inorganic phosphorus (Table 1) was manufactured at the Environmental Research Lab (ERL) in Tucson, Arizona.

2.3. Fish and lettuce culture conditions

Tilapia juveniles with an average initial body weight of 70 g were transferred to their respective tanks on 24 December 2015, 15 days before the transplanting of lettuce seedlings for acclimation of the biofilters. The biofilters were inoculated with commercial product including nitrifying bacteria (API QUICK START®) that allows for instant addition of fish in a new system as it immediately starts the natural nitrogen cycle. Four 11-day-old seedlings (Johnny's Seeds, Red Cherokee var., pelleted) were transplanted to each hydroponics channel on 7 January 2016, comprising a total of eight lettuce plants for each aquaponics experimental unit. Plants were grown for 6 weeks and harvested on day 18 February 2016 for biomass determination and tissue analysis. Fish were fed the formulated diets with the aid of automatic feeders at approximately 1.0% of body weight per day, for three weeks. All fish were harvested on day 14 January 2016.

Three aquaponics units were randomly assigned to each treatment (*Bacillus* and Control). Aquaponics units assigned to the *Bacillus* treatment received 2.41 g of a commercial *Bacillus* mixture (Sanolife® PRO-W; 5.0×10^{10} CFU g^{-1}, INVE), twice a week until the