

Harnessing Native Bacteria to Support Resilient Aquaponic Lettuce Production

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INTRODUCTION

Aquaculture and hydroponics are two of the fastest-growing sectors of agriculture.

Aquaponics combines both in a biologically coupled system with a synergy of fish, plants, and microbes. Probiotic bacteria from the genus *Bacillus* are increasingly used in both aquaculture and hydroponic applications, but introduced microbes frequently fail to establish or survive in desired environments.

Added prebiotic nutrition has improved plant growth promoting bacteria (PGPB) survival and function in soil, but a similar approach has never been applied to soilless systems like aquaponics.

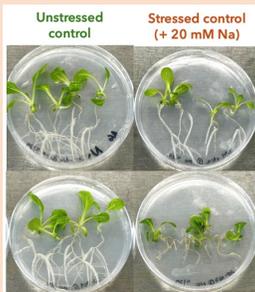
OBJECTIVES

We hypothesize that a deliberate, tailored application of probiotic bacteria can enhance system productivity and stability.

1. Identify strains isolated from aquaponic systems and AU's culture collection which possess **PGP traits in vitro**.
2. Achieve improved lettuce yield in soilless lab-scale systems under **stressors common to aquaponics**.
3. Support strain function and longevity in systems with **prebiotic nutrition and engineering support**.

EXPERIMENTAL APPROACH

1. Isolate *Bacillus* spp. and *Pseudomonas* spp. from tilapia-lettuce aquaponic systems.
2. Select proven strains from AU's culture collection.
3. Identify PGP capacity of all candidate strains: IAA and siderophore production, P and Zn solubilization.
4. Test high-performing candidates for compatibility in assays with lettuce (Fig. 1).
5. Apply winning strains in 21-day growth chamber trials with lettuce under conditions of stress common to aquaponics.



20 mM Na stress + beneficial strain (JJ-909) 20 mM Na stress + detrimental strain (AP-187)

Fig. 1 MICROBE COMPATIBILITY TEST. Compatible strains improve performance under stress; detrimental strains do not.

METHODS

Twenty-one-day batch experiments were carried out in lab-scale hydroponic systems in a Percival CU-36L5 growth chamber (Fig. 5).

Method:

1. Surface-disinfected seeds are inoculated via soaking in a 10^6 CFU/mL bacterial suspension
2. Seed are planted into autoclaved rock wool and irrigated with sterile hydroponic solution
 - a. Control: +/- weekly inoculation, no stressor
 - b. Treatments: +/- weekly inoculation, **all + stressor**
3. Grow in Percival Growth Chamber
 - a. 72-hour germination
 - b. 21-day total growth under 19:6 light:dark for 15.6 mol DLI

Analyses:

- 3x weekly pH, EC, nutrient sampling;
1. Assess growth at harvest
 - a. Survival
 - b. Shoot, root fresh and dry weight

RESULTS



Fig. 2 INOCULATION WITH *B. subtilis* STRAIN JJ-465 MITIGATES STRESS CAUSED BY PERACETIC ACID. Columns from L to R: unstressed control, PAA-stressed control, PAA-stressed + inoculation. Inoculated crops exhibit a +45.1% yield increase vs. uninoculated; crop loss vs. unstressed control is reduced from 43.6% to 18.1%. PAA applied 1x weekly at 7 mg/L.

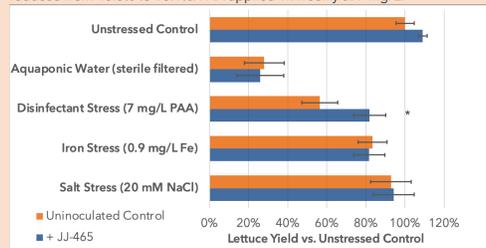


Fig. 3 INOCULATION IMPROVES PERFORMANCE UNDER SELECT STRESSORS. Negative impact of disinfectant stress from peracetic acid (PAA) is mitigated with JJ-465 inoculation; other stressor application rates sourced from literature are insufficient to significantly impact plant performance vs. unstressed control.

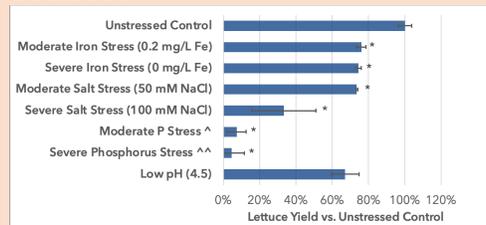


Fig. 4 ABIOTIC STRESSORS COMMON TO AQUAPONICS REDUCE THE MARKETABLE FRESH YIELD OF ROMAINE LETTUCE. Higher application rates of abiotic stressors lead to significantly reduced lettuce yield. ^ 3 mg/L soluble P and 29.5 mg/L insoluble P; ^^ 1 mg/L soluble P and 31.5 mg/L insoluble P.

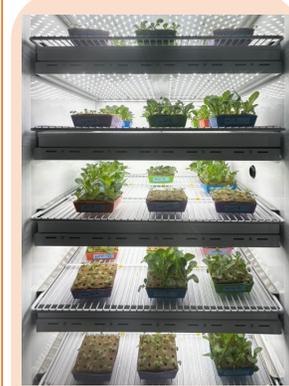


Fig. 5 LAB-SCALE HYDROPONIC SYSTEMS IN GROWTH CHAMBER EXPERIMENT

CONCLUSIONS

- Presence of beneficial bacteria *B. subtilis* strain JJ-465 can improve marketable yield of romaine lettuce under certain stressors which affect aquaponic production (Figs. 2, 3).
- PAA may act as a nutrient source for JJ-465, enhancing its PGP function.
- Crop improvement with inoculation is negligible in the absence of sufficient stressor conditions.
- Strains which exhibit PGP traits *in vitro* do not necessarily improve crop performance.

NEXT STEPS

- Evaluate JJ-465 against updated ranges of abiotic plant stressors (Fig. 4): high salt, low macro- and micronutrient load, suboptimal pH.
- Evaluate strains against biotic plant stressor: root rot caused by oomycete *Pythium* spp. (Fig. 6)
- Perform bacterial genome sequencing to identify metabolic pathways
- Assess optimal prebiotic substrates and culture conditions for JJ-465



Fig. 6 OOMYCETE INHIBITION WITH *Bacillus* spp. L: *Pythium dissotocum*, uninhibited control. R: *P. dissotocum* vs. 3 *Bacillus* spp.

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