



Application of Actinobacteria to Enhance Growth of Kale (*Brassica oleracea* var. *sabellica*) in Hydroponic System

Under Low Nutritional Stress

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Abstract

Hydroponic farming has become a popular alternative cultivation method due to its ability to control environmental factors and prevent soil contamination. Additionally, this system allows for year-round harvesting. In hydroponic cultivation, plants grow in a nutrient solution, absorbing essential nutrients directly through their roots. Kale is a highly nutritious vegetable with increasing consumer demand which requires large amount of fertilizer for growth. However, reducing the use of chemical fertilizer in the nutrient solution may affect kale growth. Actinobacteria are large group of Gram-positive bacteria, known for their plant growth-promoting properties. Therefore, this study aims to evaluate the potential of *Streptomyces thermocarboxydus* isolate S3 in promoting the growth of kale (*Brassica oleracea* var. *sabellica*) under low-nutrient hydroponic conditions. The experiment consisted of six treatments: plants were grown in 100%, 50% and 25% nutrient solution with or without *S. thermocarboxydus* isolate S3 for 45 days. *S. thermocarboxydus* isolate S3 significantly enhanced the growth of kale under low-nutrient conditions. Kale plants treated with *S. thermocarboxydus* isolate S3 exhibited higher fresh weight (169.56 ± 73.82 g), dry weight (17.20 ± 5.11 g) and total chlorophyll content (1.73 ± 0.35 mg/L) compared to the control group. Furthermore, *S. thermocarboxydus* isolate S3 reduced stress from H_2O_2 accumulation in kale grown under low-nutrient hydroponic conditions. Additionally, the presence of *S. thermocarboxydus* isolate S3 was detected in plant roots, suggesting a successful colonization.

Introduction

Queen of Green Vegetables



Good for

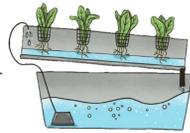


Actinobacteria

- Phylum Actinomycetota
- Filamentous or unicellular Gram-positive bacteria
- High G+C content (> 50%)
- Secondary metabolite production
- Plant growth promoting bacteria

- Hydroponic System : Widely adopted alternative farming method that allows better control of environmental factors and reduces soil contamination
- Reducing Chemical Dependency: Minimizing nutrient solution concentrations can reduce overall use of chemical fertilizers, but this may impact plant growth and productivity
- In hydroponic farming, plants absorb nutrients directly from a flowing solution, improving uptake efficiency

Nutrient Film Technique : NFT



Objective

- To investigate ability of *Streptomyces thermocarboxydus* isolate S3 to promote growth of kale (*Brassica oleracea* var. *sabellica*) cultivated in hydroponic systems under low nutritional stress

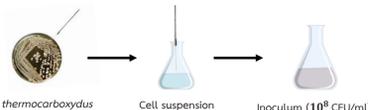
Methodology

Part I : Kale growth promotion under low nutrient

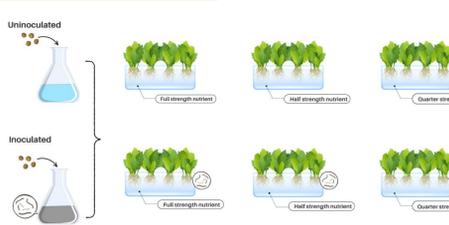
Kale seed preparation



Inoculum preparation



Hydroponic cultivation

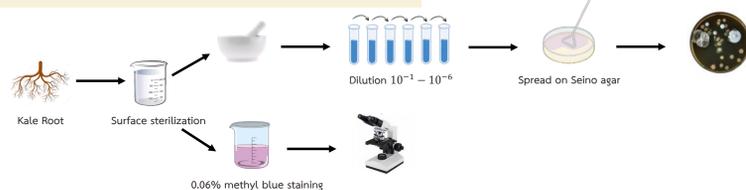


Growth Parameter

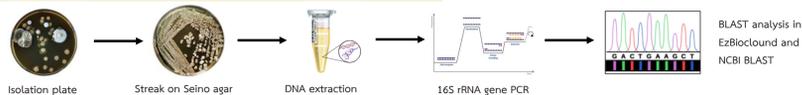
- Shoot length
- Number of leaves
- Fresh/Dry weight
- Chlorophyll content
- Carotenoid content
- Enzyme
- Total phenolic content
- Proline content
- Total soluble sugar
- Concentration of H_2O_2
- Antioxidant
- Malonaldehyde
- Electrolyte leakage
- Nutrient content

Part II : Root colonization

Re-isolation and microscopic observation



Identification by 16S rRNA gene sequencing



Results and Discussion

Part I : Kale growth promotion under low nutrient



Fig 1. Physical appearance of kale under different treatments: (A) Control without actinobacteria under 100% nutrient solution, (B) Actinobacteria-inoculated plants under 100% nutrient solution, (C) Control without actinobacteria under 50% nutrient solution, (D) Actinobacteria-inoculated plants under 50% nutrient solution, (E) Control without actinobacteria under 25% nutrient solution, and (F) Actinobacteria-inoculated plants under 25% nutrient solution

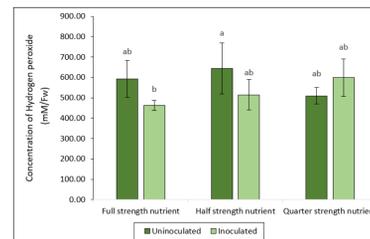


Fig 2. Effect of *S. thermocarboxydus* isolate S3 on hydrogen peroxide content in kale leaves in a hydroponic system under 50% and 25% nutrient solution.

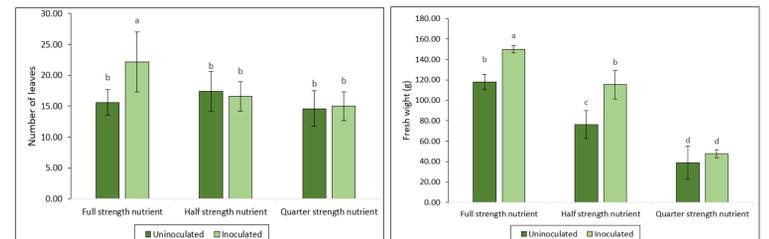


Fig 3, Fig 4. Effect of *S. thermocarboxydus* isolate S3 on number of leaves and fresh weight in kale in a hydroponic system under 50% and 25% nutrient solution

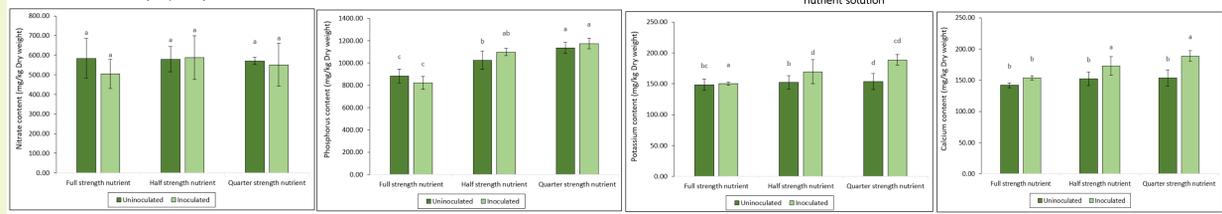


Fig 5. Effect of *S. thermocarboxydus* isolate S3 on nutrient contents in leaves of kale in a hydroponic system under 50% and 25% nutrient solution

Part II : Root colonization

Root colonization of actinobacteria



Confirmation of re-isolated strains by 16S rRNA gene sequence analysis

Tasks	Hit taxon name	Hit strain name	Accession	Similarity
○	<i>Streptomyces thermocarboxydus</i>	DSM 44293(T)	U94490	99.93
○	<i>Streptomyces lusitanus</i>	NBRC 13464(T)	AB184424	99.56
○	<i>Streptomyces indliensis</i>	NBRC 13964(T)	AB184553	99.27

Fig 6. The result of 16S rRNA gene sequence analysis using EzBioCloud for bacterial species identification.

- Microscopic examination and re-isolation confirmed the survival and colonization of *S. thermocarboxydus* S3 in kale root

Microscopic observation

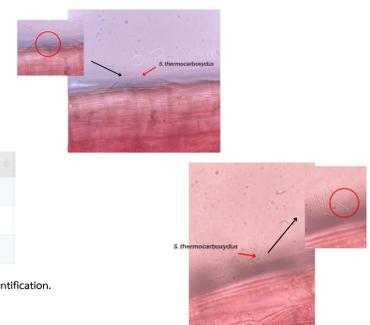


Fig 7. Root colonization of *S. thermocarboxydus* observed under a microscope

Conclusion

- *Streptomyces thermocarboxydus* S3 can enhance growth of kale cultivated in hydroponic system under low nutrient condition
- *S. thermocarboxydus* S3 mitigated detrimental effects of nutrient stress in kale by reducing hydrogen peroxide levels, presumably through the synthesis of H_2O_2 -scavenging enzymes
- The root colonization of *S. thermocarboxydus* S3 was confirmed through re-isolation and microscopic examination

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