Plant Growth Promoting Actinobacteria: Their Drought Tolerant Mechanisms and Applications in Plants

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INTRODUCTION

Kale, or curly-leaf cabbage (*Brassica oleracea* var. *sabellica*), is one of the most important plants in the Brassicaceae family. Kale is recognized as an excellent source of antioxidants due to its rich phytochemical compound. Additionally, kale contains a high amount of minerals, carotenoids, vitamins (B-complex, C, and K) and fiber Consequently, kale has been dubbed the "Queen of Greens." Kale grows well at low temperature (15-21 °C), whereas excessive heat can result in yield reduction, or complete crop loss and a bitter taste. Currently, the world is facing climate change, leading to an increase in global average temperatures and subsequent drought conditions. This in turn, causes significant damage to agricultural production. Actinobacteria are Gram-positive bacteria with a high G+C content in genome. They are known for their ability to promote plant growth and increase tolerance to adverse environmental conditions. Under drought conditions, actinobacteria several mechanisms to alleviate water stress in plants, enhancing their tolerance to unfavorable environmental conditions.

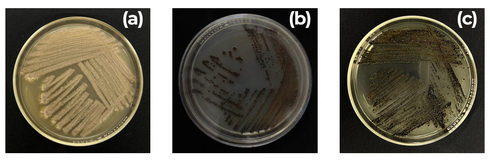
OBJECTIVES

- To study drought tolerant mechanisms in actinobacteria, which promote plant growth
- To mitigate drought stress in kale plant (*Brassica oleracea* var. *sabellica*) using selected actinobacteria

METHODOLOGY

Actinobacteria used in the experiment

Streptomyces thermocarboxydus isolate S3 (a)
Micromonospora chalcea CMU55-4 (b)
Micromonospora chalcea CMU66-1 (c)

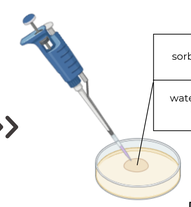


1. Drought tolerant assay

Plate assay:

Selected actinobacteria

Spore suspension (OD_{600} = 1.0, 10^8cfu/ml)



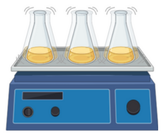
sorbitol (g/L)	0	85	175	285	405	520	605	660	780
water activity (Aw)	0.998	0.986	0.976	0.957	0.919	0.897	0.857	0.844	0.807

Drop plate onto 10% TSA with adjusted water activity

Broth assay:

Prepare 10% TSB medium supplemented with PEG 6000 at varying concentrations: 0% 10% 20% 30% 40%

Add spore suspension

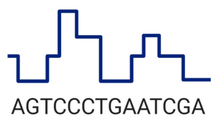


Incubate on shaker for 7 days

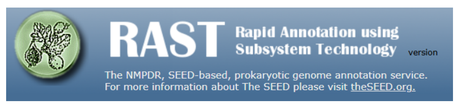
Monitor daily growth at OD_{600}

2. Drought tolerant mechanisms in plant growth-promoting actinobacteria

Whole genome analysis:

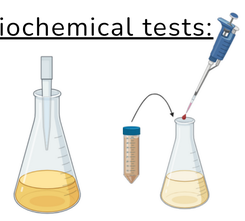


Whole genome sequences data



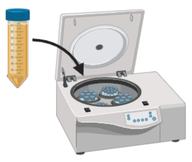
Whole genome analysis using RAST (Rapid Annotation using Subsystem Technology)

Biochemical tests:

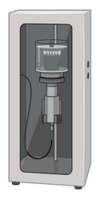


Cultivate actinobacteria under drought conditions

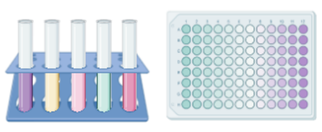
Incubate on shaker for 7 days



Centrifuge at 4°C, 7500 rpm for 10 minutes to collect the cells



Break cells using a sonicator probe for 10 minutes



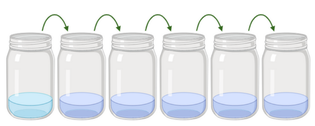
Biochemical test

- Antioxidants: DPPH assay
  ABTS assay
  MDA assay*
  Catalase*
  Superoxide dismutase*
  Glutathione peroxidase*
- Hydrogen peroxide*
- Osmolyte accumulation: Proline content*
  Total soluble sugar*

* : in progress

3. Mitigation of drought in kale

Surface sterilization of kale seeds using 0.5% NaClO for 5 minutes, wash with sterile distilled water five times, each for 1 minute



Soak seeds in spore suspension (10^8cfu/ml) and sterile distilled water (control)

Sow kale seeds in a seedling tray

Transplant kale seedlings into planting pots and cultivate them 30-45 days

1 week before harvest, induce drought stress in designated experimental groups

in progress

Harvest kale and measure growth parameters

Growth parameters

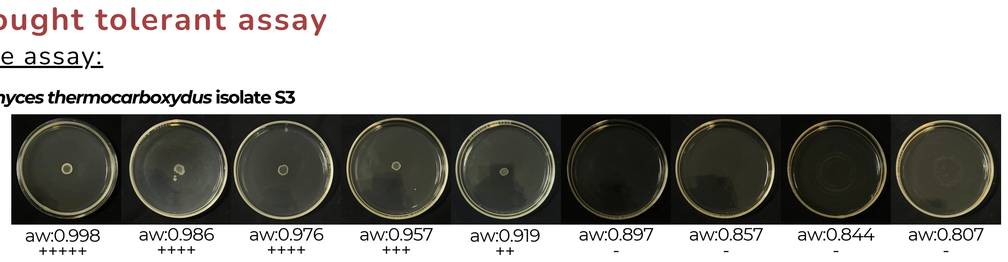
- Morphology: Root/Shoot length
  Number of leaves
  Fresh/Dry root weight
  Fresh/Dry shoot weight
- Biochemical: Chlorophyll content
  Proline content
  Total soluble sugar
  Antioxidants
  Antioxidative enzymes
  Reactive Oxygen Species (ROS)
- Physiology: Oxidative membrane damage
  Malondialdehyde (MDE)
  Electrolyte leakage (EL)

RESULTS

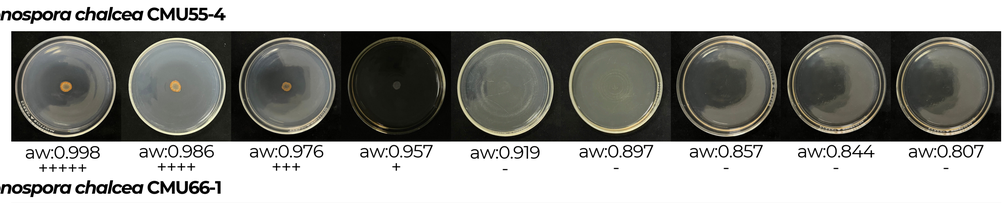
1. Drought tolerant assay

Plate assay:

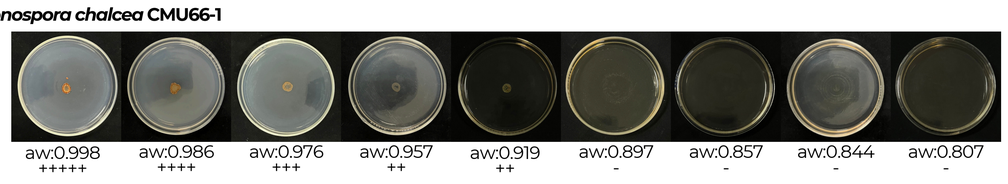
***Streptomyces thermocarboxydus* isolate S3**



***Micromonospora chalcea* CMU55-4**



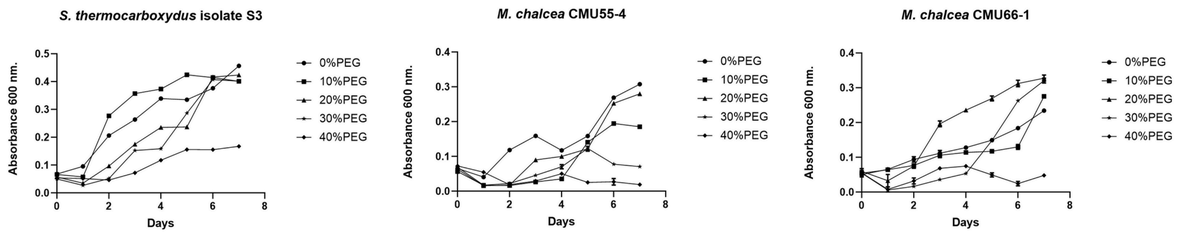
***Micromonospora chalcea* CMU66-1**



NOTE: +++++ Best growth, ++++ Very Good growth, +++ Good growth, ++ Moderate growth, + Weak growth, - No growth

Growth of *S. thermocarboxydus* isolate S3, *M. chalcea* CMU55-4 and *M. chalcea* CMU66-1 on 10%tryptic soy agar with varying water activity levels (0.998, 0.986, 0.976, 0.957, 0.919, 0.897, 0.857, 0.844 and 0.807)

Broth assay:

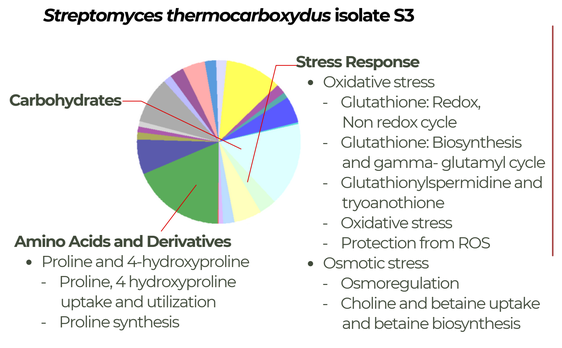


Growth of *S. thermocarboxydus* isolate S3, *M. chalcea* CMU55-4 and *M. chalcea* CMU66-1 in 10%tryptic soy broth supplemented with various concentrations of PEG6000 (0%; 10%; 20%; 30% and 40%) for 7 days optical density at 600 nm

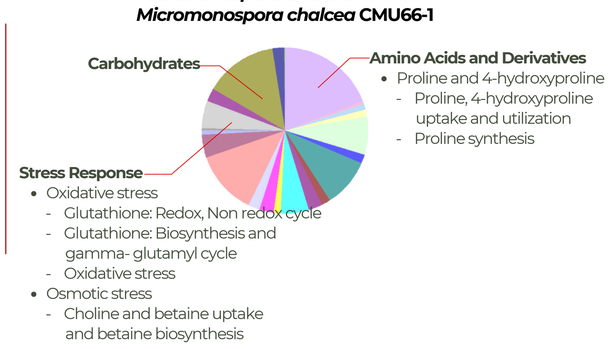
2. Drought tolerant mechanisms in plant growth-promoting actinobacteria

Whole genome analysis:

***Streptomyces thermocarboxydus* isolate S3**

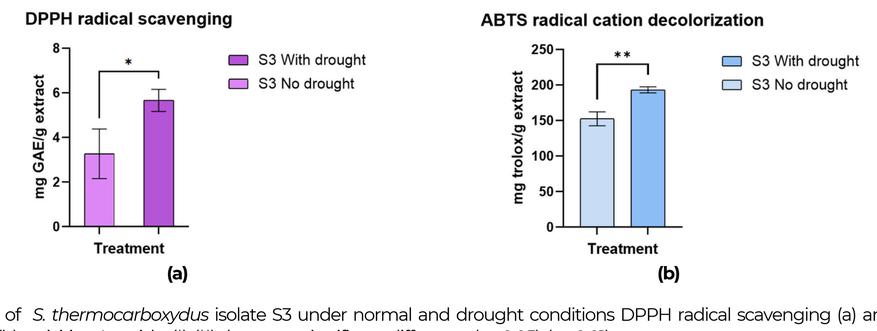


***Micromonospora chalcea* CMU55-4**
***Micromonospora chalcea* CMU66-1**



Genes associated with stress response in the genome of *S. thermocarboxydus* isolate S3, *M. chalcea* CMU55-4 and *M. chalcea* CMU66-1

Biochemical tests:



Antioxidant activity of *S. thermocarboxydus* isolate S3 under normal and drought conditions DPPH radical scavenging (a) and ABTS cation radical scavenging (b) activities. Asterisks (*), (**) denotes a significant difference ($p \leq 0.05$), ($p \leq 0.01$)

CONCLUSION

Streptomyces thermocarboxydus isolate S3 and *Micromonospora chalcea* CMU66-1 were able to grow under drought conditions. Whole genome analysis revealed that 3 strains possess stress response genes and proline biosynthesis genes, which may help alleviate drought stress. It is evident that *S. thermocarboxydus* isolate S3 mitigated drought stress using antioxidant production as seen from a significant increase in DPPH radical scavenging activity and an ABTS cation radical scavenging activity compared to normal condition.

REFERENCES

Feiyang, X. (2022). Actinobacteria From Desert For Promoting Plant Growth Under Drought. Doctoral Thesis, Chiang Mai University.
Lasudee, K., Tokuyama, S., Lumyong, S., & Pathom-aree, W. (2018). Actinobacteria associated with arbuscular mycorrhizal *Funneliformis mosseae* spores, taxonomic characterization and their beneficial traits to plants: evidence obtained from mung bean (*Vigna radiata*) and thai jasmine rice (*Oryza sativa*) *Frontiers in Microbiology*, 9, 1247.

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