Mechanisms of Drought Tolerance in Plant Growth–Promoting Actinobacteria Associated with Kale (*Brassica oleracea* var. *acephala*)

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ABSTRACT

Climate change has intensified drought conditions, which have become a major factor limiting plant growth. The use of plant growth–promoting microorganisms is one approach to enhance plant tolerance to stress. This study aimed to investigate the drought tolerance mechanisms of *Micrococcus yunnanensis* ORF15-23 and its potential to promote the growth of kale (*Brassica oleracea* var. *acephala*) under such conditions. The study encompassed genome analysis using the RAST server as well as laboratory-scale experiments. Genomic analysis revealed genes associated with osmotic and oxidative stress responses. One mechanisms were identified from laboratory-scale experiments. (1) ABTS antioxidant activity. The plant experiment consisted of four treatments: (1) kale grown under normal condition, (2) kale grown under drought condition, (3) kale inoculated with ORF15-23 grown under normal condition, and (4) kale inoculated with ORF15-23 grown under drought condition. Drought stress was applied by adding 10% PEG to 7 day-old seedling for 10 days, and plants were continued to grow for an additional 33 days. Plants inoculated with *M. yunnanensis* ORF15-23 exhibited increased accumulation of H_2O_2 and proline. These findings suggest that the *M. yunnanensis* ORF15-23 may promote accumulation of proline to mitigate H_2O_2 stress in kale.

INTRODUCTION

Climate change has increasingly resulted in more severe and prolonged drought conditions, which represent a major constraint on plant growth. Drought stress affects various physiological and metabolic processes in plants and induces the accumulation of reactive oxygen species (ROS), which can cause oxidative damage to plant cells. One promising strategy that has gained increasing attention is the use of plant growth–promoting bacteria (PGPB), particularly actinobacteria. These Gram-positive bacteria are widely distributed in soil and have shown significant potential to enhance plant tolerance to environmental stresses.

OBJECTIVES

1. To study drought tolerance mechanisms of plant growth–promoting *Micrococcus yunnanesis* ORF15-23
2. To investigate effects of *M. yunnanesis* ORF15-23 on kale growth under drought conditions

MATERIALS AND METHODS

M. yunnanesis ORF15-23 (0%PEG, 10%PEG)

- Oxidative stress markers
  - Hydrogen peroxide
  - Hydroxyl radical assay
  - Malondialdehyde (MDA) assay
- Osmolytes
  - Proline content
  - Total soluble sugar content
- Antioxidants
  - 2-2,diphenyl-1-picrylhydrazyl (DPPH) assay
  - 2,2′-azino-bis (ABTS) assay
  - Total phenolic compound
- Antioxidant enzymes
  - Catalase (CAT)
  - Ascorbate peroxidase (APX)
  - Glutathione peroxidase (GPX)
  - Superoxide dismutase (SOD)

kale (*Brassica oleracea* var. *acephala*)

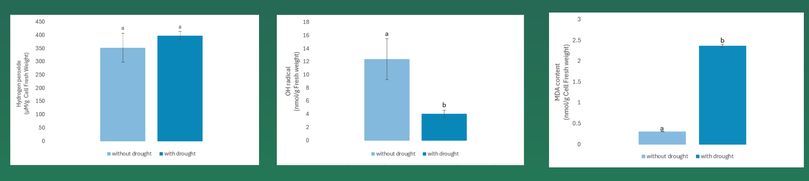
(T1) Normal condition
(T2) Drought condition (10%PEG)
(T3) Normal condition+ORF15-23 (10^8 CFU/mL)
(T4) Drought condition (10%PEG)+ORF15-23 (10^8 CFU/mL)

- Morphological parameters
  - Fresh weight
  - Dry weight
  - Number of leaves
  - Stem height
  - Root length
- Biochemical parameters
  - Chlorophyll & Carotenoid
  - Proline content
  - Total soluble sugar content
  - Hydrogen peroxide

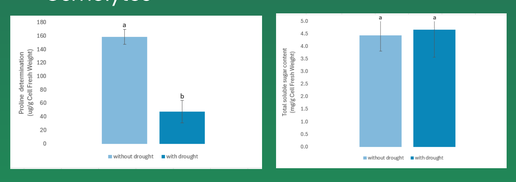
RESULTS

Drought tolerance mechanisms of *Micrococcus yunnanesis* ORF15-23

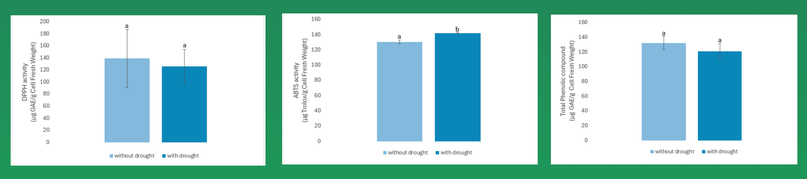
- Oxidative stress markers



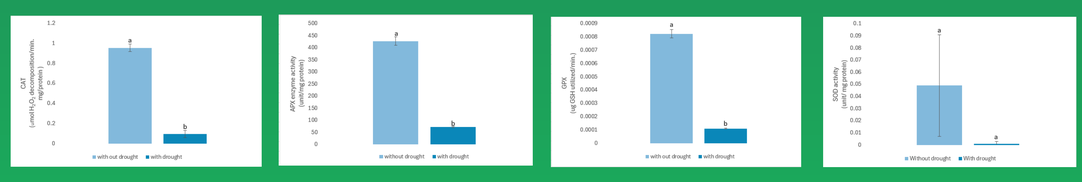
- Osmolytes



- Antioxidants

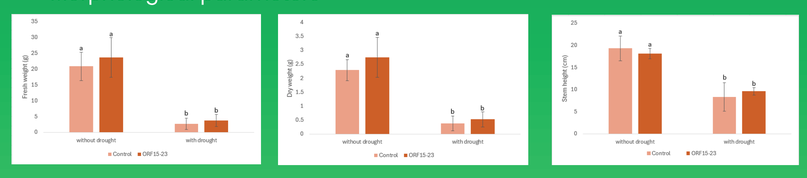


- Antioxidant enzymes

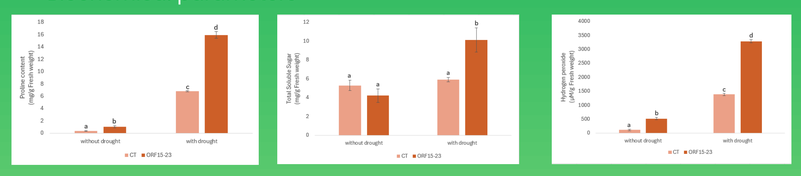


Effects of *M. yunnanesis* ORF15-23 on kale growth under drought conditions

- Morphological parameters



- Biochemical parameters



CONCLUSIONS

- Drought tolerance mechanism in *M. yunnanesis* ORF15-23 involves antioxidant production (ABTS activity)
- *M. yunnanesis* ORF15-23 mitigates oxidative stress (hydrogen peroxide) from PEG induced drought in kale via the accumulation of compatible solute (proline and total soluble sugar)

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