Title : Physiological Responses of *Streptomyces thermocarboxydus* isolate S3 to Microplastics

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ABSTRACT

Microplastics are emerging environmental pollutants that are increasingly widespread in soils and can induce oxidative stress in soil microorganisms, which disrupts physiological balance and intracellular metabolic processes. However, knowledge of the response mechanisms of actinobacteria to microplastic exposure remain limited. This study aimed to investigate the physiological responses and tolerance mechanisms of *Streptomyces thermocarboxydus* isolate S3 to microplastics and to evaluate its potential to promote seed germination under microplastic-contaminated conditions. Initially, three types of microplastics were tested at different concentrations to evaluate their effects on the growth of *S. thermocarboxydus* isolate S3. These included polyethylene terephthalate (PET), polystyrene (PS), and polypropylene (PP). Microplastic types that showed stronger inhibitory effects on growth were selected for determination of tolerance mechanisms in *S. thermocarboxydus* isolate S3. The experiment was divided into five parts. (1) Measurement of oxidative stress indicators namely superoxide dismutase (SOD), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH). (2) Measurement of antioxidant defense enzymes namely catalase (CAT), glutathione peroxidase (GPX) and ascorbate peroxidase (APX). (3) Determination of cellular damage by lipid peroxidation. (4) Phytotoxicity assay based on seed germination of lettuce (*Lactuca sativa* L. var. *longifolia*). (5) Whole-genome analysis for identification of genes associated with responses to microplastic stress. Microplastics reduced *S. thermocarboxydus* isolate S3 growth in a dose-dependent manner across all tested microplastic types with PET and PP showing the strongest inhibition. These two microplastic types were selected for further analyses. Both microplastics induced oxidative stress via the production of H_2O_2 and •OH in PP and PET exposure, respectively. PET induced more pronounced cellular damage compared to PP. In phytotoxicity assays, PET caused more severe reduction in lettuce seedling growth than PP exposure. Inoculation with *S. thermocarboxydus* isolate S3 improved seedling length, fresh weight and dry weight compared with the control. Whole-genome analysis further revealed genes associated with microplastic stress responses such as catalase and glutathione peroxidase genes.

Keywords: *Streptomyces thermocarboxydus;* microplastics; stress response; seed germination

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