DIVERSITY OF RHIZOBACTERIA ASSOCIATED TO PLANT ROOTS AS AFFECTED BY CULTURING MEDIA AND RELATED METHODOLOGY

By

MOHAMED SABRY MOHAMED SARHAN
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2013

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(Agricultural Microbiology)

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APPROVAL SHEET

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Title of Thesis: Diversity of Rhizobacteria Associated to Plant Roots as Affected by Culturing Media and Related Methodology
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ABSTRACT

The crude plant slurry homogenates, juices and saps are found to support culturability and growth of rhizobacteria. For ease of application and practicability, here we present vegetative plant materials of *Trifolium alexandrinum,* and *Paspalum vaginatum*in the form of dehydrated powders and packed in teabags to obtain liquid infusions necessary to prepare culture media for rhizobacteria. The resulting plant based culture media supported very good growth of pure isolates of *Azotobacter* spp., *Bacillus* spp., *Burkholderia* spp., *Enterobacter* spp., *Klebsiella* spp., *and Psuedomonas* spp. More importantly, they were capable to efficiently recover rhizobacteria associated to the roots of maize and clover. Quantitatively, the culturable rhizobacteria population developed on the plant based culture media represented 28-35 % of the total bacteria cells detected by quantitative real-time PCR on root samples; compared to only 16-18 % retrieved on the chemically-synthetic culture media (e.g. nutrient agar).

PCR-DGGE of 16S rDNA amplicons and sequencing were used to compare the community composition of rhizobacteria cultured on both plant-based and chemically-synthetic culture media, as well as those obtained from plant roots. Highest diversity of rhizobacteria was detected on maize roots (Shannon-Wiener index, H= 2.968), followed by CFUs cultured on plant-based culture media (H = 2.662-2.685), and chemically-synthetic culture media (H = 2.398). The PCR-DGGE band pattern composition was significantly different among rhizobacteria communities cultured on chemically-synthetic media and plant-based culture media, as well as those found on root samples. This is confirmed by sequencing and phylogenetic affiliation, where all 16S rDNA sequences obtained from the chemically-synthetic media were related to the class Gammaproteobacteria, family Enterobacteriaceae. While those of clover teabags-culture media represented not only the class Gammaproteobacteria, family Enterobacteriaceae but also and exclusively the class Alphaproteobacteria, family Rhizobiaceae.

Further, we used the teabags of plant powders with low concentrations down to 0.5 and 0.25 g l^{-1} which were experimented and proved successful. Very interestingly, the new plant medium succeeded in isolating a number of not-yet-cultured bacteria, which match closest to uncultured bacteria, grouped to *Novosphingobium* sp., *Lysobacter* sp. and *Pedobacter* sp.

It is concluded that the plant-based teabags culture media by themselves are sufficient and efficient to recover and mirror the highly diverse and complex root inhabiting rhizobacteria community.

Keywords: Plant-based culture media, Plant powder teabags, Unculturable bacteria, Rhizobacteria, PCR-DGGE, 16S rDNA

DEDICATION

I would like to dedicate this work to: My dear parents, My Father, for his endless support and inspirational continuous pushes; My Mother, for her care and prays; My brother (Ahmed) and sisters (Aya, Esraa, Asmaa, Afnan) and nephew (Aysel).Also, I dedicate this work to my uncle who was supporting and encouraging me for being better.

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CONTENTS

GENERAL INTRODUCTION

The term ‘rhizosphere’ was coined by Lorenz Hiltner in 1904 to describe the influence of root exudates on the proliferation of soil micro- organisms around and inside roots, the term that drove the locomotive of research of plant-microbe interactions (Hartmann *et al.*, 2008). The rhizobacteria, plant root-inhabiting bacteria, play a pivotal role in the functioning of plants by influencing their physiology and development. While many members of the rhizosphere microbiome are beneficial to plant growth, also plant pathogenic microorganisms colonize the rhizosphere striving to break through the protective microbial shield and to overcome the innate plant defense mechanisms in order to cause disease (Hirsch and Mauchline, 2012; Philippot *et al.*, 2013).

To enhance plant growth and health, it is essential to know which microorganism is present in the rhizosphere microbiome and what it is doing. This is very guileless to say, tremendously challenging to approach. Examining a sample of any environmental sample under the microscope and plating the same amount of sample on an agar plate will result in about 100 times more bacteria cells observed microscopically than colonies (which are assumed to grow from a single cell) counted on the agar plate. Scientists refer to this conundrum as “The Great Plate Count Anomaly”, i.e. great and significant population of bacteria are not amenable to get cultured in the laboratory conditions (Janssen *et al.*, 2002; Vartoukian *et al.*, 2010); Pham and