BIOMASS PRODUCTION OF DIAZOTROPHS FOR INOCULA PRODUCTION AND APPLICATION IN NORTH SINAI

By

EMAN NOUR EL-DEEN HAMED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2003.

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Department: Microbiology **Approval**: 20/6/2009

ABSTRACT

Increasing culturability of RMO under laboratory conditions resembles a challenge to specialists in the field, and the need arises to explore innovative culture media. The idea of using plant juices is introduced here. A number of juicy desert plants (*Mesembryanthemum crystallinum*, *Zygophyllum album*, *Carpobrotus edulis*) and cultivated (*Trifolium alexandrinum*) were tested. Pure culture studies confirmed that representatives of RMO (*A. brasilense*, *K. pneumoniae* and *E. agglomerans*) successfully developed, and exhibited characteristic colonies, on surface–inoculated agar plates prepared from crude and diluted juices. In liquid batch cultures, cells grew nicely, with doubling times very comparable to those grown in a number of tested reference culture media. *In situ* analysis proved that the plant juice–based culture agar medium, compared to commonly used agar media, successfully supported the growth and culturing of RMO populations, resident to various tested host plants. In addition, RMO associated to roots of plants were able to develop on culture media prepared from homologous and heterologous plant juices. This indicates the commonality of a significant population of RMO, and promiscuity of plant juices to support such populations. Results presented recommend fellow researchers to just sap their tested plants and prepare the necessary culture media for further RMO culturing.

Key words: Rhizosphere – plant juices – ice plant – culturable RMO – diazotrophs

DEDICATION

I dedicate this work to whom my heart felt thanks; to my husband Tarek and my daughter Laila for their patience and help, as well as to my father, mother and sisters for all the support they lovely offered along the period of my post graduation.

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INTRODUCTION

From the microbiological perspective, the rhizosphere is a cross road and a market place (Hawkes *et al.,* 2007) where microorganisms are actively involved with roots and soil. Concomitantly is the formation of both sources and sinks of diverse range of compounds, and the mediation of large fluxes of solution and gas-phase nutrient (and non-nutrient) compounds (Benlap *et al.,* 2003). Realistically, plant roots do grow into and through an extraordinary array of indigenous soil microorganisms. The phylogenetic and functional characteristics of the associated microbial community develop in concert with the plant root, and as well framed by the background and bulk soil community. Actually, this did direct the ever-going efforts to formulate culture media recommended for culturing rhizospheric microorganisms (RMO). An example is the nutrient agar-based formulas (Jensen, 1962) and the development of the soil extract agar culture medium (Parkinson *et al.,* 1971).

In fact, the development of new methods for studying the compositional and functional characteristics of rhizosphere is a real challenge for all of us. The recent development and popularity of molecular techniques to identify soil organisms has allowed us to move beyond the small subset of culturable soil organisms, less than 10% (Finlay, 2006). It is increasingly common to characterize complex microbial communities genotypically using the small subunit 16S rDNA or 16S rRNA. Such DNA-based characterization of rhizosphere microbial communities do increase our understanding of these communities and their controllers (Hawkes *et al.,* 2007).