بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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MONA MAGHRABY

DEVELOPMENT OF EFFICIENT TECHNIQUES SUITABLE FOR GROWTH AND SURVIVAL OF PLANT MICROBIOME

By

MOHAMED RAMADAN FARAG ABDELFADIL

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2016

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ABSTRACT

Long-term preservation of microorganisms is one of the top research priorities in life science. Microbial community structure is altering and going to disappear. Therefore, community level preservation should be taken into consideration in diversity conservation agenda. Therefore, we introduce the local type of clay used for pottery making in Egypt, as available, inexpensive and natural material for microbial preservation of either pure isolates or consortia of microorganisms.

Beads of pottery clay were tested for long-term preservation of the rhizobacteria; *Bacillus circulans*, *Klebsiella oxytoca*, *Sinorhizobium meliloti*, and yeast isolate under different storage temperatures, ambient, 4℃ and -20℃. The general survival patterns of examined microorganisms indicated successful maintenance on clay beads up to 11 months with very little reductions in their numbers, a phenomenon that was strain-and culture medium-dependent.

In view of the successful use of clay beads as microbial carrier and long-term preservation method, we further experimented clay chips for possible fingerprinting and preservation of consortia of microbiota associated to barley roots. The density of culturable rhizobacterial community (CFUs) captured/memorized with clay chips were significantly higher than coarse sand. DGGE analysis of culturable communities together with analysis of distance scores indicated culture media effect, the band profiles of the standard R2A was grouped distant from all those of the plant-based culture media. Both roots and clay chips were clustered together away from sand. This conclusion was further justified by PCA of bacterial DGGE banding profiles, as both plant roots and clay chips clustered together distant from sand. Diversity indices were computed and richness values were highest for bacterial population of clay chips developed on plant based culture media (PM) very much comparable to those of plant roots developed on PM. The protein spectra of all tested isolates showed that protein profiles hooked by clay chips were always and remarkably clustered together with those recovered from plant roots, depending on the culture media of developed/tested CFUs.

In conclusion, the introduced methods of clay beads and clay chips have the capacity to preserve and hook/capture, either pure isolates or microbial community. This qualifies/recommends for future core microbiome preservation and transplantation towards biotechnological application and environmental rehabilitation.

Keywords: Clay beads, Microbial Preservation, Microbial community, DGGE, MALDI-TOF

DEDICATION

Iam dedicating this thesis to four beloved people who have meant and continue to mean so much to me. To my mother,whose love for me knew no bounds, to my father, who taught me the value of hard work ; to my brothers Ahmed and Mostafa. Thank you so much , I am grateful to have you.

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INTRODUCTION

Increasing awareness about culturable diversity beside development of new cultivation approaches is constantly increasing the numbers of unculturable taxa of microbiota in culture collection (Alain and Querellou, 2009; Pace, 2009). But, cultivation and characterization of microorganisms alone seems not adequate in absence of appropriate preservation techniques that do not alter the morphology, physiology or genetics of the pure strains. Here, careful and suitable preservation is imperative for future research, teaching, and industrial applications. Actually, the long-term preservation of microorganisms is often neglected although it is of special importance for both applied and environmental microbiology (Stahl and Wagner, 2006). Janssens *et al.* (2010) mentioned that preservation of microbial resources allows, a) validation of previously obtained results, b) guarantees that strains do not lose during research, c) catalogs biodiversity for future studies and e) enables scientists to apply the documented cultures for biotechnological and/or commercial use. Long-term storage is most often achieved by either lyophilization or cryopreservation in liquid nitrogen or at -80°C (De Paoli, 2005). The former requires specialized equipment and thus is mainly used by culture collections and offers additional advantages over the latter such as ease of storage, handling and transport (Morgan *et al.*, 2006). Besides the choice and concentration of cryo- or lyo-protectant, a number of other important parameters are expected to influence the success of preservation process such as growth and preservation medium, growth rate, culture density,