AGROTECHNOLOGY OF CULTIVATION OF DESERT PLANTS

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Abstract. The exclusive use of plant juices, not as a mere supplement to synthetic culture media, for culturing rhizospheric microorganisms (RMO) is introduced here. Juices were prepared from desert (*Mesembryanthemum crystallinum* L., *Zygophyllum album* L., *Carpobrotus edulis* L.) as well as cultivated (*Trifolium alexandrinum* L., *Beta vulgaris* L.) plants. Colonies of RMO (*Azospirillum brasilense, Enterobacter agglomerans* and *Klebsiella pneumoniae*) nicely developed on surfaceinoculated agar plates prepared from crude and diluted juice of *M. crystallinum* (ice plant).
Keywords: Desert plants; Plant juices; Rhizospheric microorganisms;Diazotrophs; Culture media;

INTRODUCTION

Increasing the cultivability of RMO under laboratory conditions represents a challenge to specialists in the field. Cultivation on laboratory media has selective effects, and thus yields results that are not representative of the whole microbial community [1]. RMO communities develop in concert with the plant roots, and are, as well, framed by the background and bulk soil community [2]. This has affected the continuing efforts to formulate culture media for culturing RMO. The addition of soil extract [3] to the generally-used nutrient agar
[4] resulted in some progress, meeting some of the nutritional requirements of the soil but not of the plant. Including plant material in the composition of RMO culture media was sporadic, and originally experimented through the use of plant infusions and extracts as additional supplements for cultivation of plant/soil microorganisms. Tomato juice was included in culture media specific to *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* [5,6]. The growth of lactic acid bacteria [7] and *Leuconostoc citrovorum* [8] was stimulated through enriching the selective culture medium with the juices of tomato, cabbage, grape and orange. Potato/carrot infusions are added to the culture media of yeast, moulds and fungi [9]. Cane juice was substituted for sucrose to exert selective power in LGP culture medium for enriching *Gluconactetobacter diazotrophicus* [10].

MATERIALS AND METHODS

The major tested desert plant, *Mesembryanthemum crystallinum* (ice plant). Tested RMO were representatives of diazotrophs, *Azospirillum brasilense*, *Enterobacter agglomerans* and *Klebsiella pneumoniae*, originally isolated from desert plants [12,13], and routinely maintained on the combined carbon sources N-deficient medium, CCM [14]. The reference culture media used were nutrient agar [4], soil extract agar [3] as well as CCM.

The use of ice plant juice to prepare solid culture medium, and its ability to support growth of RMO colonies (cfu)

The first set of experiments dealt with the development of a se- lected number of RMO (diazotrophs) pure cultures. They were initially inoculated into liquid CCM medium (100 ml in 250 ml capacity flasks), supplemented with NH_4Cl (0.5 g^{-1}) and yeast extract (0.2 g^{-1}), then incubated in a rotary shaker (100 rpm) at 30 °C for 24 h. Serial dilutions prepared from the resulting liquid batch cultures were surface-inoculated on agar plates prepared from the ice plant juice (crude and further successive dilutions) and CCM for comparison. Inoculated plates were incubated at 30 °C for 2–5 days and colony forming units (cfu) were counted.

RESULTS AND DISCUSSION

The second set of experiments tested the ability of agar plates prepared from plant juices to replicate and support the growth of a wide array of RMO colonies originally developed on agar plates of reference culture media. For this purpose, RMO associated to roots of maize and sugar beet were cultured, using the surface-inoculation method and agar plates prepared from tested reference culture media [13]. The conventional replica technique of Lederberg and Lederberg [15] was adjusted and employed. Hundreds of 72-h-old RMO colonies developed on master agar plates, those prepared from all tested reference culture media with an average of 20–50 cfu plate^{-1}, were progressively stamped (5–7 times) onto agar plates of the tested plant juices. During a week of incubation at 30 °C, the successfully replicated colonies were monitored on the various combinations of plant juice agar plates and percentage recovery was calculated.

The rhizosphere of four host desert plants (*Hordeum murinum, M. crystallinum, Z. album* and *Stipagrostis scoparia*) and one cultivated Nile valley crop (*Hordeum vulgare*) was examined. Total RMO in the ectoand endorhizospheres were determined using the ice plant juice (crude juice diluted 1:40 distilled water, v/v)-based agar medium and were compared with the reference media of nutrient agar, soil extract agar CCM. Ecto-rhizosphere samples were prepared [13] by transferring sufficient portions of root systems with closely adhering soil into sampling bottles containing the basal salt solution of CCM, as diluent. Bottles were shaken for 30 min and serial dilutions were prepared. The endo-rhizosphere samples were prepared [17] by washing another set of roots with tap water, then with 95% ethanol for 5–10 s, followed by 3% sodium hypochlorite for 1.5 h. Surface sterilized roots were then thoroughly washed with sterile water and crushed for 5 min in a Waring blender with adequate volume of basal salts of CCM medium. Further serial dilutions were prepared, and suitable dilutions of both spheres were surface-inoculated on agar plates prepared from all tested culture media. Incubation took place at 30 °C for 2–7 days and cfu were counted. Dry weights for suspended roots (80 °C) and rhizosphere soil (105 °C) were determined.

RMO of *T. alexandrinum* were assayed on culture agar plates of the homologous plant juice (diluted 1:40) as well as nutrient and soil extract agar. The culturing method was further modified by trying to adjust the oxygen diffusion at the surfaces of inoculated agar plates.

CONCLUSION

In conclusion, the results demonstrated the successful use of plant juice-based culture media for RMO cultivation. However, the presented qualitative approach remains to be further investigated quantitatively, using DNA-based methods, to distinguish the phylogenetic differences between cfu developed on plant juices and on reference culture media, to ascertain whether new types of bacteria are possibly cultured. Towards more standardization, the effect of plant variety and growth stage, previously established with tomato plants and lactic acid bacteria [8], remains to be experimented and verified.

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