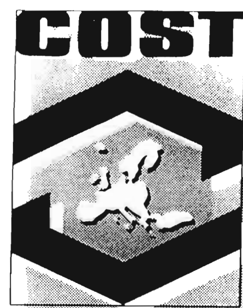
Granada
11 to 14 July 1994

COST action 821 on arbuscular mycorrhizas: A link with other types of mycorrhizal association



Mycorrhizas in integrated systems from genes to plant development

Proceedings of the fourth European Symposium on Mycorrhizas

Final report

European Commission
Directorate-General XII
Science, Research and Development

1996

EUR 16728 EN

Purification of an Arbuscular Mycorrhizal Endoglucanase from Colonized Roots

J. M. García-Garrido, I. García-Romera and J. A. Ocampo

Department of Microbiology, Estación Experimental del Zaidín, C.S.I.C.,
Prof Albareda 1, 18008 Granada, Spain.

Summary.- An arbuscular mycorrhizal endoglucanase (EC 3.2.1.4) was purified, for the first time, from roots of onion (*Allium cepa* cv. Babosa) colonized with *Glomus mosseae*. The stepwise purification procedure consisted of filtron concentration (10 kD), anion-exchange chromatography, anion-exchange Fast Protein Liquid Chromatography, and electroelution from polyacrylamide gel. The endoglucanase has a relative molecular weight of about 27 kD.

Keywords: Allium cepa, *cellulases, endoglucanases,* Glomus mosseae, *arbuscular mycorrhiza*

Introduction

The colonization of plant roots by arbuscular mycorrhizal (AM) fungi involves the formation of intercellular hyphae and intracellular arbuscules in the root (1). Electron microscopic observations suggest that the establishment of an intracellular symbiosis between AM fungus and plant roots requires penetration of the host cell by the AM fungi. Cell wall–hydrolyzing enzymes must be involved in this process (9).

The infection of roots by pathogenic and mutualistic microorganisms such as *Rhizobium* and *Azospirillum* appears to be mediated by cell wall–hydrolyzing enzymes (5, 13, 16). In spite of the low production of hydrolytic enzymes by these mutualistic microorganisms (14), endo– and exoglucanase, which belong to the cellulase system (6), seem to be involved in the dissolution of the cell wall which permits *Rhizobium* to enter the host cell (4, 17). The sequence of endoglucanase activity observed in the AM association, and the fact that the spores and external mycelia of *Glomus fasciculatum* and *Glomus mosseae* showed endoglucanase activity, suggest that this enzyme may be involved in the mycorrhizal colonization of plant roots (7). To date no AM endoglucanase has ever been purified; the availability of purified preparations of AM endoglucanase would make possible inmunological studies.

The aim of this work was to purify endoglucanase proteins found in AM–colonized plants that have the same electrophoretic mobility as the external mycelium of *G. mosseae.*

Materials and Methods

Plants were grown in 300 ml capacity open pots of soil collected from the Province of Granada, Spain. The soil was a calcexerollic xerochrept type (pH 7.6) (10). It was steam–sterilized and mixed with sterilized quartz sand at a proportion of 1:1 (V:V). Onion (*Allium cepa* cv. Babosa) was used as the test plant. Seeds were sown in moistened sand, and after two weeks seedlings were transplanted to the pots and grown under greenhouse conditions. Natural light was supplemented by Silvania incandescent and cool–white lamps, 400 nmol m^{-2} s^{-1} (400–700 nm), with a 16–8 h light–dark cycle at 25–19°°C and 50% relative humidity. Plants were watered from below using a capillary system, and were fed with a nutrient solution (12) lacking phosphate for AM–inoculated plants.

The AM inoculum consisted of 5 g of rhizosphere soil from alfalfa plant pot cultures of an isolate of *G. mosseae* (Nicol. & Gerd.) Gerd. and Trappe, which contained spores (15 sporocarps per g with 1 to 5 spores per sporocarp), mycelium and colonized root fragments. Soil filtrate (Whatman No.1 filter paper) from the rhizosphere of mycorrhizal plants was added to the uninoculated treatment. The filtrate contained common soil microorganisms, but no propagules of *G. mosseae.*

Plants were harvested after 35 days. Part of the root system (2 g fresh weight) was cleared and stained (15), and the percentage of total root length which was colonized with arbuscular mycorrhiza, was measured by the gridline intersect method (11).

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