Plant Physiology and Biochemistry 109 (2016) 308–318

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Characterization and purification of a bacterial chlorogenic acid esterase detected during the extraction of chlorogenic acid from arbuscular mycorrhizal tomato roots

Jonathan Negrel [a, *], Francine Javelle [a], Dominique Morandi [a], Géraldine Lucchi [b]

[a] Agroécologie, AgroSup Dijon, CNRS, INRA, Univ. Bourgogne Franche-Comté, F-21000, Dijon, France
[b] Clinical Innovation Proteomic Platform, Univ. Bourgogne Franche-Comté, F-21070, Dijon, France

A R T I C L E I N F O

Article history:
Received 30 August 2016
Received in revised form
14 October 2016
Accepted 15 October 2016
Available online 17 October 2016

Keywords:
Solanum lycopersicum
Arbuscular mycorrhizal fungi
Root-associated bacteria
Root exudates
Chlorogenic acid
Chlorogenase

A B S T R A C T

A Gram-negative bacterium able to grow using chlorogenic acid (5-caffeoylquinic acid) as sole carbon source has been isolated from the roots of tomato plants inoculated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. An intracellular esterase exhibiting very high affinity ($K_m = 2$ μM) for chlorogenic acid has been extracted and purified by FPLC from the chlorogenate-grown cultures of this bacterium. The molecular mass of the purified esterase determined by SDS-PAGE was 61 kDa and its isoelectric point determined by chromatofocusing was 7.75. The esterase hydrolysed chlorogenic acid analogues (caffeoylshikimate, and the 4- and 3-caffeoylquinic acid isomers), feruloyl esterases substrates (methyl caffeate and methyl ferulate), and even caffeoyl-CoA *in vitro* but all of them were less active than chlorogenic acid, demonstrating that the esterase is a genuine chlorogenic acid esterase. It was also induced when the bacterial strain was cultured in the presence of hydroxycinnamic acids (caffeic, p-coumaric or ferulic acid) as sole carbon source, but not in the presence of simple phenolics such as catechol or protocatechuic acid, nor in the presence of organic acids such as succinic or quinic acids. The purified esterase was remarkably stable in the presence of methanol, rapid formation of methyl caffeate occurring when its activity was measured in aqueous solutions containing 10–60% methanol. Our results therefore show that this bacterial chlorogenase can catalyse the transesterification reaction previously detected during the methanolic extraction of chlorogenic acid from arbuscular mycorrhizal tomato roots. Data are presented suggesting that colonisation by *Rhizophagus irregularis* could increase chlorogenic acid exudation from tomato roots, especially in nutrient-deprived plants, and thus favour the growth of chlorogenate-metabolizing bacteria on the root surface or in the mycorhizosphere.

© 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

We recently reported the occurrence in arbuscular mycorrhizal tomato roots of an enzyme exhibiting a remarkable stability in the presence of methanol (MeOH) and catalysing the trans-esterification of chlorogenic acid (CGA) to methyl caffeate during the extraction of phenolic compounds from these roots (Negrel et al., 2013). This enzyme was especially active in mycorrhizal roots of nutrient-deprived plants, but its activity could also be detected in the roots of non-mycorrhizal plants belonging to various species and grown under different mineral supplies (Negrel et al., 2013). Only preliminary characterization of this enzyme could be achieved because of its very low activity in soluble protein extracts but it was nevertheless possible to demonstrate unambiguously that it was not a fungal enzyme and that it exhibited a distinct substrate preference for CGA, suggesting that it could be a plant enzyme playing a role in CGA catabolism (Negrel et al., 2013). While attempting to purify this enzyme from tomato roots inoculated with *Rhizophagus irregularis*, we later noticed that a significant activity was always associated with the centrifugation pellets after repeated extraction of soluble proteins. Unexpectedly part of this activity could be released from these pellets after incubation with lysozyme. This observation led us to attempt to isolate bacteria associated with arbuscular mycorrhizal tomato roots and able to produce this enzyme in liquid cultures. We report here the isolation of a bacterial strain, tentatively identified as a *Variovorax* sp. strain,

* Corresponding author.
E-mail address: jonathan.negrel@inra.fr (J. Negrel).

http://dx.doi.org/10.1016/j.plaphy.2016.10.015
0981-9428/© 2016 Elsevier Masson SAS. All rights reserved.

from tomato roots colonized with *Rhizophagus irregularis*. When grown in the presence of CGA as sole carbon source, this bacterium produces an intracellular chlorogenic acid esterase (CGAE, also known as chlorogenase), which can be readily purified, and exhibits the same properties as the enzyme detected in arbuscular mycorrhizal tomato roots. In particular, it is able to catalyse the transesterification of CGA to methyl caffeate in MeOH-containing aqueous solutions. Our results therefore suggest that the transesterification of CGA detected during its extraction from tomato roots is due to the production of CGAEs by bacteria closely associated with roots. These results highlight the role of secondary products released in root exudates and confirm that they can act as specific substrates able to modulate the activity of bacteria in the rhizosphere (Doornbos et al., 2012).

2. Material and methods

2.1. Plant material and inoculation by AM fungi

Tomato cv. Marmande seeds were surface-disinfected for 10 min in 3.5% (w/v) calcium hypochlorite, pregerminated for a week in autoclaved vermiculite before being transferred to 150 cm^3 pots (one seedling per pot) filled with a mixture of 75% gamma irradiated clay loam soil (Epoisses soil) and 25% zeolite (v/v). Inoculation of *Rhizophagus irregularis* was performed by replacing zeolite by a commercial zeolite-based inoculum as previously described [Negrel et al., 2013). Plants were cultivated in growth chambers (day/night temperature 18/23 °C, 16 h photoperiod, light intensity 300 μmol photons m^{-2} s^{-1}) and watered everyday with reverse osmosis-purified water. After 4 weeks, plants were harvested and the root system was washed in water, dried, frozen in liquid nitrogen, and stored at −80 °C until required. Plants used to isolate bacterial strains were used immediately after harvesting. To monitor the colonisation of the root system after inoculation with *Rhizophagus irregularis*, two plants per experiment were collected and roots were observed after KOH digestion (10%, 45 min at 90 °C) and ink staining, as previously described (Negrel et al., 2013). AM colonisation parameters of the mycorrhizal tomato plants were in line with those previously reported, *i.e.* 10–20% for global intensity, and 70–90% for the percentage of cortical cells containing arbuscules in the mycorrhizal part of the root.

In vitro grown tomato plants used to attempt to detect CGAE activity in sterile roots were grown on hormone-free Murashige and Skoog medium for 1 month from sterile seeds.

2.2. Chemicals, substrates and biochemicals

CGA, 4-caffeoylquinic acid, 3-caffeoylquinic acid and lysozyme, from chicken egg white, were purchased from Sigma-Aldrich. Methyl caffeate was prepared from caffeic acid and MeOH using H_2SO_4 as catalyst and purified by column chromatography on silica gel as previously described (Negrel et al., 2013). Methyl ferulate was synthesized and purified using the same protocol. Caffeoylputrescine was available in our laboratory. Caffeoyl-CoA was prepared enzymatically from caffeic acid, using recombinant tobacco 4-coumarate:coenzyme A ligase (Beuerle and Pichersky, 2002). Caffeoylshikimate was synthesized enzymatically from caffeoyl-CoA and shikimic acid using a recombinant *Arabidopsis thaliana* HCT (Hoffmann et al., 2004) and purified before use by HPLC.

The green coffee extract used to test the activity of CGAE in the presence of dicaffeoylquinic isomers was prepared from green coffee beans (Clifford, 1986). After extraction of the different quinic acid esters in MeOH, the extract was evaporated *in vacuo* and partitioned between water and ethyl acetate. The ethyl acetate phase was then evaporated and taken up in a small volume of MeOH. HPLC analysis confirmed that the extract contained high concentrations of dicaffeoylquinic acids, and it was therefore used to test the specificity of CGAE.

2.3. Culture conditions for bacteria

Bacteria were cultivated using a basal mineral medium containing 6 g l^{-1} K_2HPO_4, 3 g l^{-1} KH_2PO_4, 1 g l^{-1} $(NH_4)_2SO_4$, 0.2 g l^{-1} $MgSO_4.7$ H_2O and 10 μL of a 10% $FeCl_3$ solution (final concentration 1 mg l^{-1} Fe^{3+}) (Meyer and Abdallah, 1978). The pH was adjusted to 7 by addition of NaOH prior to sterilisation. CGA (0.35 mg/ml) was added just prior to use after dissolution in a small volume of mineral medium and sterilisation by filtration through 0.22 μm Millipore filters. The other substrates tested as carbon source to try to induce CGAE activity (Fig. 3) were also added to the medium just prior to use by filtration through Millipore filters. Growth on CGA medium was relatively slow. In some experiments requiring a rapid growth of the bacterial strain (identification of bacteria by MALDI-TOF), chlorogenate was replaced by succinate at the same concentration (1 mM).

2.4. Enrichment and isolation from tomato roots of bacterial strains capable of using CGA as sole source of carbon

Tomato plants were harvested 1 month after inoculation with *Rhizophagus irregularis*. The root system was gently removed from the substrate, thoroughly washed under tap water, followed by sterile water. Root samples (c.a. 1 g fresh weight) were then introduced into sterile 30 ml polypropylene tubes filled with 10 ml sterile 10 mM $MgSO_4$ and were vortexed for 30 s. 100 μL aliquots of the $MgSO_4$ solution were then used to inoculate liquid culture tubes containing 10 ml mineral medium supplemented with 1 mM CGA. After 5 days of growth at 30 °C, 100 μL aliquots were subcultured into 10 ml of the same medium. After another 3 days, 10^{-1} to 10^{-5} dilutions of the medium were spread out on Petri dishes with the same medium solidified with 2% (w/v) agar. Bacteria were cultivated at 30 °C for 5 days. The isolated colonies were re-streaked to attempt to obtain axenic monocultures. The best 4 growing colonies were chosen for further studies.

Preliminary characterization of the isolated colonies included colony morphology, Gram staining, mobility, oxidase and catalase reaction and biochemical profiling using API 20 NE galleries (Biomérieux, Lyon, France). Since the API 20 NE test system did not allow the identification of the isolated colonies, one of the colonies was chosen for further studies.

The bacterium was tentatively identified by MALDI—TOF mass spectrometry (Singhal et al., 2015) and MALDI Biotyper. After 24 h of incubation at 30 °C on succinate medium, a small amount of biological material was applied directly onto the MALDI target plate in a thin film. After drying, the thin microbial film was overlaid with 2 μl of matrix solution (10 mg/ml alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile 2.5% trifluoroacetic acid) and air dried. MALDI-TOF MS analyses were performed with an autoFlex Speed in linear and positive mode in a range of 2000 to 18,000 Da, and the MALDI BioTyper 4 software (Bruker Daltonics). The identification scores were used as recommended by the manufacturer; a score >2 means identification to the species level, a score between 1.7 and 1.9 means identification to the genus level, two successive scores (first and second best matches) between 1.7 and 1.9 corresponding to the same species also allowing identification to the species level. Any other cases did not allow identification to the species level.

2.5. CGAE purification

The CGAE producing strain was grown in the standard mineral

Download English Version:

https://daneshyari.com/en/article/5515648

Download Persian Version:

https://daneshyari.com/article/5515648

Daneshyari.com