Systematic and Applied Microbiology 33 (2010) 232–235

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Short communication

Genotypic and phenotypic variation among *Lysobacter capsici* strains isolated from *Rhizoctonia* suppressive soils

J. Postma[a,*], E.H. Nijhuis[a], A.F. Yassin[b,**]

[a] *Plant Research International, P.O. Box 69, 6700 AB Wageningen, The Netherlands*
[b] *Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, 53127 Bonn, Germany*

ARTICLE INFO

Article history:
Received 27 August 2009

Keywords:
Lysobacter capsici
16S rRNA gene sequence
Fatty acids
DNA–DNA hybridization
BOX-PCR
Rhizoctonia solani inhibition

ABSTRACT

Four Gram-negative bacterial strains, recovered from clay soils cultivated with different crops in the Netherland, were subjected to a polyphasic taxonomic study in order to clarify their taxonomic status. Comparative analysis of the 16S rRNA gene sequences revealed that they belong to the genus *Lysobacter* and to be highly related to the type strains of *L. antibioticus* DSM 2044^T, *L. gummosus* DSM 6980^T, and *L. capsici* DSM 19286^T, displaying 99.1–99.3%, 99.2–99.6% and 99.4–100% sequence similarities, respectively, to these species. The results of DNA–DNA hybridization studies unambigiously indicated that the four strains belonged to the species *L. capsici*. Nevertheless, DNA fingerprinting and phenotypic characterization indicated that there was a considerable diversification and niche differentiation among the strains belonging to *L. capsici*. The newly identified *L. capsici* strains strongly inhibit *Rhizoctonia solani* AG2 and originate from *Rhizoctonia*-suppressive soils where also populations of *L. antibioticus* and *L. gummosus* were present. This is the first report of the presence of combined populations of closely related *Lysobacter* spp. within agricultural soils.

Introduction

The genus *Lysobacter* was first proposed by Christensen & Cook (1978) for highly mucoid, cream, pink or yellow-brown coloured, gliding organisms with high G+C contents (62–70.1%). The genus *Lysobacter* belongs to the family *Xanthomonadaceae* in the γ-proteobacteria and currently comprises more than 15 species. *Lysobacter* species are characterized by strong proteolytic activity and propensity to lyse a variety of microorganisms such as Gram-positive and Gram-negative bacteria (including actinomycetes), fungi, green algae and nematodes [2]. Many strains are of considerable ecological and biotechnological interest as producers of exoenzymes and of antibiotics [17]. The genus also contains several species with high potential as biocontrol agent [6].

During a screening program of bacteria inhibiting growth of *Rhizoctonia solani* AG2, an important pathogen of several vegetable crops, a large number of *Lysobacter* isolates was obtained from soil samples collected from different places in the Netherlands. *Lysobacter* species were isolated more frequently from soils that showed disease suppression of *Rhizoctonia* in sugar beet than from conducive soils [13]. These *Lysobacter* species showed strong inhibition of *Rhizoctonia* growth on nutrient media. They belonged mainly to the species *L. gummosus* (55%) and *L. antibioticus* (33%). However, 12% of the isolates were related to these species, but could not be identified up to species level at that time. In another study, similar isolates were recovered from a *Rhizoctonia*-suppressive soil derived from a continuous cauliflower cropping system [14].

Several new species have been described within the genus *Lysobacter* in the past few years. One of these is *L. capsici*, which is closely related to *L. gummosus* and *L. antibioticus* [12]. The type strain of *L. capsici* YC5194 (= DSM 19286^T) was isolated from the rhizosphere of pepper and was found to strongly inhibit fungi as well as oomycetes. Until now, only one strain of this new species has been described in detail.

In the current study, four of the unidentified *Lysobacter* strains, which had been isolated from four different *Rhizoctonia*-suppressive soils, were subjected to a polyphasic approach in order to clarify their taxonomic affiliation. A proper identification of these strains is important for further ecological studies on *Lysobacter* spp. occurring in disease suppressive soils.

Materials and methods

Bacterial strains

Four strains from different locations in the Netherlands were selected for identification (Table 1). The strains were stored at

* Corresponding author. Tel.: +31 317 480664.
** Corresponding author. Tel.: +49 228 2871 9071.
E-mail addresses: joeke.postma@wur.nl (J. Postma), yassin@mibi03.meb.uni-bonn.de (A.F. Yassin).

0723-2020/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2010.03.002

Table 1
Origin of the *Lysobacter* strains.

Isolate nr.	EMBL accession nr. for the 16S rRNA sequence	Year of isolation	Location	Crop	Niche	Reference
1.3.3	FN357195	2003	Strijen, NL	Grass-clover	Clay soil	[13]
6.2.3	FN357196	2003	Hensbroek, NL	Grass	Clay soil	[13]
10.4.5[a]	FN357197	2003	Marknesse, NL	Grass	Clay soil	[13]
55	FN357198	2003	Zwaagdijk, NL	Cauliflower	Clay soil	[14]

[a] The strain is deposited in the culture collection of DSMZ (Braunschweig, Germany) under the accession number DSM23109.

−80 °C in 10% glycerol, and cultured on the nutrient poor agar medium R2A (Difco Laboratories, Detroit, MI, USA).

16S rRNA amplification and sequencing

Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene were carried out using established procedures [16]. The purified PCR products were sequenced using Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Germany). An automatic DNA sequencer (model 310; Applied Biosystems) was used for electrophoresis of the sequence reaction products. The 16S rRNA gene sequence of the four strains as well as those of valid species of the genus *Lysobacter* (retrieved from GenBank) were added to the ARB-database [11] and aligned using the respective tool from the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony, neighbour-joining and maximum-likelihood methods. The evolutionary distance matrix for the neighbour-joining method was calculated using the correction of Jukes and Cantor [10]. The topologies of the neighbour-joining tree were evaluated using bootstrap analyses [4] based on 1000 resamplings.

DNA–DNA hybridization

DNA–DNA relatedness studies were performed between strain 10.4.5, and *L. antibioticus* DSM 2044^T, *L. gummosus* DSM 6980^T, and *L. capsici* DSM 19286^T, as well as between strains 1.3.3, 6.2.3, 55 and *L. capsici* DSM 19286^T. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite [1]. DNA–DNA hybridization was carried out as described by De Ley et al. [3] under consideration of the modifications described by Huss et al. [7] using a model Cary 100 Bio UV/vis-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

BOX-PCR patterns

BOX-PCR fingerprints of the four strains 1.3.3, 6.2.3, 10.4.5 and 55, as well as the type strains *L. capsici* DSM 19286^T, *L. antibioticus* DSM 2044^T, and *L. gummosus* DSM 6980^T were performed according to Rademaker et al. [15] using primer BOX A1R (5′-CTACGGCAAGGCGACGCTGACG- 3′.

Phenotypic characteristics

The biochemical properties of the four strains 1.3.3, 6.2.3, 10.4.5 and 55, as well as the type strain *L. capsici* DSM 19286^T, were determined by using the API 20NE, API 50 CHE and API ZYM systems according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France). Hydrolysis of casein, elastin, guanine, xanthine, hypoxanthine, tyrosine, and uric acid was investigated by using the methods described previously [5].

Biomass for fatty acid analysis was derived from 7-day-old Brain Heart Infusion broth (Oxoid) shake cultures incubated at 34 °C, harvested by centrifugation and washed with distilled water. Fatty acids were extracted, purified and analysed by using standard procedures as described previously [21].

In vitro inhibition of *R. solani* AG 2-2IIIB strain 02-337 by the four strains 1.3.3, 6.2.3, 10.4.5 and 55, as well as type strain *L. capsici* DSM 19286^T was tested in triplicate in a dual plate assay on R2A as described previously [13]. Growth of *R. solani* was compared in the presence and absence of *Lysobacter* strains.

Results

16S rRNA sequence analysis

Strains 1.3.3, 6.2.3, 10.4.5 and 55 were found to be genetically highly related, displaying 99.4–100% 16S rRNA sequence similarity. Phylogenetically the closest relative of the strains is *L. capsici* DSM 19286^T. The four strains formed a robust cluster with this species, supported by a bootstrap resampling value of 100% (Fig. 1). The association of these strains and *L. capsici* with *L. antibioticus* and *L. gummosus* is supported by all of the tree-making algorithms. In terms of sequence similarities, the four strains displayed highest relatedness to *L. capsici* (99.4–100% sequence similarities), *L. antibioticus* (99.1–99.3%) and *L. gummosus* (99.2–99.6%), with other species showing lower levels of similarity.

DNA–DNA hybridization

Chromosomal DNA–DNA hybridizations were performed between the four strains and related type strains. Reassociation values for strain 10.4.5 with respect to *L. antibioticus* DSM 2044^T, *L. gummosus* DSM 6980^T and *L. capsici* DSM 19286^T were 22.1%, 20.1% and 89.3%, respectively. Reassociation of strains 1.3.3, 6.2.3, 55 with *L. capsici* DSM 19286^T were 100.6%, 74.1% and 97.5%, respectively. These results show that our strains should be assigned to the species *L. capsici* [20].

BOX-PCR patterns

The fingerprints prepared by BOX-PCR of strains 1.3.3, 6.2.3, 10.4.5, 55, and *L. capsici* DSM 19286^T were all different from each other (Supplementary Fig. S1), as well as from the fingerprints of type strains *L. antibioticus* DSM 2044^T and *L. gummosus* DSM 6980^T.

Phenotypic characteristics

The four strains possessed similar physiological characteristics; results of strain 55 and 6.2.3 are presented in Table 2. They resemble *L. capsici* DSM 19286^T in being able to hydrolyse aesculin, casein, elastin, gelatin and hippurate and their ability to assimilate N-acetylglucosamine, D-glucose and D-mannose. The studied strains displayed similar enzymatic activities with *L. capsici* DSM 19286^T. The latter species differs from the studied strains by its inability to assimilate citrate, malate and D-maltose and a delayed reaction for adenine hydrolysis.

Analysis of the non-hydroxylated fraction of the cellular fatty acids from the studied strains and *L. capsici* DSM 19286^T revealed that they were nearly identical (Supplementary Table S1). Their