Dechlorosoma suillum Achenbach et al. 2001 is a later subjective synonym of Azospira oryzae
Reinhold-Hurek and Hurek 2000

In order to clarify the taxonomic position of Dechlorosoma suillum, which shares 99.9 % 16S rDNA sequence identity (1433 of 1435 bp) with Azospira oryzae, we compared the two species in a polyphasic taxonomic approach. Results of 122 physiological and biochemical tests for D. suillum DSM 13638T and Azospira oryzae 6a3T were identical, except for the lack of growth of Azospira oryzae 6a3T with perchlorate as the terminal electron acceptor. Presence of a nifH gene and nitrogenase activity, a key feature of Azospira, were also detected in D. suillum by Southern hybridization and by the acetylene reduction assay, respectively. Whole-cell SDS-PAGE profiles of SDS-soluble proteins of strains DSM 13638T and 6a3T were almost identical. DNA–DNA hybridization studies showed more than 90 % binding between D. suillum and two strains of Azospira oryzae. These data provide evidence that the two bacteria belong to the same species and that D. suillum is a later subjective synonym of Azospira oryzae.

The type strain of D. suillum, DSM 13638T, was received from the DSMZ. Azospira oryzae strains 6a3T (= LMG 9096T) and OM8A-5 and Azovibrio restrictus OSB2-4 were revived from stocks stored in liquid nitrogen in our laboratory. Unless stated otherwise, all strains were grown at 37 °C on VM ethanol medium (Reinhold-Hurek & Hurek, 2000).

Biotype 100 strips and API 20E systems with medium 2 (bioMérieux) were used to determine substrate utilization patterns and enzyme profiles. Both strips were inoculated according to the manufacturer’s instructions. Growth was recorded after 1, 2, 4 and 7 days incubation at 30 °C (Reinhold-Hurek & Hurek, 2000). Data recorded at day 4 are given; they did not differ from recordings at day 7. Perchlorate reduction was tested by anaerobic culturing techniques as described previously (Bruce et al., 1999; Coates et al., 1999). For the medium, solutions A (1000 ml), B (10 ml) and C (10 ml) (http://www.dsmz.de/media/med908.htm) were autoclaved separately and anaerobically under N2/CO2 (90 : 10). Dissolved O2 was removed from the medium by boiling. Vitamin solution (5 ml) and trace element solution SL-10 (1 ml) (http://www.dsmz.de/media/med908.htm), as well as sodium salts of acetate and chlorate (10 mM each) as the electron donor and acceptor (Coates et al., 1999), were added.
For protein patterns, cells were grown on VM ethanol plates for 36 h at 37 °C. Extraction of SDS-soluble proteins and electrophoresis conditions were described before (Reinhold-Hurek & Hurek, 2000).

For Southern hybridization, genomic DNA was extracted (Hurek et al., 1993) from D. suillum DSM 13638 T and Azospira oryzae 6a3 T and digested separately with restriction enzymes EcoRI and PstI. Fragments were separated by agarose gel electrophoresis, transferred onto nylon filter membranes and hybridized to a DNA probe labelled with digoxigenin (Roche). The nifH gene probe was generated by PCR with primer pair TH25/TH26 from a plasmid carrying nifH of strain BH72 (Hurek et al., 1997b). Hybridization was carried out at medium stringency as described by Hurek et al. (1997b).

For gas-chromatographic detection of nitrogenase activity, strains were grown on semi-solid nitrogen-free SM medium (Reinhold et al., 1986) at 28 °C for 4 days. The acetylene reduction assay was carried out with 1% (v/v) acetylene and the ethylene formed was quantified by using a gas chromatograph with flame-ionization detector (Karg & Reinhold-Hurek, 1996).

For DNA–DNA hybridization, DNAs were extracted and purified according to the method of Marmur (1961). Levels of DNA relatedness were determined by the initial renaturation rate method (De Ley et al., 1970) as described previously (Reinhold-Hurek & Hurek, 1996).

165 rDNA sequences obtained from the GenBank database were aligned by RDP (Ribosomal Database Project) (Maidak et al., 1999). Distances of the aligned sequences (corresponding to positions 56–1492 of the Escherichia coli 16S RNA gene) were calculated by the method of Jukes & Cantor (1969). The tree topology was inferred by the neighbour-joining method (Saitou & Nei, 1987) and the phylogenetic tree was constructed by using the TRECON software package (Van de Peer & de Wachter, 1994).

As the description of D. suillum did not include a detailed analysis of carbon sources for growth or enzymic activities (Achenbach et al., 2001), we subjected D. suillum DSM 13638 T and Azospira oryzae strains 6a3 T and OM8A-5 to a comparative analysis of 122 physiological and biochemical tests. All three strains showed identical characteristics. They used D(-)tartrate, L(-)-malate, D(-)-lactate, succinate, fumarate, D,L-β-hydroxybutyrate (3-hydroxybutyrate), L-aspartate, L-glutamate, propionate, 2-oxoglutarate, butyrate, acetate and ethanol as sole carbon sources. Except for these 13 positive reactions (butyrate, acetate and ethanol tests were not included in Biotype 100 strip), all strains tested could not use the other 89 carbon sources supplied in the Biotype 100 strip. These physiological features are in agreement with previous observations for Azospira oryzae (Reinhold-Hurek & Hurek, 2000). All tested strains showed catalase, cytochrome c oxidase and denitrification activity, but no gelatinase, tryptophan deaminase, urease, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase or β-galactosidase activity. No fermentation or oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin or arabinose occurred. These data demonstrated that D. suillum and Azospira oryzae have many physiological and biochemical characteristics in common. However, perchlorate reduction was only observed in D. suillum. In contrast to strain DSM 13638 T, Azospira oryzae strains 6a3 T and OM8A-5 did not show significant growth within 4 days in the presence of perchlorate.

For comparison of profiles of SDS-soluble proteins, three strains (D. suillum DSM 13638 T, Azospira oryzae 6a3 T and Azovibrio restrictus OSB2-4) were used (Fig. 1a). D. suillum DSM 13638 T and Azospira oryzae 6a3 T had almost identical

![Fig. 1. (a) SDS-PAGE of SDS-soluble whole-cell proteins of D. suillum, Azospira oryzae and Azovibrio restrictus strains. Lanes: 1, molecular-mass standards; 2, Azovibrio restrictus OSB2-4; 3, D. suillum DSM 13638 T; 4, Azospira oryzae 6a3 T.](image-url)
protein patterns. As a negative control, extracts of cells of a
different diazotrophic member of the Rhodocyclus-group,
Azovibrio restrictus OSB2-4, were significantly different.

Strains of Azospira oryzae were originally isolated from roots
of Kallar grass and several species of rice under conditions
of nitrogen fixation. Diazotrophy is a main feature of this
genus and species. However, this feature had not been
tested for D. suillum (Achenbach et al., 2001). Southern
hybridization of D. suillum DSM 13638T with a nifH gene
probe (Fig. 1b) indicated that this strain also harbours a
nifH-homologous gene, albeit with a slight restriction
fragment length polymorphism in comparison with
Azospira oryzae 6a3T. Reduction of acetylene (C2H2) to ethylene
(C2H4) was detected by gas chromatography after 2–4 days
of growth of strain DSM 13638T in semi-solid N-free
medium. This suggests that the nifH gene is functional and
nitrogenase is present in D. suillum.

A phylogenetic analysis of the 16S rRNA genes of Azospira
oryzae, D. suillum and related genera showed that Azospira
oryzae and D. suillum were located in the same branch
and formed a lineage distinct from others, supported by
bootstrap values of 100 or 61% (Fig. 2). The similarity of
the 16S rDNA sequences of Azospira oryzae and D. suillum
was 99–9%, with only 2 bp different in 1435 bp. These data
suggested that Dechlorosoma is highly related to Azospira.

The similarity level of DNA–DNA hybridization is crucial
for assessment of bacterial species identity (Stackebrandt &
Goebel, 1994). DNA–DNA hybridizations were performed
between DNA of strains DSM 13638T, 6a3T and OM8A-5.
Respective similarity levels of 90 and 92% were recorded
between strains DSM 13638T and OM8A-5 and strains
DSM 13638T and 6a3T. The high DNA relatedness, of more
than 70%, strongly suggested that D. suillum and Azospira
oryzae belong to the same species.

In conclusion, the following observations, based on the
analysis of type strains, demonstrate that D. suillum and
Azospira oryzae belong to the same species: (i) the 16S rDNA
shows 99–9% similarity; (ii) the SDS-soluble proteins show almost identical patterns in electrophoregrams,
which indicates a high degree of genomic similarity
(Kersters, 1985); (iii) the two bacteria share most of the
phenotypic characteristics tested, such as carbon source
utilization, enzymic activities and diazotrophy, a key
feature of the genus Azospira [the difference in a single
characteristic, dissimilatory (per)chloride reduction, would
not justify separation into two species or general]; and (iv)
the DNA–DNA binding level of 90–92% is well above the
suggested limit for species identity.

The name Azospira oryzae was validly published in 2000
(Reinhold-Hurek & Hurek, 2000), the taxon being first
described as Azorarcus sensu lato group D in 1993 (Reinhold-
Hurek et al., 1993), and 16S rDNA sequences were available
in GenBank and published as a fragment in 1995
(Hurek & Reinhold-Hurek, 1995) and as almost complete
sequences in 1997 (Hurek et al., 1997a). However, neither
Azorarcus sp. D nor Azospira oryzae was included in
phylogenetic analyses of D. suillum (Achenbach et al., 2001;
Coates et al., 1999, 2001). Our data, however, provide
evidence that D. suillum (Achenbach et al., 2001) is a later
subjective synonym of Azospira oryzae (Reinhold-Hurek &
Hurek, 2000) within the β-subclass of the Proteobacteria.
This extends the known habitats for Azospira oryzae, which
was previously thought to harbour exclusively plant-
and fungus-associated strains.

**Emended description of Azospira oryzae**

**Reinhold-Hurek and Hurek 2000**

Azospira oryzae (o.ry’zae. N.L. fem. n. Oryza genus name of
rice; N.L. gen. n. oryzae from rice, referring to its frequent
occurrence in association with rice roots).

The following description is based on the data of
Reinhold-Hurek et al. (1993), Reinhold-Hurek & Hurek
(2000) and Achenbach et al. (2001) and the data presented
in this paper. On VM ethanol medium, colonies are
translucent, pinkish to salmon-coloured, smooth, convex
with an entire margin. Growth optimum at 37°C. Sources of isolates are surface-sterilized roots of Gramineae such as
Kallar grass [Leptochloa fusca (L.) Kunth] or rice (Oryza)
species, grown in the Punjab of Pakistan or Nepal,
Philippines and Italy, respectively, resting stages (sclerotia)
of basidiomycetes (Pakistan) and (per)chlorate-reducing
enrichments from samples collected from a primary
treatment lagoon of swine waste in the USA.

The type strain is strain 6a3T (=LMG 9096T), which has a
G+C content of 65.2 mol% and was isolated from
Kallar grass.

**Fig. 2.** Phylogenetic tree of Azospira oryzae, D. suillum
and related genera. The tree was constructed by the neighbour-
joining method from 16S rDNA sequences. Bootstrap support
percentages from 1000 replications are indicated for each
node. GenBank accession numbers are shown in parentheses.
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