Minutes

Kristina Lindström, Secretary
J. P. W. Young, Acting Chairperson

Minutes of the meeting, 7 September 2010, Geneva, Switzerland

International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of Agrobacterium and Rhizobium

The closed meeting opened at 21:00 on 7 September 2010, during the 9th European Nitrogen Fixation Conference in Geneva, Switzerland. J. P. W. Young was elected to serve as a chairperson in the absence of E. Martínez-Romero, the usual chairperson.

Minute 2. Record of attendance. The members present were P. de Lajudie, G. Laguerre, K. Lindström and J. P. W. Young. All subcommittee members had the opportunity to participate in the online discussions.

Minute 3. Acceptance of the previous minutes. The minutes from the meeting on 31 August 2008, Gent, Belgium, were accepted (Lindström & Young, 2009).

Minute 4. Membership issues. The recruitment of new members was discussed. A wider geographical representation of subcommittee members would be valuable.

Minute 5. New taxa described since the last meeting. The new taxa are listed in Table 1.

Minute 6. Current status of Agrobacterium spp. The inclusion of all Agrobacterium species in the genus Rhizobium, proposed by Young et al. (2001) since neither genus was monophyletic, was contested by Farrand et al. (2003) who proposed to maintain the two genera. This introduced uncertainties in taxonomic papers where A. tumefaciens is sometimes called A. radiobacter or R. radiobacter. Nomenclatural uncertainties also surround the classification of strain K84, a famous non-pathogenic Agrobacterium strain used as a biocontrol agent. Strain K84 is variously called A. radiobacter, A. rhizogenes or R. rhizogenes.

i) To resolve the polyphyletic status of the genus Agrobacterium, it is sufficient to transfer the one most remotely related species – the so-called biovar 2 of Agrobacterium (Keane et al., 1970) – into the genus Rhizobium. As a result, Rhizobium rhizogenes is a valid designation for biovar 2 (Costechareyre et al., 2010). Notably, as strain K84 is a biovar 2 strain, strain K84 should be called R. rhizogenes instead of A. radiobacter (Vélázquez et al., 2010).

ii) The emended genus Agrobacterium is monophyletic and can be conserved as a genus (Costechareyre et al., 2010). It contains several biovars, but it should be noted that, in the Agrobacterium literature, the term ‘biovar’ does not have the usual meaning of a specific phenotypic form within a species. Instead, it is generally agreed that biovars correspond to biological species in this genus (Kersters & De Ley, 1984). In this respect, A. vitis (i.e. biovar 3), A. rubi and A. larrymoorei are acceptable designations for three homogeneous genomic species (Ophe & Kerr, 1990; Popoff et al., 1984; Bouzar & Jones, 2001). Hybridization studies have revealed, however, that biovar 1 is not a homogeneous species but a complex of several genomic species or genomovars (Popoff et al., 1984; Kersters & De Ley, 1984).

iii) Most biovar 1 genomovars have not yet received accepted Latin binomials and are currently designated genomovar G1 to G9 or G13. Genomovar G4 is an exception because it includes the type strains of both A. radiobacter and A. tumefaciens (i.e. ATCC 19358T and B6, respectively), causing nomenclatural uncertainties (Mougel et al., 2002; Portier et al., 2006; Costechareyre et al., 2010). Although Bouzar (1994) proposed that A. tumefaciens should be retained because it is the type species of the genus, Young et al. (2006) stipulated that, despite this, the epithet radiobacter has priority over tumefaciens. For this reason, A. radiobacter is a valid name for genomovar G4, with ATCC 19358T as the type strain (Costechareyre et al., 2010).

iv) The name A. radiobacter is only valid for genomovar G4, but not for other biovar 1 genomovar members, such as the completely sequenced strains C58 and H13-3, which belong to genomovars G8 and G1, respectively. For this reason, it was proposed that the biovar 1 species complex should be collectively called the Agrobacterium tumefaciens species complex (Costechareyre et al., 2010). This seems a good interim solution until genomovars can be formally named. Strains C58, H13-3, B6 and ATCC 19358T all belong to the A. tumefaciens species complex, but only the latter two belong to the bona fide species A. radiobacter. The type strain of A. radiobacter is ATCC 19358T (=CFBP 2414T =LMG 1407T). Strain B6 was the type strain of A. tumefaciens but as this is no longer a species, strain B6 should no longer be a type strain.

v) Although Allorhizobium undicola appears to be related to the genus Agrobacterium (Costechareyre et al., 2010), its generic status is not yet clearly ascertained.
Table 1. Novel species described since the last meeting of the committee in August 2008

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>Host plants nodulated</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Devesia yakushimensis</td>
<td>Bautista et al. [Int J Syst Evol Microbiol 60 (2010) 627–632]</td>
<td>Isolated from nodules of Pueraria lobata; no nodulation reported</td>
<td></td>
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<tr>
<td>Rhizobium alkalosilis</td>
<td>Lu et al. [Int J Syst Evol Microbiol 59 (2009) 3006–3011]</td>
<td>Isolated from Phaseolus vulgaris. No nodulation reported</td>
<td>New comparative studies including strains of both taxa must be performed in order to confirm that R. alamii and R. mesosinicum are two really different species.</td>
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</table>
vi) *Rhizobium galegae* and related species cluster with the genus *Agrobacterium* in some phylogenetic analyses (e.g. Young *et al.*, 2001) but not others (e.g. Velázquez *et al.*, 2010). The phylogenetic position of these species relative to the genera *Rhizobium*, *Agrobacterium* and *Ensifer* (*Sinorhizobium*) is currently uncertain. While they may eventually be removed from the genus *Rhizobium*, it would be premature to suggest that they should be transferred to the genus *Agrobacterium*.

**Minute 7. Aeschynomene photosynthetic bradyrhizobia.**

Photosynthetic bradyrhizobia were isolated from *Aeschynomene* spp. plant root and stem nodules in Africa (Alazard, 1990) and Central America (Miché *et al.*, 2010), from African wild rice roots (Chaintreuil *et al.*, 2000) and from lake water (Hirsch & Muller, 1985). *Aeschynomene* spp. plants are nodulated by photosynthetic (PB) and non-photosynthetic (NPB) bradyrhizobial strains with distinct host ranges on *Aeschynomene* spp. (Alazard, 1990). Some NPB strains lack the canonical nodulation genes and nodulate *Aeschynomene* spp. plants via a Nod factor-independent system; among these are the two sequenced model strains BTAi1 and ORS278 (Giraud *et al.*, 2007). Nod gene-independent symbiosis is linked to *Aeschynomene* host species but not strictly to photosynthetic ability (Miché *et al.*, 2010). Photosynthesis is active in bacteroids (Eaglesham & Szalay, 1983) and is generally reported as playing a role in symbiotic infectivity and effectiveness (Evans *et al.*, 1990; Yurkov & Beatty, 1998; Giraud *et al.*, 2000; Giraud & Fleischman, 2004), although efficient *Aeschynomene* stem nodulating NPB strains have also been isolated (Montecchia *et al.*, 2002; Miché *et al.*, 2010). Miché *et al.* (2010) hypothesized that stem nodulating bradyrhizobial evolution may involve an ancestral nod-independent nodulation coupled with a photosynthetic trait, followed by occasional lateral acquisitions of *nod* genes and loss of photosynthetic ability.

Early 16S rRNA gene-based phylogenetic studies indicated that PB strains were separate from NPB (van Berkum *et al.*, 1995; Molouba *et al.*, 1999). This was later confirmed by ribosomal 16S–23S rRNA internal transcribed spacer (ITS) region phylogenies (Willems *et al.*, 2003), AFLP and MLSA (Nzoué *et al.*, 2009; Miché *et al.*, 2010). However PB strains may harbour multiple rRNA operons that are heterogeneous in ITS size and sequence (Willems *et al.*, 2003). The phylogenetic distance between PB and other *Bradyrhizobium* spp. is such that several authors (So *et al.*, 1994; Fleischman & Kramer, 1998; Nzoué *et al.*, 2009) even discussed the possibility that the PB clade should be considered to be a separate genus. Several authors have highlighted the genetic diversity of photosynthetic bradyrhizobia (Willems *et al.*, 2000; Miché *et al.*, 2010).

Comparative genomics of the two model photosynthetic bradyrhizobium strains ORS278 and BTAi1 has demonstrated high plasticity within genomes, as reflected by large variations in genome sizes and composition (Giraud *et al.*, 2007).

According to DNA–DNA hybridization data, Willems *et al.* (2001) distinguished two genospecies, VI and VIII, among PB strains. However, genospecies VI, comprising the model *Bradyrhizobium* strains ORS278 and BTAi1 was described before the transfer of *Sinorhizobium* to *Ensifer* (Young, 2003).

**Table 1. cont.**

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<tr>
<td>‘<em>Sinorhizobium abri</em>’</td>
<td>Ogasawara <em>et al.</em> [Symbiosis 34 (2003) 53–68]</td>
<td><em>Abrus precatorius</em></td>
<td>Not a validly published name. This species belongs to the <em>Ensifer</em> phylogenetic cluster but was described before the transfer of <em>Sinorhizobium</em> to <em>Ensifer</em> (Young, 2003).</td>
</tr>
<tr>
<td>‘<em>Sinorhizobium indaense</em>’</td>
<td>Ogasawara <em>et al.</em> [Symbiosis 34 (2003) 53–68]</td>
<td><em>Sesbania rostrata</em></td>
<td>Not a validly published name. This species belongs to the <em>Ensifer</em> phylogenetic cluster but was described before the transfer of <em>Sinorhizobium</em> to <em>Ensifer</em> (Young, 2003).</td>
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strain ORS 278, is heterogeneous, with strains having DNA–DNA hybridization values ranging from 43 to 100% between them and belonging to several AFLP groups (Willems et al., 2000). Photosynthetic bradyrhizobia genospecies VI may represent several emerging separate genospecies (Rivas et al., 2009; Nzoué et al., 2009). The species status of ORS 278 remains unclear.

van Berkum et al. (2006) proposed the inclusion of Blastobacter denitrificans in the genus Bradyrhizobium and Blastobacter denitrificans as a synonym of Bradyrhizobium denitrificans. Later MLSA (3–9 housekeeping genes) data of Rivas et al. (2009) and Nzoué et al. (2009) added further arguments to this proposal, including photosynthetic bradyrhizobia genospecies VIII, comprising the genome sequenced BTAi1 strain in Bradyrhizobium denitrificans.

References


nod-independent stem nodulation is not restricted to photosynthetic bradyrhizobia. Environ Microbiol 12, 2152–2164.


