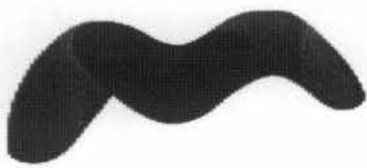
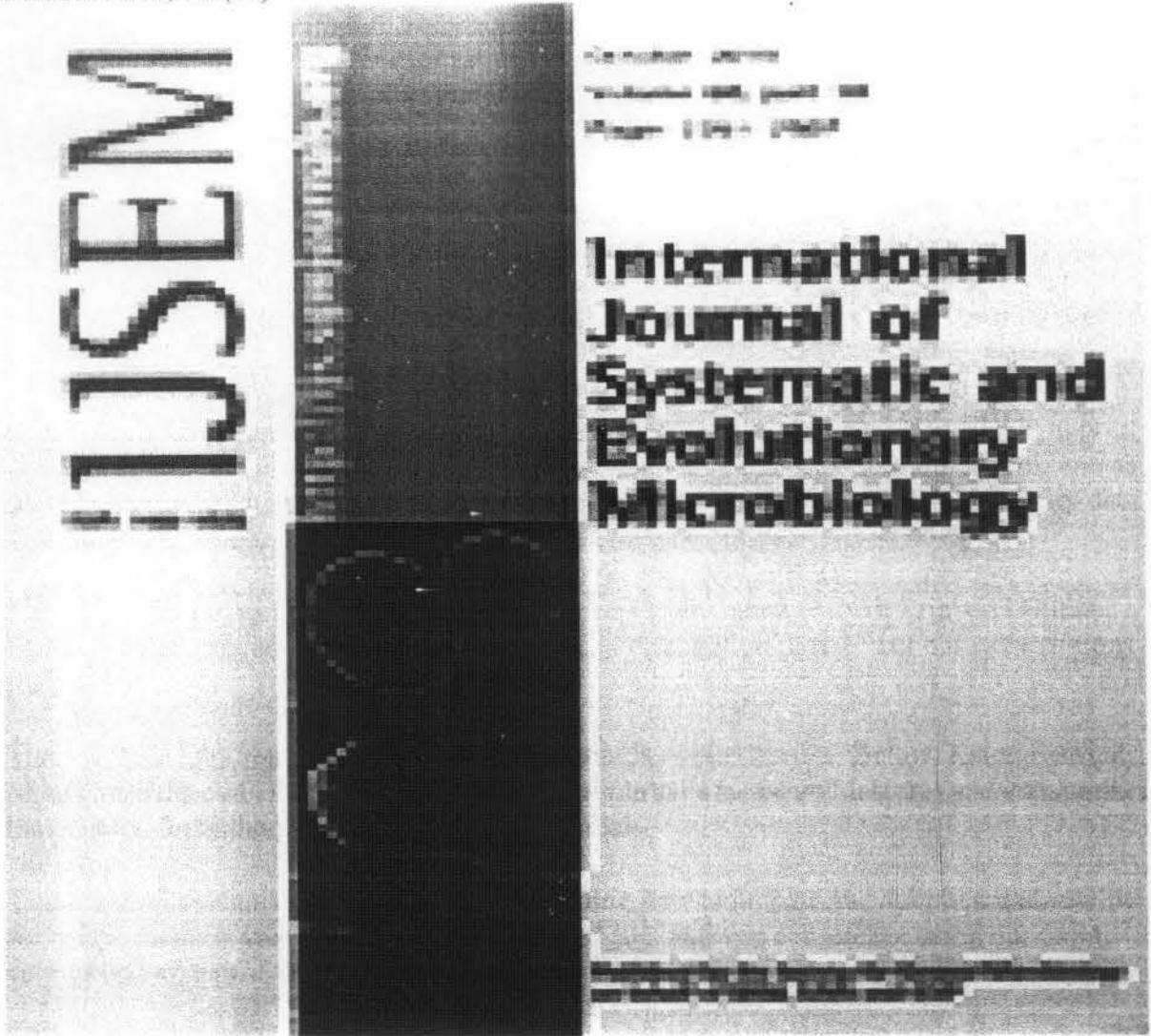


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Actinokineospora baliensis sp. nov., *Actinokineospora cibodasensis* sp. nov. and *Actinokineospora cianjurenensis* sp. nov., isolated from soil and plant litter

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Six actinomycete strains isolated from soil and plant-litter samples in Indonesia were studied for their taxonomic position by using a polyphasic approach. Phylogenetically, all the strains were located in the broad cluster of the genus *Actinokineospora*. Chemotaxonomic data [cell-wall diamino acid, meso-diaminopimelic acid; cell-wall peptidoglycan, type III (A1_γ); major sugars, galactose and arabinose; major menaquinone, MK-9(H₄); major fatty acid, iso-C_{16:0}; major phospholipid, phosphatidylethanolamine] supported the affiliation of all six strains to the genus *Actinokineospora*. The results of DNA–DNA hybridization with DNA from type strains of *Actinokineospora* species with validly published names revealed three DNA–DNA relatedness groups. Group I (ID03-0561^T) showed low relatedness to the other strains studied. The three strains in group II (ID03-0784^T, ID03-0808 and ID03-0809) formed a group with high relatedness (98–100%) and showed low relatedness to the other strains studied. The two strains in group III (ID03-0810^T and ID03-0813) showed 58–68% relatedness to *Actinokineospora terrae* NBRC 15668^T and showed low relatedness (2–24%) to the other strains studied. The description of three novel species is proposed: *Actinokineospora baliensis* sp. nov., for the single strain in group I (type strain ID03-0561^T = BTCC B-554^T = NBRC 104211^T), *Actinokineospora cibodasensis* sp. nov., for the strains in group II (type strain ID03-0784^T = BTCC B-555^T = NBRC 104212^T), and *Actinokineospora cianjurenensis* sp. nov., for the strains in group III (type strain ID03-0810^T = BTCC B-558^T = NBRC 105526^T).

The genus *Actinokineospora* was proposed by Hasegawa (1988) for motile, arthrospore-bearing actinomycetes. Recently, Labeda *et al.* (2010) emended the description of the genus to accommodate species that have not been observed to produce motile spores, and transferred

Amycolatopsis fastidiosa to the genus as *Actinokineospora fastidiosa*. At the time of writing, the genus contains eight species: *Actinokineospora riparia* (the type species), *Actinokineospora inagensis*, *Actinokineospora globicatena*, *Actinokineospora terrae*, *Actinokineospora diospyrosa*, *Actinokineospora auranticolor*, *Actinokineospora enzanensis* and *Actinokineospora fastidiosa* (Hasegawa, 1988; Tamura *et al.*, 1995; Otoguro *et al.*, 2001b; Labeda *et al.*, 2010). These actinomycetes have meso-diaminopimelic acid as a cell-wall diamino acid, galactose and arabinose as diagnostic whole-cell sugars, MK-9(H₄) as the predominant menaquinone, phospholipid type II, iso-C_{16:0} fatty acid as

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains ID03-0561^T, ID03-0784^T and ID03-0810^T are AB447488, AB447489 and AB473945, respectively.

Minimum-evolution and maximum-parsimony 16S rRNA gene sequence-based trees are available as supplementary material with the online version of this paper.

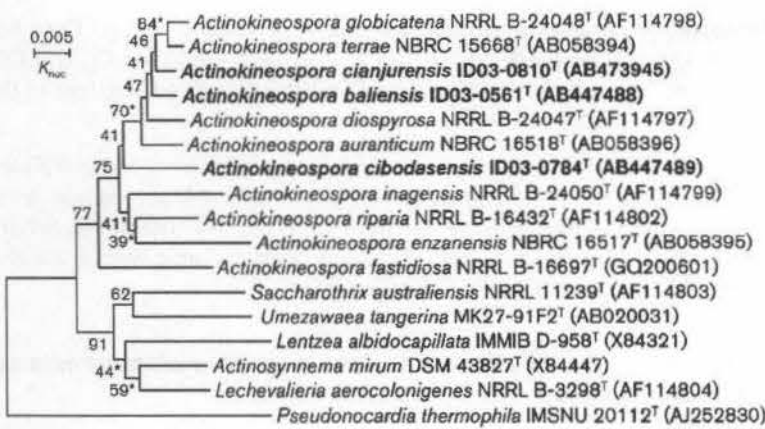


Fig. 1. 16S rRNA gene sequence dendrogram reconstructed by the neighbour-joining method using the software MEGA version 4 (Tamura *et al.*, 2007) displaying the relatedness of the novel strains and other members of the genus *Actinokineospora*. The sequence of *Pseudonocardia thermophila* IMSNU 20112^T was used as the outgroup. Bar, 0.005 substitutions per nucleotide position. Asterisks indicate branches of the tree that were also recovered using the minimum-evolution and maximum-parsimony methods (these trees are available as Supplementary Figs S1 and S2 in IJSEM Online).

Table 2. The range of G+C content of the isolates was 70.2–71.9 mol%. DNA–DNA hybridization revealed that the six Indonesian isolates were divided into three DNA–DNA relatedness groups. Group I contained a single isolate (ID03-0561^T), group II contained three isolates (ID03-0784^T, ID03-0808 and ID03-0809) and group III contained two isolates (ID03-0810^T and ID03-0813). Group I showed low relatedness to the other Indonesian strains and the reference strains used. Strains of group II showed high relatedness to each other (98–100%) and low relatedness to the other tested strains. The strains of group III showed 58–68% relatedness to *Actinokineospora terrae* NBRC 15668^T and low relatedness (2–24%) to the other reference strains.

The results of phenotypic characterization, performed as described previously (Seino *et al.*, 1985; Shirling & Gottlieb, 1966; Otoguro *et al.*, 2001b), are given in the species descriptions. The isolates used for phenotypic tests

were grown in yeast extract/glucose broth medium as described for chemotaxonomic analysis and resuspended in distilled water. DNA–DNA relatedness groups I–III could be distinguished from the type strains of other species of the genus *Actinokineospora* by using a combination of phenotypic properties. Strain ID03-561^T in group I was positive for utilization of mannose and sucrose and negative for utilization of arabinose, galactose, fructose and rhamnose. The isolates in group II were positive for utilization of galactose, mannose, fructose, sucrose and maltose and negative for utilization of arabinose and rhamnose as sole carbon sources. The two isolates in group III were distinguished from *Actinokineospora terrae* by being positive for utilization of galactose and negative for utilization of arabinose and rhamnose.

It is clear from the genotypic, chemotaxonomic and phenotypic data that the six Indonesian strains represent three novel species in the genus *Actinokineospora*. The

Table 2. DNA base composition and DNA–DNA hybridization of *Actinokineospora* strains

Strain	G+C content (mol%)	DNA–DNA hybridization (%) with labelled DNA from strain:									
		1	2	3	4	5	6	7	8	9	10
1. ID03-0561 ^T	71.4	(100)	12	13	16	27	10	24	37	17	2
2. ID03-0784 ^T	71.3	12	(100)	ND	14	ND	6	15	17	17	14
ID03-0808	71.9	15	93	ND	17	ND	11	23	35	32	9
3. ID03-0809	71.5	13	94	(100)	15	ND	ND	52	ND	2	ND
4. ID03-0810 ^T	70.2	25	23	12	(100)	ND	3	26	25	58	16
ID03-0813	70.3	31	10	13	91	ND	9	ND	ND	65	25
5. <i>A. riparia</i> NBRC 14541 ^T	ND	7	7	7	8	(100)	19	25	2	7	38
6. <i>A. inagensis</i> NBRC 15663 ^T	ND	ND	20	ND	ND	ND	(100)	27	30	25	30
7. <i>A. globicatena</i> NBRC 15664 ^T	ND	26	24	ND	ND	28	1	(100)	13	26	32
8. <i>A. diospyrosa</i> NBRC 15665 ^T	ND	20	7	9	12	16	9	20	(100)	24	39
9. <i>A. terrae</i> NBRC 15668 ^T	ND	29	15	19	68	20	4	30	10	(100)	20
10. <i>A. enzaensis</i> NBRC 16517 ^T	ND	13	13	ND	ND	27	17	33	4	24	(100)
<i>A. auranticolor</i> NBRC 16518 ^T	ND	6	5	8	8	17	12	16	5	10	2

ND, Not determined.

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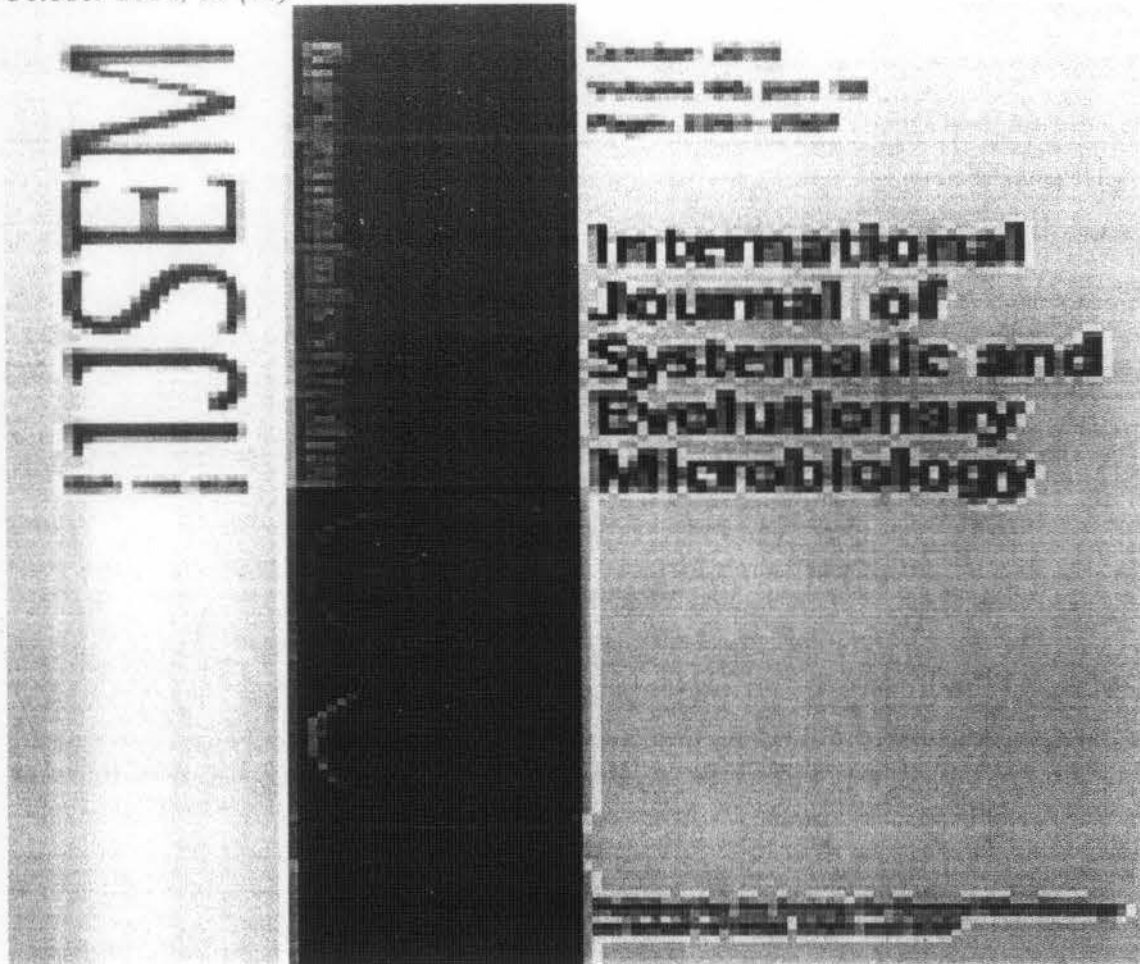
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