Reclassification of *Promicromonospora pachnodae* Cazemier *et al.* 2004 as *Xylanimicrobium pachnodae* gen. nov., comb. nov.

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The recently described facultatively anaerobic *Promicromonospora pachnodae* is phylogenetically only moderately related to authentic members of *Promicromonospora*. *P. pachnodae* is closely related to *Xylanibacterium ulmi* and slightly less closely related to *Xylanimonas cellulosilytica* and *Isoptericola variabilis* (basonym *Cellulosimicrobium variabile*). Members of the different genera of *Promicromonosporaceae* have similar chemotaxonomic properties; they share the same peptidoglycan type (A4 α) and have similar profiles of polar lipids, menaquinones, fatty acids and whole cell sugars. However, they differ from each other in the detailed amino acid composition of peptidoglycan, a taxonomically significant character that has previously been used in the delineation of actinobacterial genera. Recognized *Promicromonospora* species and *Xylanibacterium ulmi* exhibit the L-Lys–L-Ala–D-Glu type, *Xylanimonas cellulosilytica* and *I. variabilis* show the L-Lys–D-Asp type, whereas *P. pachnodae* has the L-Lys–L-Ser–D-Glu type. This property, together with the distinct phylogenetic position of *Promicromonospora pachnodae*, suggests a novel genus for the xylanolytic organism *Xylanimicrobium pachnodae* (Cazemier *et al.* 2004) gen. nov., comb. nov.

Owing to the order of descriptions of *Cellulosimicrobium variabile* (Bakalidou *et al.*, 2002), *Xylanimonas cellulosilytica* (Rivas *et al.*, 2003), *Xylanibacterium ulmi* (Rivas *et al.*, 2004), *Promicromonospora pachnodae* (Cazemier *et al.*, 2003) and two novel authentic species of *Promicromonospora* (Busse *et al.*, 2003), several of these strains were not or could not be included in studies on the description of most of the other novel species. Only when the 16S rRNA gene sequences of these species became available were the phylogenetic relationships unravelled. As a consequence, *Cellulosimicrobium variabile* was reclassified as *Isoptericola variabilis* (Stackebrandt *et al.*, 2004). Fig. 1 displays a 16S rRNA gene sequence neighbour-joining tree of these organisms and their closest relatives. The similarity values of almost complete sequences of *Promicromonosporaceae* type strains range between 94.6 and 99.2 %, with the highest value shared between authentic members of *Promicromonospora* (98.4–99.2 %). *P. pachnodae* DSM 12657^T and *Xylanibacterium ulmi* XIL08^T show 98 % sequence similarity, whereas values determined for *P. pachnodae* and the four authentic species of *Promicromonospora* are slightly lower, ranging between 95.5 and 96.1 %.

The generic affiliation of *P. pachnodae* was based on the presence of the following characters: phylogenetic position, presence of a fragmenting mycelium, matching 16S rRNA gene sequence signatures (Stackebrandt *et al.*, 1997), biochemical properties that fall into the range of those of

Promicromonospora species and the presence of peptidoglycan type A4 α [L-Lys as diamino acid and a dicarboxylic amino acid (Glu or Asp) as interpeptide bridge constituent]. However, the composition of peptidoglycan amino acids in *Promicromonospora* species needs further discussion. The presence of the Lys–Ala–Glu type was determined in *Promicromonospora sukumoe* and confirmed in this study for *Promicromonospora citrea* as originally stated by Evtushenko *et al.* (1984), using the methods of Schleifer & Kandler (1972) and Groth *et al.* (1999). By contrast, Kalakoutskii *et al.* (1989) reported the type A3 α , L-Lys–Ala–Ala, for *P. citrea*, and Busse *et al.* (2003) indicated the presence of glycine in the peptidoglycan of *Promicromonospora aerolata* and *Promicromonospora vindobonensis*. As actinobacterial genera are usually coherent with respect to peptidoglycan type A4 α , the peptidoglycan composition was redetermined for the two latter species. Indeed, the composition must be corrected to the composition Lys–Ala–Glu as found in the other two recognized *Promicromonospora* species.

Menaquinone composition and polar lipids were not reported by Cazemier *et al.* (2003) for *P. pachnodae*; these compounds were analysed in this study using the methods of Minnikin *et al.* (1979), Collins & Jones (1980) and Groth *et al.* (1999). The chemotaxonomic properties of *P. pachnodae* and related organisms are shown in Table 1. The menaquinone MK-9(H₄) together with phosphatidylglycerol,

Fig. 1. Phylogenetic relatedness among members of *Promicromonospora*, *Xylanimonas*, *Xylanibacterium*, *Isoptericola* and *Cellulosimicrobium* based upon 16S rDNA sequence comparison. *Beutenbergia cavernae* DSM 12333^T (Y18378) and *Jonesia denitrificans* DSM 20603^T (X83811) served as a root. The dendrogram was generated by neighbour-joining analysis (Felsenstein, 1993). Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 100 bootstrapped trees (only values of 50% and above are shown). Bar, 5 nucleotide substitutions per 100 nucleotides.

diphosphatidylglycerol, phosphatidylinositol and unknown phospholipids are commonly present in all these organisms, and thus are not discriminatory. This is also the case for the fatty acid pattern (Cazemier *et al.*, 2003).

P. pachnodae shares with *Xylanibacterium ulmi* and *Xylanimonas cellulosilytica* the ability to produce xylanases. Comparison of metabolic properties of members of *Promicromonosporaceae* reveals a significant number of negative reactions for *P. pachnodae* in the API 50CHE substrate panel (Table 2).

The topology of the 16S rDNA dendrogram, recovered by different phylogenetic methods, suggests that *P. pachnodae* is not a member of the recognized genus *Promicromonospora*. However, as pointed out previously in the literature (Schumann *et al.*, 2001; Stackebrandt *et al.*, 2004), and consistent with a polyphasic approach to classification, branching points, chemotaxonomic, morphological and cultural properties should be used for reclassification purposes. The rationale for the reclassification of *P. pachnodae* as the nucleus of a new genus, adjacent to the genera *Xylanimonas* and *Xylanibacterium*, is based upon the presence of the peptidoglycan type L-Lys-L-Ser-D-Glu, which is not found in recognized species of *Promicromonosporaceae* or in phylogenetically neighbouring species.

Table 1. Morphological and chemotaxonomic characteristics that differentiate strains of the genera *Cellulosimicrobium*, *Xylanimonas*, *Promicromonospora* and related species

Data from Rivas *et al.* (2004), Busse *et al.* (2003) and our own data. Abbreviations: Ara, arabinose; Gal, galactose; Rha, rhamnose; Fuc, fucose; Man, mannose; Glc, glucose; Xyl, xylose; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid; PGL, unknown phosphoglycolipid; GL, unknown glycolipid. –, Negative; w, weak; +, positive.

Property	<i>P. pachnodae</i> DSM 12657 ^T	<i>Xylanibacterium</i> <i>ulmi</i> XIL08 ^T	<i>Xylanimonas</i> <i>cellulosilytica</i> XIL07 ^T	<i>I. variabilis</i> DSM 10177 ^T	Authentic <i>Promicromonospora</i> species
Shape	Irregular shaped, single or in pairs	Small rods	Coccoid	Short rods, V-shaped or coccoid	Short rods, V- or Y-shaped or coccoid
Mycelium	Mycelia-like fringes	–	–	Primary mycelium	Mycelium
Aerial hyphae	–	–	–	–	+
Fermentation	+	+	w	+	Rarely
Peptidoglycan composition	L-Lys-L-Ser-D-Glu	L-Lys-L-Ala-D-Glu	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-L-Ala-D-Glu*
Cell-wall sugars	Rha, Gal, Glc	Rha, Fuc, Man, Gal, Ara, Glc	Gal, Rha	Rha, Gal, Glc	Rha, Gal, Glc or Glc, Gal, Xyl
Phospholipid composition	PG, DPG, PI, 3 PLs	PG, DPG, PI, PIM	PG, DPG, PI, PIM	PG, DPG, PI, 2 PLs	PG, DPG, 2 GLs, 2 or 3 PGLs, PL or PG, DPG, PI
Major menaquinones	MK-9(H ₄)	MK-9(H ₄), MK-8(H ₄)	MK-9(H ₄), MK-8(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Predominant fatty acids	ai-C _{15:0} , i-C _{15:0} , C _{16:0} , C _{14:0}	ai-C _{15:0} , i-C _{15:0} , C _{16:0} , C _{14:0}	ai-C _{15:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{15:0} , C _{14:0} , C _{16:0} , i-C _{16:0} , ai-C _{17:0}	ai-C _{15:0}

*Conflicting reports on the peptidoglycan type are given in the literature: L-Lys-Ala₂ (Kalakoutsii *et al.*, 1989) and Lys-Ala-Glu (Evtushenko *et al.*, 1984).

Table 2. Physiological characteristics of closely related members of the family *Promicromonosporaceae*

Strains: 1, *P. citrea* DSM 43110^T; 2, *P. sukumoe* DSM 44121^T; 3, *P. vindobonensis* V45^T; 4, *P. aerolata* V54A^T; 5, *P. pachnodae* DSM 12657^T; 6, *Xylanibacterium ulmi* XIL08^T; 7, *Xylanimonas cellulosilytica* XIL07^T. The following compounds were used by all strains in API 50 CHE: glycerol, L-arabinose, D-xylose, D-lyxose, galactose, D-fructose, D-mannose, aesculin, cellobiose, sucrose, trehalose, β -gentiobiose and D-turanose. None of the strains utilized dulcitol, inositol, sorbitol, D-raffinose, inulin or L-arabitol. –, Negative; +, positive; ND, not done.

Reactions for:	1	2	3	4	5	6	7
Erythritol, adonitol	–	–	–	+	–	–	–
L-Sorbose	–	–	–	+	–	–*	–
Acetate	–	–	–	–	+	+	–
D-Arabinose, mannitol, D-arabitol, methyl α -D-mannoside, methyl β -xyloside	+	+	+	+	–	–	–
Salicin, glycogen, D-fucose	+	+	+	+	–	–	+
Starch	+	+	+	+	–	ND	+
Ribose	–	+	+	+	–	+	–
L-Xylose	–	–	+	–	–	–	–
Rhamnose	+	–	+	+	–	–	+
Methyl α -D-glucoside, melezitose	+	–	–	–	–	–	–
N-Acetylglucosamine	–	–	+	+	+	–*	–
Arbutin, L-fucose	–	+	+	+	–	–	+
Melibiose	+	+	–	+	–	–	–
Amygdalin, lactose, maltose, tagatose	+	+	+	+	+	–	+
Xylitol	–	–	–	–	–	+	–
5-Ketogluconate	–	–	+	+	–	+	+
Gluconate, 2-ketogluconate	–	–	+	+	–	+	–

*Reactions for L-sorbose, N-acetylglucosamine and gluconate differ from those given in the species description of *Xylanibacterium ulmi* (Rivas *et al.*, 2004).

The reclassification is also based on morphological criteria. Similarities in polar lipids, fatty acid composition, isoprenoid composition (Table 1) and base composition of DNA are family-specific rather than genus-specific characters, whereas physiological differences are species-specific (Table 2). The generic name *Xylanimicrobium* is suggested for the novel combination in order to highlight the phylogenetic similarity of the xylanase-producing type strain DSM 12657^T (Cazemier *et al.*, 1999) to members of the genera *Xylanimonas* and *Xylanibacterium*.

Description of *Xylanimicrobium* gen. nov.

Xylanimicrobium (Xy.la.ni.mi.cro'bi.um. N.L. neut. n. xylan xylan a polysaccharide; Gr. adj. mikros small; Gr. masc. n. bios life; N.L. neut. n. *Xylanimicrobium* xylan-hydrolysing microbe).

The description is based on the original description of the species *Promicromonospora pachnodae* DSM 12657^T and data from Cazemier *et al.* (2003) and our own data. Gram-positive, non-spore-forming, irregular rod-shaped cells, non-motile, occurring singly or in pairs. Aerial mycelium not formed. The murein contains the amino acids lysine, glutamic acid and serine (D-Lys–L-Ser–D-Glu), belonging to the peptidoglycan type A4 α , variation A11.48. N-glycolylmuramic acid, mycolic acids and hydroxy fatty acids are absent. Whole cell sugars are rhamnose, galactose

and glucose. The main menaquinone is MK9 (H₄). Major fatty acid is aiC_{15:0}. Phospholipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and three unknown phospholipids. Catalase, oxidase and aminopeptidase positive. Xylanolytic; facultative anaerobic, acid is produced from some carbohydrates. DNA G + C content is 70 mol%. Phylogenetically related to *Xylanimonas cellulosilytica*, *Xylanibacterium ulmi* and *I. variabilis*.

The type species is *Xylanimicrobium pachnodae* (Cazemier *et al.* 2004).

Description of *Xylanimicrobium pachnodae* (Cazemier *et al.* 2004) comb. nov.

Xylanimicrobium pachnodae (pach.no'dae. N.L. gen. n. *pachnodae* of *Pachnoda*, referring to the source of the micro-organism, *Pachnoda marginata*).

Basonym: *Promicromonospora pachnodae* Cazemier *et al.* 2004

The description is that of *P. pachnodae* and data included in the publication of Cazemier *et al.* (2003), supplemented with our own data. In the exponential phase of growth, cells are pleomorphic rods (0.4–0.6 $\times$ 0.6–3.0 μ m), whereas, in the stationary phase, spherical cells dominate (diameter 0.5–0.7 μ m). Under aerobic conditions in basal medium with glucose as carbon source, nocardioform mycelia-like

fringes dominate; these forms are absent under anaerobic conditions. In rich media, cells are more homogeneously distributed. Aerobic to facultatively anaerobic, fermenting glucose, xylose, maltose, lactose (weak) and sucrose; fermentation products of glucose or xylose are formate, lactate, ethanol and acetate but not succinate. Aerobically, cells grow with high densities with beach litter, NaOH-pretreated beach litter, xylan, carboxymethylcellulose, cellobiose, glucose, xylose and brain heart infusion medium. Optimum growth at pH 7.5 and 35 °C. Similar pH and temperature optima are found under anaerobic conditions. Nitrate reduction is positive, gelatin is hydrolysed. Major fatty acids are aiC_{15:0} (58.7 mol%), iC_{16:0} (8.5 mol%), C_{14:0} (8.0 mol%), iC_{15:0} (6.8 mol%), C_{16:0} (6.1 mol%) and C_{15:0} (5.5 mol%). iC_{14:0} (3.9 mol%) and aiC_{17:0} (1.9 mol%) occur in smaller amounts. In addition to the reaction indicated in Table 2, the following reactions are observed in the Biolog GP2 microtitre plate substrate panel (only strong positive reactions that are not already listed in Table 2 are listed): glycogen, mannan, Tween 40, N-acetyl-D-glucosamine, amygdalin, D-lactose, D-glucose, lactulose, D-mannose, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, palatinose, D-psicose, D-ribose, D-tagatose, D-trehalose, methyl pyruvate, 2'-deoxyadenosine, inosine, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, fructose 6-phosphate, glucose 1-phosphate and glucose 6-phosphate. Isolated from the intestine of the rose chafer *Pachnoda marginata* (Scarabaeidae, Coleoptera).

The type strain is VPCX2^T (=DSM 12657^T=NCCB 100020^T).

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