Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae

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A Gram-positive, aerobic, spore-forming actinomycete strain, HR1-2^T, was isolated from a peat bog near Gifhorn, Lower Saxony, Germany. Comparative analysis of the 16S rDNA sequence indicated that HR1-2^T was phylogenetically related to members of the family *Micromonosporaceae*, branching adjacent to *Spirilliplanes yamanashiensis*, *Couchioplanes caeruleus*, *Catenuloplanes japonicus* and members of the genus *Micromonospora*. The affiliation to the family was supported by the presence of family-specific 16S rDNA signature nucleotides, DNA G+C content of 70 mol %, peptidoglycan of type A1 γ ' (directly crossed-linked, presence of glycine, alanine, glutamic acid and meso-diaminopimelic acid in the peptide side-chain), menaquinone MK-9(H₄) as the major respiratory lipoquinone, polar lipid composition PII (phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylserine and phosphatidylinositolmannosides) and a glycolyl type of muramic acid. It differed from genera of the family by the lack of arabinose in whole-cell sugars and a unique nucleotide signature stretch between positions 1132 and 1143 (*Escherichia coli* numbering), 5' CAAUUCGGUUG 3'. Morphologically strain HR1-2^T resembles *Micromonospora* species but can be distinguished from them by the lack of arabinose in whole-cell sugars, the presence of 10-methyl C_{17:0} fatty acids and a distinct 16S rDNA sequence. Based on the unique combination of morphological, chemotaxonomic and phylogenetic properties a new genus, *Verrucosispora* gen. nov., is proposed. The type species of this genus is *Verrucosispora gifhornensis* sp. nov., and the type strain of *V. gifhornensis* is strain HR1-2^T (= DSM 44337^T).

Keywords: *Verrucosispora gifhornensis*, *Micromonosporaceae*, 16S rDNA analysis, phylogeny, chemotaxonomy

INTRODUCTION

The family *Micromonosporaceae* has recently been placed in the suborder *Microsporineae* as one of several suborders of the order *Actinomycetales*, subclass *Actinobacteridae*, class *Actinobacteria* (Stackebrandt *et al.*, 1997). The recognition as a suborder was based on the distinct phylogenetic position of members of the family as determined by 16S rDNA analysis as well as the presence of a family-specific pattern of 16S rDNA nucleotides which distinguished its members from all

other actinomycete species. The family comprises the genera *Micromonospora*, *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium* and *Pilimelia* (Koch *et al.*, 1996a) and the recently described genus *Spirilliplanes* (Tamura *et al.*, 1997).

Although most of these genera are phylogenetically closely related (Koch *et al.*, 1996a; Tamura *et al.*, 1997), members of the genera can be distinguished by morphological features such as the presence or absence of sporangia, spore motility, chemotaxonomic properties including fatty acid profiles, chemical composition of cell wall and whole cells and phospholipid types. The decision whether a new isolate should be affiliated to one of the established genera or whether it represents

The EMBL accession number for the 16S rDNA sequence of strain HR1-2^T is Y15523.

a novel genus must therefore take into account the phylogenetic position and the combination of phenotypic properties. In this paper we describe the characteristics and the resulting classification of strain HR1-2^T (T = type strain). On the basis of a unique combination of genotypic and phenotypic characteristics we propose that this organism is placed in a new genus and species of the family *Micromonosporaceae* designated *Verrucosipora gifhornensis*.

METHODS

Bacterial strains and cultural conditions. Strain HR1-2^T was isolated from a peat sample taken in October 1995 from a peat bog near Gifhorn, Lower Saxony, Germany (10°33'E, 52°30'N). Samples were taken from a depth of 20–40 cm after removal of the top peat layer. Ten grams of an aged peat sample, stored at 4 °C for 12 months, were suspended in a flask with sterile tap water to a final volume of 100 ml. Ten millilitres of this suspension was added to another 90 ml of sterile tap water which was repeated to set up a tenfold dilution series to 10⁻⁶. Of the final dilution step, aliquots of 200 µl were spread-plated onto Actinomycete isolation agar, pH 8.2 (Difco), prepared without the addition of glycerol. Following sterilization of the medium, the antibiotics cycloheximide and nystatin were added to a final concentration of 100 µg ml⁻¹ each. After 11 d aerobic incubation at 30 °C, growth of isolate HR1-2^T was indicated by an expanding clear circular zone in the medium. Further incubation for another 17 d allowed development of an orange-red colony which was transferred to fresh Actinomycete isolation agar. Incubation was for 14 d until a well-developed mycelium occurred on the plate and purity of the isolate could be confirmed.

Short-term storage was carried out by inoculating 5 ml tryptic soy broth (Difco), with the addition of 3.0 g l⁻¹ yeast extract prior to autoclaving, with a loopful of cell material for 7 d at 28 °C, shaking. Aliquots of 1 ml of the suspension were thoroughly mixed with 1 ml glycerol and immediately frozen at -70 °C. Long-term storage was carried out at the DSMZ by freeze-drying and storage in liquid nitrogen.

The organisms used for comparison of the physiological and chemotaxonomic properties were: *Spirilliplanes yamashiensis* IFO 15828^T (= DSM 44325^T) (Tamura *et al.*, 1997) and *Micromonospora olivasterospora* DSM 43868^T (Kawamoto *et al.*, 1983).

Determination of physiological characteristics. A pre-culture of isolate HR1-2^T was grown in tryptic soy broth with yeast extract at 28 °C for 7 d. One millilitre of the cell suspension was pelleted by centrifugation. The pellet was washed twice by resuspension and centrifugation in mineral base medium (Stanier *et al.*, 1966) and finally suspended in 10 ml of this medium. Aliquots of 100 µl of this solution were taken to inoculate the following physiological tests.

The temperature and pH ranges for the growth of isolate HR1-2^T were determined in tryptic soy broth with yeast extract. Temperature range tested was from 8 to 60 °C; pH range tested was from 3.0 to 8.5. Carbon source utilization was tested at 35 °C in standard mineral base medium (Stanier *et al.*, 1966) containing 0.2% of the carbon source. The tests were set up daily and allowed to grow for 18 and 15 d, respectively, until no further growth in the test tubes was observed.

Growth was also tested on the International *Streptomyces* Project (ISP) media #1–#7 (Shirling & Gottlieb, 1966) at

35 °C. The utilization of amino acids as nitrogen source was tested as described by Collins *et al.* (1989). Halotolerance was tested by the use of tryptic soy broth (prepared as described above) with the addition of 0, 1, 2, 3, 4, 5, 7 and 10% NaCl, respectively.

Gelatin liquefaction was tested according to the methods of Gerhardt *et al.* (1981) in that plates of tryptic soy broth (prepared as described above) containing 0.4% gelatin were incubated with strain HR1-2^T for 14 d. For the evaluation the plates were flooded with warm (55 °C) 1 M sulfuric acid, saturated with Na₂SO₄. A resulting clear circular zone around the colony indicates digestion of the gelatin.

Peptonization of milk, starch hydrolysis and nitrite production were tested by procedures described by Gordon *et al.* (1973). Cellulose decomposition was tested by the use of two-layer agar plates incubated for 2 weeks. The bottom layer contained the growth medium (tryptic soy broth, prepared as described above), while the top layer consisted of 1.5% water-agar with 0.6% CM-cellulose. Decomposition of the cellulose is indicated by the formation of clear zones around the colonies.

Growth in the presence of different antibiotics was tested according to the procedure of DIN 58940 (Anonymous, 1991). A pre-culture grown for 2 d in tryptic soy broth was streaked out on plates of Müller–Hinton Agar (Oxoid). Disks containing the different antibiotics (Susceptibility discs, Oxoid) were applied to the plates with a Disk Dispenser (Oxoid), incubated for 4 d at 35 °C and the resulting inhibition zones measured.

To determine whether isolate HR1-2^T produces antibiotic substances, a selection of reference strains was streaked out against the isolate. Reference strains were *Escherichia coli* DSM 498, *Saccharomyces cerevisiae* DSM 70449^T, *Streptomyces hygroscopicus* DSM 40632, *Lactobacillus plantarum* DSM 20174^T, *Enterobacter cloacae* DSM 30054^T and *Staphylococcus aureus* subsp. *aureus* DSM 20231^T. With an inoculation loop a 1 cm broad line of strain HR1-2^T was streaked along the diameter of an agar plate of tryptic soy broth with yeast extract and grown for 5 d at 35 °C. The reference strains were then streaked out perpendicular to strain HR1-2^T, as close as 0.5 cm. Further incubation was at 28 °C for 8 d.

Electron microscopy. For scanning electron microscopy, agar blocks with cells grown on ISP#4 were cut out, fixed in 2.5% (w/v) glutaraldehyde and dehydrated in a series of increasing acetone concentrations (10, 30, 50, 70, 90 and 100%). Preparations were dried at the critical point of liquid CO₂, sputter-coated with gold (10 nm) and examined in a Zeiss model DSM 982 scanning electron microscope.

Analysis of chemotaxonomic characteristics. Cell wall, sugar and lipid analysis were carried out by the methods of Schleifer & Kandler (1972), Saddler *et al.* (1991), MacKenzie (1987), Uchida & Aida (1984), Stead *et al.* (1992) Collins *et al.* (1977), Minnikin *et al.* (1975, 1979) and Collins & Jones (1980), as described previously (Groth *et al.*, 1996).

Isolation of DNA, determination of the G + C content and DNA–DNA hybridization. A volume of 300 ml tryptic soy broth was inoculated with isolate HR1-2^T and incubated aerobically at 28 °C until dense growth had occurred. Cells were harvested by centrifugation and disrupted with a French pressure cell. The DNA was purified by chromatography on hydroxyapatite according to the procedure of Cashion *et al.* (1977). G + C contents were determined by

HPLC (Mesbah *et al.*, 1989). DNA–DNA hybridizations for isolate HR1-2^T were carried out by using the thermal renaturation method (DeLey *et al.*, 1970) with modifications as described by Huß *et al.* (1983) and Escara & Hutton (1980) using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter. Renaturation rates were computed by the program TRANSFER.BAS (Jahnke, 1992).

PCR amplification of the 16S rRNA gene and sequence analysis. Genomic DNA was extracted by an enzymic lysis procedure and used for PCR-mediated amplification of the 16S rDNA (Rainey *et al.*, 1996). The purified PCR products were directly sequenced by using previously described protocols (Rainey *et al.*, 1996). The sequence reaction mixtures were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems).

Phylogenetic analysis. The 16S rDNA sequence of isolate HR1-2^T was initially aligned to the sequences of the type species of the class *Actinobacteria* (Stackebrandt *et al.*, 1997) and subsequently with members of the family *Micromonosporaceae* (Koch *et al.*, 1996b; Tamura *et al.*, 1997) using the ae2 editor (Maidak *et al.*, 1997). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms (DeSoete, 1983) were constructed using sequences from different sets of reference strains. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 500 resamplings (Felsenstein, 1985).

RESULTS AND DISCUSSION

Morphology

Isolate HR1-2^T produced a well-developed substrate mycelium on all media, approximately 0.4 µm in diameter. An aerial mycelium or sporangia were not formed. On older parts (approx. 14 d), granular structures on the mycelial surface were observed by scanning electron microscopy. Spores were borne singly from the substrate mycelium having a diameter of 0.8 µm. The spore surface appeared warty in younger states and changed to a hairy appearance with increased age (Fig. 1). Spores were non-motile.

Physiological characteristics

Isolate HR1-2^T grew strictly aerobically. On all the media tested orange colonies, which became brownish with increased age, developed. Best growth was observed on agar plates made with tryptic soy broth. No growth was observed below 20 °C or above 40 °C. Good growth was observed between 30 and 40 °C, with an optimum at 35 °C. The pH range for growth was from 6.5 to 8.2, with an optimum at 7.5.

Good growth was observed on ISP#1 (tryptone-yeast extract broth), ISP#4 (inorganic salts-starch agar), and ISP#5 (glycerol-asparagine agar); moderate growth was observed on ISP#2 (yeast extract-malt extract agar), ISP#6 (peptone-yeast extract iron agar), and ISP#7 (tyrosine agar); no growth was observed on ISP#3. Growth was slow on HV agar (Hayakawa & Nonomura, 1987).

Isolate HR1-2^T grew well between 0 and 2% NaCl,

moderately between 2 and 4%, but failed to grow above 4% NaCl.

The results of further physiological tests are summarized in Table 1. Also included are the literature data of the closest phylogenetic relatives to isolate HR1-2^T as judged by 16S rDNA analysis.

Antibiotic susceptibility

Growth of isolate HR1-2^T was strongly inhibited (diameter of the inhibition zone: 31 to 50 mm) by amikazin (content per disc: 30 µg), bacitracin (10 µg), cephalozin (30 µg), doxycycline (30 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), neomycin (30 µg), polymyxin B (300 µg) and tetracycline (30 µg). Good inhibition (21–30 mm) was observed with aztreonam (30 µg), cefotaxime (30 µg), cephalothin (30 µg), norfloxacin (10 µg), penicillin G (6 µg) and ticarcillin (75 µg). Poor inhibition (10–20 mm) was observed with ampicillin (10 µg), azlocillin (30 µg), chloramphenicol (30 µg), colistin sulfate (10 µg), erythromycin (15 µg), mezlocillin (30 µg) and ofloxacin (5 µg). No inhibition was observed with lincomycin (15 µg), nitrofurantoin (100 µg), oxacillin (5 µg) and pipemidic acid (20 µg).

Production of antibiotic substances

As judged by growth of all reference organisms in the presence of strain HR1-2^T, antibiotic substances were not produced under the conditions employed in this experiment.

Phylogenetic analysis

The almost complete 16S rDNA sequence, comprising 1462 nucleotides [94.8% of the *E. coli* sequence (Brosius *et al.*, 1978)] was determined for strain HR1-2^T. Similarity values were calculated for this strain compared to a representative selection of actinomycete species (not shown). Strain HR1-2^T was found to be more than 95% similar to members of the family *Micromonosporaceae*, the range in which members of the genera of this family are related among themselves (Koch *et al.*, 1996a, b). Of the species of the *Micromonosporaceae*, *M. olivasterospora* (98.0%), *S. yamanashiensis* (97.4%) and *Catenuloplanes japonicus* (97.3%; Yokota *et al.*, 1993) showed the highest 16S rDNA similarity values to strain HR1-2^T. The membership of strain HR1-2^T in the family *Micromonosporaceae* is also supported by the presence of an almost complete set of family-specific signature oligonucleotides (Stackebrandt *et al.*, 1997) in its 16S rDNA sequence. The only deviation is the occurrence of an A–U pair at positions 1133–1141. All other members of the family exhibit a G–C pair at these positions, therefore, this signature is not family-specific and should be excluded from the list of signatures. Screening the 16S rDNA sequence of strain HR1-2^T for the presence of genus-specific signatures revealed a high similarity with members of the genus *Micromonospora* (11 of 12 common signatures; Koch *et al.*,

Fig. 1. Scanning electron microscopic images of isolate HR1-2^T. (a) Young mycelium with smooth surface, partially grown into the agar surface; bar, 20 µm. (b) Overview of older mycelium, spores are already formed, parts of the mycelium show granulous structures on the surface; bar, 5 µm. (c) Mycelium with warty spores (overview); bar, 2 µm. (d) Old mycelium with spores; the mycelial surface developed a granular surface; bar, 2 µm. (e) Young spores in detail; bar, 2 µm. (f) Old spores in detail; the spore surface has become hairy; bar, 1 µm.

Table 1. Physiological properties of strain HR1-2^T and related organisms

++, Good utilization; +, normal utilization; ±, poor utilization; —, no utilization; P, positive; N, negative; ND, not determined.

Characteristic	Strain HR1-2 ^T	<i>Micromonospora olivasterospora</i> *	<i>Spirilliplanes yamanashiensis</i> †
Utilization as carbon source:			
D-Xylose	++	++	+
D-Ribose	—	++	ND
D-Fructose	—	++	—
D-Glucose	++	++	++
D-Galactose	+	++	ND
D-Mannose	±	++	ND
Maltose	+	++	ND
Sucrose	+	++	+
α-Trehalose	+	++	ND
D-Arabinose	++	—	±
L-Rhamnose	—	—	++
L-Sorbose	—	—	ND
Lactose	—	—	ND
α-Melibiose	—	—	ND
Melezitose	—	—	ND
Raffinose	—	—	+
Glycerol	—	—	ND
Dulcitol	—	—	—
myo-Inositol	—	—	ND
Sorbitol	—	—	ND
Mannitol	—	—	++
Salicin	+	—	ND
Utilization as nitrogen source:			
L-Serine	+	++	ND
L-Aspartic acid	+	++	ND
L-Glutamic acid	+	++	ND
L-Histidine	+	++	ND
L-Arginine	+	++	ND
L-Alanine	±	—	ND
L-Valine	±	—	ND
L-Methionine	—	—	ND
L-Phenylalanine	+	—	ND
L-Tryptophan	—	—	ND
Gelatin liquefaction	P	Weak P	P
Peptonization of milk	P	P	N
Starch hydrolysis	P	P	P
Nitrite produced from nitrate	N	P	N
Cellulose decomposition	N	Weak P	N

* According to Kawamoto *et al.* (1983).

† According to Tamura *et al.* (1997).

1996a). The only deviation is a C residue at position 998, which is either a U or a G residue in members of the other genera. However, the C residue at this position is also present in the closest phylogenetic neighbours, *C. japonicus*, *S. yamanashiensis* and *M. olivasterospora* (see below). Strain HR1-2^T exhibits a unique stretch of nucleotides between positions 1132 and 1142 (*E. coli* numbering), which contains a 3 bp helical region (pos. 1132–1134/1140–1142) and a loop

region (pos. 1135–1139), 5' CAAUUCGGUUG 3' (the loop region is underlined), which can be considered to be the 16S rDNA signature for this strain.

Phylogenetic analysis of the 16S rDNA sequence of strain HR1-2^T and members of the family *Micro-monosporaceae* indicated that irrespective of the number and selection of reference organisms chosen, strain HR1-2^T formed an individual subline of descent within

Fig. 2. Phylogenetic tree showing the position of isolate HR1-2^T within the family Micromonosporaceae, based upon 16S rDNA analysis. *M. olivasterospora* (DSM 43868^T) is shown as an individual lineage as this strain was found to be more closely related to strain HR1-2^T than other strains of the genus *Micromonospora*. The number of species of the different genera taken into account is given in parentheses. The sequence of *Streptomyces ambifaciens* served as an outgroup sequence. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 500 bootstrapped trees. The bar represents 1 nucleotide substitution per 100 nucleotides.

the family (Fig. 2). The branching point of this line, as well as those of its phylogenetically closest neighbours, members of *Actinoplanes*, *Catenuloplanes*, *Couchioplanes*, *Micromonospora* and *Spirilliplanes*, changed their position in the 16S rDNA dendrogram without, however, changing the genus affiliation of the species. Low bootstrap values found for the branching points of deeply branching lineages are yet another indication of the uncertainty about the order in which these lineages emerged during evolution. Despite strain HR1-2^T showing the highest 16S rDNA similarity percentage with *M. olivasterospora*, the branching point of strain HR1-2^T indicates that this isolate is not a member of the genus *Micromonospora*. Similar high similarity values were also found for *S. yamanashiensis* and *M. olivasterospora* (97.9%) and between the latter species and *C. japonicus* (97.3%).

DNA base composition and DNA–DNA relatedness

The G + C content of the DNA of isolate HR1-2^T was 70 mol %. To verify the species status of strain HR1-2^T, DNA–DNA hybridization was carried out between

this strain and its two phylogenetically closest relatives, *M. olivasterospora* and *S. yamanashiensis*. The level of DNA–DNA relatedness between isolate HR1-2^T and *M. olivasterospora* and *S. yamanashiensis* was 41.4 and 40.7%, respectively. *M. olivasterospora* and *S. yamanashiensis* showed 30.8% DNA similarity. These values exclude the affiliation of strain HR1-2^T to either species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994) and confirms the isolated phylogenetic position as indicated in Fig. 2.

Chemotaxonomic properties

The decision whether or not strain HR1-2^T constitutes the nucleus of a new genus or whether it should be affiliated to one of the described genera of the family should not be done solely on the basis of the phylogenetic analysis but should include analysis of chemotaxonomic data. The application of this combination of data, together with morphological properties, has been the most successful approach in the delineation of actinomycete genera during the past 20 years (Embley & Stackebrandt, 1994).

Fatty acid profiles. The fatty acid profile of isolate HR1-2^T (Table 2) is characterized by the predominance of iso-C_{15:0}, followed by iso-C_{16:0} and anteiso-C_{17:0}. The predominance of iso-C_{15:0} and iso-C_{17:0} fatty acids over anteiso-C_{15:0} and anteiso-C_{17:0} fatty acids, the occurrence of significant amounts of unsaturated fatty acids and the absence of 10-methyl- and 2-OH-fatty acids matches the diagnostic fatty acid type 2d (Kroppenstedt, 1985). A different composition is found in the phylogenetic neighbours of strain HR1-2^T such as *S. yamanashiensis*, *M. olivasterospora* and *C. japonicus* (Table 3).

Peptidoglycan. The directly cross-linked peptidoglycan of isolate HR1-2^T contained glycine in position 1 of the peptide subunit and *meso*-diaminopimelic acid as the diagnostic amino acid. Isolate HR1-2^T therefore represents peptidoglycan type A1γ' as described by Schleifer & Seidel (1985). This type is represented in all members of the family. The acyl type of muramic acid was glycolyl.

Whole-cell sugars. Characteristic whole-cell sugars are xylose, mannose and minor amounts of ribose, arabinose is absent. An additional sugar of unknown composition was also present. *S. yamanashiensis* and *C. japonicus* also lack arabinose, while the remaining members of the family contain this sugar in whole cells (Table 3).

Isoprenoid quinones. Isoprenoid quinones were MK-9(H₄), MK-9(H₆), MK-10(H₂) and MK-9(H₂) at a peak area ratio of 77:7:5:4. This pattern is common among species of the family.

Polar lipids. The polar lipid pattern was type II (Lechevalier *et al.*, 1977), phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol mannosides, consisting of phosphatidylserine and an un-

Table 2. Chemotaxonomic characteristics of strain HR1-2^T and related organisms

Values less than 1 % not shown. Abbreviations: i, iso; ai, anteiso; A₂pm, diaminopimelic acid; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; 3-*O*-Man, 3-*O*-methylmannose; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; PIM, phosphatidylinositol mannoside; PSer, phosphatidylserine; PI, phosphatidylinositol.

Characteristic	Strain HR1-2 ^T	<i>Micromonospora</i> <i>olivasterospora</i> *	<i>Spirilliplanes</i> <i>yamanashiensis</i> †
Cellular fatty acids			
i-C _{14:0}		1.6	2.7
i-C _{15:0}	31.1	21.2	12.9
ai-C _{15:0}	2.2	2.9	14.6
C _{15:0}	1.0		4.8
i-C _{16:0}	18.6	29.9	12.9
i-C _{16:1}		7.0	
C _{16:0}	1.0		2.6
C _{16:1}	1.0	1.7	1.5
i-C _{17:0}	8.3	3.5	1.2
ai-C _{17:0}	16.0	5.8	5.3
i-C _{17:1}	4.0	6.9	
ai-C _{17:1}		3.1	
C _{17:0}	2.9		15.0
C _{17:1}			17.5
i-C _{18:0}	4.4	1.6	
C _{18:0}			3.3
i-C _{18:1}		1.1	
C _{18:1}	1.8	1.6	2.2
C _{19:0}			2.0
Unidentified			1.5
Sugar composition of whole cells	Man, Xyl, l unknown	Xyl, Ara, Glc	3- <i>O</i> -Man, Man, Gal, Xyl, Glc
Cell wall chemotype‡	II	II	II
Major menaquinones	MK-9(H ₄)	MK-10(H ₄) MK-9(H ₄)	MK-10(H ₄)
Polar lipids	PE, DPG, PIM, PSer, l unknown	PE, DPG, PIM	PI, PE
Peptidoglycan	meso-A ₂ pm	meso-, hydroxy-A ₂ pm	meso-A ₂ pm

* According to Kawamoto *et al.* (1983) and our own investigations.

† According to Tamura *et al.* (1997).

‡ According to Lechevalier & Lechevalier (1970).

known phospholipid, a pattern common among species of the family.

On the basis of the combination of morphological, chemotaxonomic, physiological and phylogenetic factors, strain HR1-2^T is a member of the family *Micromonosporaceae* but can be readily distinguished from the genera presently affiliated to this family (Table 3). Therefore, we propose that strain HR1-2^T (= DSM 44337^T) be classified as the type strain of the type species of the new genus, designated *Verrucosipora* gen. nov. as *Verrucosipora giffhornensis* sp. nov.

Description of *Verrucosipora* gen. nov.

Verrucosipora (Ver.ru'co.si.spo.ra. L. fem. n. *verruca* wart; Gr. n. *spora* a seed; M.L. fem. n. *Verrucosipora* an organism with warty spores).

Gram-positive, non-acid fast, aerobic organism with branching hyphae. Well-developed septate mycelium averaging 0.4 µm in diameter. Non-motile spores borne singly, sessile, or on short or long sporophores. Warty spore surface changes to a hairy appearance with increasing age. Aerial mycelium absent. The peptide side-chain of peptidoglycan contains meso-diaminopimelic acid, glycine, alanine and glutamic

Table 3. Differential characteristics of the genus *Verrucosipora* and related genera

+, Present; —, absent; ND, not determined. Abbreviations: Man, mannose; Xyl, xylose; Gal, galactose; Ara, arabinose.

Genus	DNA G + C content (mol %)	Motility of spores	Sporangium formation	Fatty acid type*	Cell wall type†	Major menaquinone(s)	Phospholipid type‡	Characteristic sugar(s) in whole cells
<i>Verrucosipora</i>	70	—	—	2b	II	MK-9(H ₄)	P11	Man, Xyl
<i>Spirilliplanes</i>	69	+	—	2d	II	MK-10(H ₄)	P11	Xyl, Gal
<i>Actinoplanes</i>	72–73	+	+	2d	II	MK-9(H ₄), MK-10(H ₄)	P11	Ara, Xyl
<i>Dactylosporangium</i>	72–73	+	+	3b	II	MK-9(H ₄ , H ₆ , H ₈)	P11	Ara, Xyl
<i>Pilimelia</i>	ND	+	+	2d	II	MK-9(H ₂ , H ₄)	P11	Ara, Xyl
<i>Micromonospora</i>	71–73	—	—	3b	II	MK-10(H ₄ , H ₆), MK-9(H ₄ , H ₆)	P11	Ara, Xyl
<i>Catenuloplanes</i>	71–73	+	—	2c	VI	MK-9(H ₆), MK-10(H ₈)	P111	Xyl
<i>Couchioplanes</i>	70–72	+	—	2c	VI	MK-9(H ₄)	P11	Xyl, Ara, Gal
<i>Catellatospora</i>	71–72	—	—	ND	II	MK-10(H ₄ , H ₆), MK-9(H ₄ , H ₆)	P11	Ara, Xyl
<i>Glycomyces</i>	71–73	—	—	2c	II	MK-9(H ₄), MK-10(H ₂ , H ₄)	P1	Ara, Xyl

* According to the classification of Kroppenstedt (1985).

† According to the classification of Lechevalier & Lechevalier (1970).

‡ According to the classification of Lechevalier *et al.* (1977).

acid. The peptide side-chains are directly cross-linked (type A1γ'). The acyl type of the muramyl residue is glycolyl. Mannose, xylose and minor amounts of ribose are present in whole-cell hydrolysates. Characteristic phospholipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylserine. The major menaquinone is MK-9(H₄), with minor amounts of MK-9(H₆), MK-10(H₄) and MK-9(H₂). Major fatty acids (> 15% of total) are iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}, with minor amounts (> 4 to < 15%) of iso-C_{17:0}, iso-C_{17:1} and iso-C_{18:0}. Strictly aerobic. Chemo-organotroph. Good growth occurs at temperatures between 30 and 40 °C. The G + C content of the DNA is 70 mol% (determined by HPLC). A member of the family *Micromonosporaceae*. Type species: *Verrucosipora gifhornensis*.

Description of *Verrucosipora gifhornensis* sp. nov.

Verrucosipora gifhornensis (gif'horn.en.sis. L. -ensis ending to denote a locality; M.L. fem. n. *gifhornensis* referring to the city of Gifhorn, adjacent to the peat bog from which the organism was isolated).

The description of morphological, chemotaxonomic and general characteristics are as described for the genus. An orange pigment is produced on all media tested, including ISP media 1, 2, 4, 5, 6 and 7. Gelatin liquefaction and peptonization of milk are positive. Hydrolyses starch. Nitrite not produced from nitrate. Cellulose decomposition is negative. D-Xylose, D-glucose, D-galactose, maltose, sucrose, D-arabinose and α-trehalose are utilized; D-ribose, D-fructose, L-rhamnose, L-sorbose, lactose, α-melibiose, melizitose, raffinose, glycerol, dulcitol, *myo*-inositol and salicin are not utilized. L-Serine, L-aspartic acid, L-glutamic acid, L-histidine, L-arginine and L-phenylalanine are used as nitrogen source. Highly sensitive to a wide range of antibiotics. A stretch of the 16S rDNA sequence between positions 1132–1142 (5' CAAUU-

CGGUUG 3') is the molecular signature of the species. The G + C content of the DNA is 70 mol% (determined by HPLC). The type strain is HR1-2^T (DSM 44337^T).

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