Actinomadura nitritigenes sp. nov., Isolated from Experimental Biofilters

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We describe a new species of the genus *Actinomadura* for four nitrite-producing strains which were isolated from experimental biofilters that had been supplied with ammonia. The whole cells of these strains contain meso-diaminopimelic acid, ribose, madurose, glucose, and galactose. The polar lipids include diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The main menaquinone is MK-9(H₈). The fatty acids include hexadecanoic acid and 10-methyloctadecanoic acid. The guanine-plus-cytosine content of the DNA is 74 mol%. The fatty acid composition, morphology, and physiological reaction profiles of these strains differentiate them from previously described species. The name proposed for the new species is *Actinomadura nitritigenes*.

Treatment of ammonia-containing waste gases with biofilters leads to the production of nitrite and nitrate, which are products of the nitrification process. Accumulation of these nonvolatile end products and the resulting decrease in the pH cause a decrease in filter activity. Accumulation of the more toxic product nitrite is often discussed as an effect of disturbed nitrification. A second possible process, which has not been investigated fully, is the reduction of nitrate under aerobic conditions. Detection and characterization of organisms responsible for this reaction should lead to a better understanding of the nitrogen transformations that occur in biofilters, which is essential for future optimization and control of this economical method of waste gas treatment.

During an examination of heterotrophic bacteria isolated from biofilters used for treatment of ammonia-containing waste gases, we found that some strains were able to reduce nitrate to nitrite in a peptone-meat extract medium under aerobic conditions. Four of these strains produced nonfragmenting substrate and aerial mycelia with short spore chains. These characteristics placed the strains in the sporoactinomycete group as defined by Prauser (32). Initially, the genera of this group were defined on the basis of morphological criteria, in contrast to the second broad group of actinomycetes, the nocardioform bacteria (8). Now, most of the sporoactinomycete genera are clearly defined by chemotaxonomic criteria, such as peptidoglycan structure, cell sugar and quinone contents, composition of the polar lipids, and fatty acid profiles. The morphological and chemotaxonomic characteristics of the strains which we isolated were consistent with the definition of the genus *Actinomadura* (14). The chemotaxonomic, physiological, and morphological properties of these strains led to the proposal of a new species, *Actinomadura nitritigenes*.

MATERIALS AND METHODS

Bacterial strains. Strains L20, L34, L46^T (T = type strain), and L81 were isolated from laboratory scale biofilters which contained tree bark compost as the packing material. Strain L81 was isolated from a biofilter supplied with ammonia, and strains L20, L34, and L46^T were isolated from a biofilter supplied with dimethyl disulfide and ammonia. The strains were isolated on DNB agar (0.03 g of meat extract per liter, 0.05 g of peptone from meat per liter [31]) and were subsequently cultivated on oatmeal agar (ISP 3 agar [33]) and GYM agar (ISP 2 agar [33]) which was supplemented with 0.2% CaCO₃. All media were incubated for 2 weeks at 30°C.

Colony characterization. The following media were used for colony charac-

terization (33): oatmeal agar, GYM agar supplemented with 0.2% CaCO₃, inorganic salts-starch agar (ISP 4 agar), glycerol-asparagine agar (ISP 5 agar), peptone-yeast extract-iron agar (ISP 6 agar), and tyrosine agar (ISP 7 agar). Inoculated media were incubated at 30°C for 21 days before observations were made. The colors of the mycelia were determined by comparing the mycelia with color chips from the *Color Harmony Manual* (4). Colonial morphology and pigmentation were also determined by the method of Athalye et al. (2) by using glucose-yeast extract agar.

Physiological tests. Degradation of hypoxanthine, degradation of testosterone, degradation of starch, degradation of L-tyrosine, and degradation of xylan were determined as described by Athalye et al. (2). Degradation of adenine, guanine, xanthine, and casein and hydrolysis of esculin were determined by the method of Gordon et al. (9). Sierra medium (34) was used to test for hydrolysis of Tween 20 and Tween 80. Nitrate reduction was determined by the method of Gordon and Mihm (10). H₂S production was tested on ISP 6 medium with lead acetate strips (Merck, Darmstadt, Germany). Resistance to various inhibitors, antibiotics, and temperatures was determined on GYE agar (2). The basal medium of Stevenson (36) was used for carbon source utilization tests. The media used for carbon source utilization tests were inoculated with spore suspensions, and all other media were inoculated with 1 drop of a preculture grown in GYE broth. All test preparations were incubated at 30°C, and results were recorded after 7, 14, 21, and 28 days.

Cultivation for chemotaxonomic analyses. For fatty acid analyses cells were grown in Trypticase soy broth (Becton Dickinson, Heidelberg, Germany) for 15 days at 30°C, harvested by filtration, and washed twice with deionized water. *Actinomadura cremea* DSM 43676^T was incubated at 37°C. For all other analyses cells were grown in nutrient broth (3.0 g of meat extract [Merck] per liter, 5.0 g of peptone from meat [Merck] per liter) at 30°C for 15 days, harvested by filtration, and washed twice with deionized water. For the quinone, polar lipid, and peptidoglycan analyses the washed cells were lyophilized.

Fatty acid analyses. Saponification, methylation, extraction, and identification of fatty acids were performed as described by Kroppenstedt et al. (15) by using a model 5898A microbial identification system (Hewlett-Packard Co., Palo Alto, Calif.). The identities of fatty acids were verified by performing gas chromatography-mass spectrometry with a Hewlett-Packard model 5890 series II gas chromatograph equipped with a 5% phenyl methyl silicone capillary column (0.25 mm by 30 m) and a model 5972 mass selective detector. Helium was used as the carrier gas, the injection volume was 2 µl, the injector temperature was 250°C, the column temperature was increased from 120 to 240°C at a rate of 5°C/min, and the gas chromatography-mass spectrometry transfer line temperature was 280°C. Fatty acid methyl esters were separated on the basis of their retention times and were identified by their mass spectra. The positions of unsaturated fatty acid double bonds were determined by analyzing the dimethyl disulfide adducts (28).

Analyses of isoprenoid quinones and polar lipids. Lipids were extracted by using the small-scale integrated procedure of Minnikin et al. (24). Polar lipids were also analyzed by this method by using two-dimensional silica gel thin-layer chromatography. Menaquinones were analyzed by high-performance liquid chromatography (HPLC) by using the method of Kroppenstedt et al. (15).

Analyses of peptidoglycan and cell sugars. We used the method of Becker et al. (3) to determine the diaminopimelic acid isomer in the cell wall. The acyl type of peptidoglycan was determined by the method of Uchida and Aida (39). For the cell sugar analyses we used whole-cell hydrolysates and the method of Lechevalier (17), as modified by Stanek and Roberts (35).

DNA base composition. The guanine-plus-cytosine content of the DNA was determined by workers at the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, who used the HPLC method described by Mesbah et al. (21).

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TABLE 1. Colonial characteristics of the isolates on various media

Medium	Growth	Reverse color	Aerial mycelium	Soluble pigment
ISP 2 agar	Good	Colorless	Brown mahogany (6pn) ^a	None
ISP 3 agar	Good	Maple sugar (3ie)	Oyster white (b)	None
ISP 4 agar	Good	Clove brown (3ni)	Oyster white (b)	None
ISP 5 agar	Good	Colorless	Maple sugar (3ie)	None
ISP 6 agar	Moderate	Colorless	Colorless	None
ISP 7 agar	Good	Colorless	Brown mahogany (6pn)	None

^a The color codes are the color codes used in the *Color Harmony Manual* (4).

Microscopy. Cells for light microscopy were grown for 2 weeks at 30°C on ISP 2 medium prepared with a slanted cover glass. Mycelia and spore chains were characterized by using an Axioscope instrument (Zeiss, Oberkochen, Germany). The samples used for scanning electron microscopy were 2-week-old mycelia that were grown on oatmeal agar at 30°C, dehydrated with a graded ethanol series, dried by critical point drying with liquid carbon dioxide, and coated with gold. A Cambridge Stereoscan model 250 microscope was used for scanning electron microscopy.

Comparison with reference strains. Three *Actinomadura* species were found to be similar to the new isolates in fatty acid composition and physiological reaction profiles when previously published data were examined. Therefore, the type strains of these species, *Actinomadura atramentaria* DSM 43919, *A. cremea* DSM 43676, and *Actinomadura macra* DSM 43962, were included in this study.

RESULTS

Colony and morphological characterization. Table 1 shows the macroscopic appearance of isolates L20, L34, L46^T, and L81 on various culture media. All four strains exhibited the same growth and colors. Our microscopic examination revealed oval to ellipsoidal spores that had smooth surfaces and were 0.5 to 0.6 by 0.7 to 1.0 µm. The spore chains were up to seven spores long and straight to hook-like (Fig. 1 and 2).

Chemotaxonomy. The four strains were identical for all of the chemotaxonomic markers which we investigated. The fatty acid profiles revealed that hexadecanoic acid (16:0) and tuberculostearic acid (18:0 10-methyl) were the predominant fatty acids, and 2-hydroxyhexadecanoic acid (16:0 2OH) was a characteristic marker. The fatty acid compositions of the isolates examined are shown in Table 2. *meso*-Diaminopimelic acid was the diamino acid found. The sugars detected in whole-cell hydrolysates were ribose, madurose, glucose, and galactose. Diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides were the polar lipids and were identified by their chromatographic and staining properties; no nitrogenous compound was found in any polar lipid pattern. The main isoprenoid quinone was MK-9(H₈); in addition, there were minor amounts of MK-9(H₆) and MK-9(H₄). The peptidoglycan of the four strains was of the acetyl type. The guanine-plus-cytosine content of strain L46^T was 74 mol%.

Physiology. The physiological reaction profiles of the new isolates and reference strains are shown in Table 3. A remarkable feature of the new isolates was the fact that nitrite accumulated in nutrient broth under aerobic incubation conditions. The final nitrite concentration corresponded to the initial nitrate concentration in nutrient broth when 5 mM KNO₃ was added to the medium. The oxygen concentrations of the cultures did not fall below 90% saturation during incubation as determined with a Clarke type of oxygen electrode.

DISCUSSION

Identity of the genus. The presence of *meso*-diaminopimelic acid, the presence of *N*-acetylated muramic acid, the presence of madurose in whole-cell hydrolysates, the presence of a hexahydrogenated menaquinone with nine isoprene units as the main quinone, the lack of any nitrogenous compound in the

polar lipids, and the presence of a fatty acid pattern containing 10-methyloctadecanoic acid are consistent with the definition of the genus *Actinomadura* given by Kroppenstedt and Goodfellow (14). The guanine-plus-cytosine content of isolate L46^T was 74 mol%; the previously published values for the guanine-plus-cytosine contents of members of the genus *Actinomadura* are 65 to 69 mol% (23) and 70 to 78 mol% (2). Our strains could be clearly separated from the related genus *Microtetraspora* by their phospholipid type (type PI, lacking nitrogenous compounds [18]) and from the genus *Nocardiopsis* by their isoprenoid quinone type. Spore chain length distinguished our strains from the genus *Thermomonospora*, which is characterized by the presence of single spores.

FIG. 1. Scanning electron micrographs of a 2-week-old culture of strain L46^T grown on oatmeal agar at 30°C.

FIG. 2. Phase-contrast micrograph of a 2-week-old culture of strain L46^T grown on GYM agar at 30°C. Bar = 20 μm.

Identity of the species. A characteristic marker of our isolates was the presence of 2-hydroxyhexadecanoic acid. According to the results of extensive investigations of Kroppenstedt et al. (15) and other authors (1, 5, 25–27), this marker has been found previously only in *A. atramentaria* DSM 43919^T. However, despite this common marker our isolates can be distinguished from *A. atramentaria* DSM 43919^T by some clear differences. The major differences are the amounts of 14:0, 16:0 iso, and 16:0, the presence of 16:0 10-methyl, and the lack of 16:0 iso 2OH in the fatty acid profiles of the new isolates, as shown in Table 2. Although we found quantitative differences for some straight-chain and 10-methyl fatty acids of the refer-

ence strains between our data and previously published data, the amount of the diagnostic compound 2-hydroxyhexadecanoic acid which we found was in good agreement with previously published data (15, 25). On the basis of the data for this diagnostic marker, the new isolates could not be assigned to any of the reference species included in this study or any species described previously (1, 5, 15, 25–27).

The physiological and morphological properties of the new isolates were compared with the properties of all previously validly described *Actinomadura* species. Selected morphological data are shown in Table 4. These data show that the new isolates could be separated from all previously described species on the basis of spore chain form and length, spore surface type, aerial and substrate mycelium colors, and the lack of any soluble pigment. These differences confirmed the results of the fatty acid analyses. In addition, we found differences in the physiological properties of the new isolates and most of the previously described species, as follows: *A. atramentaria* is negative for growth on D-xylose, L-arabinose, and sucrose (25), negative for growth on galactose, negative for degradation of hypoxanthine, and positive for degradation of adenine (this study); *Actinomadura aurantiaca* is negative for degradation of casein, hypoxanthine, and esculin (23); *Actinomadura citrea* is positive for starch degradation (23), negative for growth in the presence of 0.01% potassium tellurite, positive for growth in the presence of 5% NaCl, and positive for growth on maltose and pyruvate (2); *Actinomadura coerulesa* is negative for esculin degradation (23) and positive for degradation of guanine (14); *A. cremea* is positive for growth on maltose, negative for growth on cellobiose, mannose, galactose, and starch, negative for degradation of hypoxanthine, negative for growth at 45°C, and susceptible to 3 μg of neomycin per ml (2; this study); *Actinomadura echinospora* is negative for nitrate reduction to nitrite and for growth on L-rhamnose (26, 29) and negative for growth at 45°C (29); *Actinomadura fibrosa* is positive for growth in the presence of 5% NaCl, positive for growth on propionate and pyruvate, and negative for nitrate reduction to nitrite (20); *Actinomadura hibisca* is positive for degradation of adenine and negative for growth at 45°C (38); *Actinomadura*

TABLE 2. Fatty acid profiles of the new isolates and reference strains

Fatty acid ^a	% in:			
	Strains L20, L34, L46 ^T , and L81		<i>A. atramentaria</i> DSM 43919 ^T	<i>A. cremea</i> DSM 43676 ^T
	Mean	Range		<i>A. macra</i> DSM 43862 ^T
12:0	1.3	0.9–1.7		2.0
14:0 iso	0.5	0.0–1.3	2.2	1.6
14:0	10.9	9.3–13.2	3.8	19.2
15:0	2.0	1.5–2.5	5.8	2.8
16:0 iso	2.2	1.9–2.8	22.7	5.2
16:1 <i>cis</i> 9	5.4	4.2–6.6		2.7
16:0	35.8	33.7–37.4	16.8	47.1
16:0 10-methyl	2.2	2.0–2.4		1.3
17:1 <i>cis</i> 9	0.7	0.4–1.0	2.8	
16:0 iso 2OH			4.4	
17:0	1.2	0.7–1.8	8.6	5.9
16:0 2OH	4.0	3.6–4.4	3.3	1.6
17:0 10-methyl	0.9	0.5–1.2	4.9	2.2
18:0 iso	0.3	0.0–0.3	5.1	0.9
18:1 <i>cis</i> 9	11.2	9.6–13.0	4.7	5.5
18:0	3.7	2.8–4.8	6.0	7.0
18:0 10-methyl	17.2	16.3–18.4	8.9	15.6
				7.6

^a Examples of abbreviations for fatty acids: 16:0, hexadecanoic acid; 18:0 10-methyl, 10-methyloctadecanoic acid (tuberculosteric acid); 18:1 *cis*9, *cis*-9-octadecenoic acid; 16:0 2OH, 2-hydroxyhexadecanoic acid.

TABLE 3. Colonial characteristics and physiological reaction profiles of the new isolates and reference strains

Characteristic	Strains L20, L34, L46 ^T , and L81 ^a	<i>A. cremea</i> DSM 43676 ^T	<i>A. atramentaria</i> DSM 43919 ^T	<i>A. macra</i> DSM 43862 ^T
Colonial characteristics				
Aerial mycelium sparse	0	+	—	—
Aerial mycelium moderate	0	—	—	+
Aerial mycelium abundant	100	—	+	—
Colonies white, cream, or yellow	100	+	+	+
Diffusible pigment brown	0	—	+	—
Colony elevation crateriform	100	+	+	—
Colony elevation irregular	0	—	—	+
Colony margin entire	0	+	—	+
Colony margin filamentous	100	—	+	—
Formation of:				
Nitrite from nitrate	100	+	+	+
H ₂ S	0	+	—	+
Degradation of:				
Casein	100	+	+	+
Esculin	100	+	+	—
Hypoxanthine	100	—	—	—
Starch	0	—	—	—
Tween 20	100	+	+	+
Tween 80	100	+	+	+
L-Tyrosine	50	+	—	—
Testosterone	100	+	+	—
Adenine	0	—	+	—
Guanine	0	—	—	—
Xanthine	0	—	—	—
Xylan	0	—	—	—
Growth in the presence of:				
Adenine (0.4%, wt/vol)	0	—	—	—
Crystal violet (0.0001%, wt/vol)	100	—	+	+
Crystal violet (0.001%, wt/vol)	0	—	—	—
Phenol (0.01%, wt/vol)	100	+	+	+
Phenol (0.1%, wt/vol)	0	—	—	—
Potassium tellurite (0.001%, wt/vol)	100	+	+	+
Potassium tellurite (0.005%, wt/vol)	100	+	+	+
Potassium tellurite (0.01%, wt/vol)	100	+	+	+
Sodium azide (0.001%, wt/vol)	100	+	+	+
Sodium azide (0.01%, wt/vol)	50	—	—	—
Sodium azide (0.02%, wt/vol)	0	—	—	—
Sodium chloride (3%, wt/vol)	100	+	+	—
Sodium chloride (5%, wt/vol)	0	+	—	—
Sodium chloride (7%, wt/vol)	0	—	—	—
Thallous acetate (0.0001%, wt/vol)	100	+	+	+
Thallous acetate (0.001%, wt/vol)	75	—	+	—
Thallous acetate (0.01%, wt/vol)	0	—	—	—
Gentamicin (4 µg/ml)	0	—	—	—
Gentamicin (16 µg/ml)	0	—	—	—
Streptomycin (4 µg/ml)	0	—	—	+
Streptomycin (16 µg/ml)	0	—	—	+
Neomycin (3 µg/ml)	100	—	+	+
Neomycin (10 µg/ml)	50	—	—	—
Growth on the following compounds as sole carbon sources (1%, wt/vol)				
Glucose	100	+	+	—
Arabinose	100	+	—	—
Cellobiose	100	—	+	—
Fructose	100	+	+	—
Galactose	100	—	—	+
Glycerol	100	+	+	+
Lactose	0	—	+	—
Maltose	0	+	—	—
Mannose	100	—	+	—
Rhamnose	100	+	+	—
Starch	100	—	—	—
Sucrose	100	+	—	+
Trehalose	100	+	+	—
Xylose	100	+	—	—

Continued on following page

TABLE 3—Continued

Characteristic	Strains L20, L34, L46 ^T , and L81 ^a	<i>A. crema</i> DSM 43676 ^T	<i>A. atramentaria</i> DSM 43919 ^T	<i>A. macra</i> DSM 43862 ^T
Growth on the following compounds as sole carbon sources (0.01%, wt/vol)				
L-Alanine	0	—	—	—
L-Proline	100	+	—	—
Sodium propionate	0	—	—	—
Protocatechuic acid	75	+	—	—
Sodium pyruvate	0	—	—	—
L-Serine	0	—	—	—
L-Tyrosine	100	+	—	—
Growth at 45°C	100	—	+	—

^a The values are the percentages of positive strains. Strain L46^T was negative for degradation of L-tyrosine, negative for growth in the presence of 0.01% sodium azide and 0.001% thallos acetate, resistant to 10 µg of neomycin per ml, and positive for growth on protocatechuic acid.

kijaniata is positive for growth on maltose, positive for hydrolysis of adenine, xanthine, xylan, and starch, resistant to gentamicin and streptomycin (11), and negative for degradation of testosterone (23); *Actinomadura libanotica* is positive for growth on maltose, positive for hydrolysis of starch, negative for degradation of casein and hypoxanthine, negative for growth at 45°C and for growth on mannose, fructose, and glycerol (22), positive for growth on L-alanine and propionate, positive for growth in the presence of 0.001% crystal violet, and susceptible to 3 µg neomycin per ml (2); *Actinomadura*

livida is negative for degradation of hypoxanthine and positive for degradation of guanine (14); *Actinomadura luteofluorescens* is positive for starch degradation, positive for growth on lactose, maltose, L-alanine, pyruvate, and L-serine, negative for growth at 45°C, and resistant to 3 µg of gentamicin per ml and 16 µg of streptomycin per ml (2); *A. macra* is positive for H₂S formation, negative for growth at 45°C, negative for growth on arabinose, rhamnose, fructose, and mannose (12), negative for degradation of esculin and testosterone, and resistant to 16 µg of streptomycin per ml (this study); *Actinomadura madurae* is

TABLE 4. Morphological characteristics of strain L46^T and previously described *Actinomadura* species

Species or strain	Spore chains		Spore surface ^a	Interspace pads	Color of aerial mycelium on ^b :				Color of substrate mycelium on ^b :				Color of soluble pigment on ^b :				Reference(s)
	Form ^c	No. of spores			ISP 2 agar	ISP 3 agar	ISP 4 agar	ISP 5 agar	ISP 2 agar	ISP 3 agar	ISP 4 agar	ISP 5 agar	ISP 2 agar	ISP 3 agar	ISP 4 agar	ISP 5 agar	
<i>A. atramentaria</i>	str	3–5	sm	— ^d	wh	wh	wh	wh	br	cl	cl	br	br	—	—	br	25
<i>A. aurantiaca</i>	ho-sp	4–8	wa	+	wh	cr-pi	wh	wh	ye-br	ye	cr-or	—	—	—	—	—	23
<i>A. carminata</i>	psp	6–14	sm	—	—	pi	—	—	—	re-vi	—	—	—	—	—	—	7
<i>A. citrea</i>	ho	3–9	wa	—	—	wh-bl	ye-bl	—	ye-bl	ye	ye-br	—	—	ye	ye	—	23
<i>A. coerulea</i>	ho-sp	—	wa	—	—	pi-bl	bl	—	br	cl	cl	—	—	—	—	—	23
<i>A. crema</i>	ho-sp	—	wa	—	wh	wh-cr	pi	—	br	cl	cl	—	—	—	—	—	23
<i>A. echinospora</i>	—	2	spi	—	—	ye-pi	—	—	br	ye-or	tr	tr	ye-br	ye-br	—	—	14, 29
<i>A. fibrosa</i>	—	—	—	—	wh	pi	pi	—	br	or	br	or	br	—	—	—	20
<i>A. fulvescens</i>	sp	—	sm	—	—	—	—	—	—	cl	—	ye	—	—	—	—	37
<i>A. hibisca</i>	str	10–50	sm	—	wh	—	—	wh	re	—	—	cl-pi	re	—	—	ye-pi	38
<i>A. kijaniata</i>	sp	>10	sm	—	wh	wh	—	—	gr	gr	gr	gr	—	—	gr	gy-br	11
<i>A. libanotica</i>	ho	5–12	wa	+	pi	wh-pi	—	—	ye-br	ye-br	—	—	—	—	—	—	22
<i>A. livida</i>	ho-sp	—	irr	—	—	tr	—	—	br	gy-br	ye-br	—	—	vi	vi	—	23
<i>A. luteofluorescens</i>	ho	—	wa	—	wh-ye	ye-bl	tr	—	ye	ye-gr	or-br	—	ye	ye-gr	ye	—	23
<i>A. macra</i>	str-ho	4–15	sm	—	wh-gy	cr-pi	—	cr	bk	cr-pi	cl	cr	br	—	—	—	12
<i>A. madurae</i>	ho-sp	3–12	wa	—	—	tr	—	—	pi-br	cl	wh-gy	—	—	—	—	—	23
<i>A. oligospora</i>	ho	10–50	sm	—	—	wh	wh	—	gy	gy	gy	gy	br	—	—	—	19
<i>A. pelletieri</i>	ho-sp	2–6	wa	—	tr	—	—	—	pi-br	pi-br	—	—	—	—	—	—	23
<i>A. rubra</i>	sp	2–20	spi	—	—	gr-bl	—	—	—	or	—	—	—	—	—	—	14
<i>A. rugatobispora</i>	—	2	ru	—	—	—	—	—	—	—	—	—	—	—	—	—	26
<i>A. spadix</i>	psp	5–10	sm	—	—	—	—	—	br	gy-br	gy-br	gy-br	br	gy-br	—	gy-br	30
<i>A. umbrina</i>	—	—	sm	—	—	wh	—	—	—	cl	—	tr	—	pi-br	—	—	6
<i>A. verrucospora</i>	ho	5–15	wa	—	—	bl	gy	—	ye	tr	pi-or	pi-or	—	—	—	—	30
<i>A. vinacea</i>	str	—	irr	—	—	—	wh-gy	—	br-re	pi-re	cl	—	re	—	pi	—	23
<i>A. viridis</i>	str	4	sm	—	—	—	—	—	—	—	—	—	—	—	—	—	27
<i>A. yumanensis</i>	ho	~20	sm	—	—	wh	wh	wh	ye-br	gy-ye	cl	ye	or	—	—	ye	16
L46 ^T	str-ho	2–7	sm	—	br	wh	wh	br	cl	br	br	cl	—	—	—	—	—

^a sm, smooth; wa, warty; spi, spiny; irr, irregular; ru, rugose.

^b bk, black; bl, blue; br, brown; cl, colorless; cr, cream; gr, green; gy, gray; or, orange; pi, pink; re, red; vi, violet; wh, white; ye, yellow; tr, trace.

^c str, straight; ho, hooked; sp, spiral; psp, pseudosporangia.

^d —, absent; +, present.

positive for growth in the presence of 5% NaCl, positive for growth on maltose, and positive for starch hydrolysis (2); *Actinomadura oligospora* is negative for growth on galactose, sucrose, arabinose, mannose, trehalose, and rhamnose, negative for nitrate reduction to nitrite, and negative for testosterone degradation (19); *Actinomadura pelletieri* is negative for testosterone degradation, negative for growth on arabinose, glycerol, L-rhamnose, starch, sucrose, trehalose, and xylose, susceptible to 3 µg of neomycin per ml, and positive for growth in the presence of 0.001% crystal violet (2); *Actinomadura rugatobispora* is positive for starch hydrolysis and negative for growth on D-xylose (26); *Actinomadura spadix* is positive for starch hydrolysis (23) and negative for degradation of testosterone and hypoxanthine (14); *Actinomadura verrucosospora* is negative for growth at 45°C, negative for nitrate reduction to nitrite (30), and positive for guanine hydrolysis (20); *Actinomadura vinacea* is negative for nitrate reduction to nitrite and negative for degradation of testosterone and hypoxanthine (23); *Actinomadura viridis* is positive for growth in the presence of 5% NaCl and negative for nitrate reduction to nitrite (27); and *Actinomadura yumanensis* is positive for growth on maltose, pyruvate, and propionate, positive for degradation of starch and xanthin, and negative for growth on arabinose, L-rhamnose, and D-xylose (16).

Recently, the conclusions based on chemotaxonomic, physiological, and morphological data were confirmed by the sequence of 450 nucleotides from the 5' end of the 16S rRNA of isolate L46^T (13). Comparisons with the sequences of *A. marduae* DSM 43067^T, *A. atramentaria* DSM 43919^T, *A. verrucosospora* DSM 43358^T, *A. luteofluorescens* DSM 40398^T, and *Thermomonospora ferrosensis* DSM 43997^T, resulted in levels of homology of less than 96%. Thus, strain L46^T could not be assigned to one of these species. Assignment to the genus *Actinomadura* was confirmed by the sequence data (12a).

Description of *Actinomadura nitritigenes*. We propose the name *Actinomadura nitritigenes* (ni.tri.i'ge.nes. L. n. *nitritum*, nitrite; Gr. v. suff.-genes, producing; L. adj. *nitritigenes*, nitrite producing) for isolates L20, L34, L46^T, and L81. The type strain is strain L46. *A. nitritigenes* forms branching vegetative and aerial mycelia. Spores are produced in straight or hook-like chains that are up to seven spores long. The spore surface is smooth. The aerial mycelium is white. No soluble pigment is produced. Degrades casein, esculin, Tween 20, Tween 80, hypoxanthine, and testosterone. Does not degrade adenine, guanine, xanthine, and xylan and does not form H₂S. Nitrate is reduced to nitrite even under aerobic incubation conditions. Grows on glucose, arabinose, cellobiose, fructose, galactose, glycerol, mannose, rhamnose, starch, sucrose, trehalose, xylose, L-proline, and L-tyrosine. Does not grow on lactose, maltose, L-alanine, propionate, pyruvate, and L-serine. Grows at 45°C. Contains *meso*-diaminopimelic acid, an acetyl type of peptidoglycan, and madurose. Phospholipid type PI. MK-9(H₆) is the major menaquinone. The major fatty acids are hexadecanoic acid and tuberculostearic acid; minor amounts of 2-hydroxyhexadecanoic acid occur. Aerobic and mesophilic. All strains were isolated from experimental biofilters which were filled with tree bark compost as filter material and supplied with ammonia. Isolate L46^T (= DSM 44137^T) has been deposited in the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

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