40 The Family *Segniliparaceae*

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Abstract
Segniliparaceae, a family within the order *Actinomycetales* and suborder *Corynebacterineae*, comprises the sole genus *Segniliparus* with two species. The two type strains have been isolated from human sputum and are described to be opportunistic pathogens. They are aerobic, mesophilic, and chemoorganotroph. The most distinctive characteristic is the presence of ultralong C60–C100 mycolic acids. These are non-oxygenated α-mycolates with high levels of *cis* unsaturation, a feature solely present on *Segniliparus* species Hong (PLoS One 7: e39017, 2012). The type strain of *Segniliparus rotundus* is CDC 1076^T (=ATCC BAA-972^T = CIP 108378^T = DSM 44985^T) and of *Segniliparus rugosus* CDC 945^T (=ATCC BAA-974^T = CIP 108380^T = DSM 45245^T). Of each species, further strains have been isolated, mainly from the human habitat.

Taxonomy, Historical and Current

Short Description of the Family

Segniliparaceae Butler et al. 2005

Segniliparaceae (Seg'ni.li.par.a'ce.ae. N.L. masc. n. *Segniliparus* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Segniliparaceae*, the *Segniliparus* family).

Members of the family are non-spore forming and nonmotile. The genus *Segniliparus* is the only genus in the family with the two species *Segniliparus rotundus* and *S. rugosus* (Butler et al. 2005). The family belongs to the suborder *Corynebacterineae*, order *Actinomycetales*, subclass *Actinobacteridae*, class *Actinobacteria*, and phylum Actinobacteria (Garrity and Holt 2001; Ludwig et al. 2012; Stackebrandt et al. 1997; Zhi et al. 2009). The cells are rod-shaped without any branching. They are aerobic, strongly acid-fast, and produce multiple chemical functional groups of high-molecular-mass, nonpolar, mycolic acids.

Molecular Analyses

The DNA–DNA association value between the type strains of *Segniliparus rotundus* and *S. rugosus* was <28 % using the hydroxyapatite method with an optimum reassociation temperature of 70 °C (Butler et al. 2005). A complete genome sequence has been obtained for *Segniliparus rotundus* (Sikorski et al. 2010) and a high-quality draft genome sequence for *S. rugosus* has been published by Earl et al (2011), revealing genome sizes of 3.16 and 3.64 megabases and a DNA coding region of 92.3 % and 86.4 %, respectively. The number of genes associated with the general COG functional categories is similar in both strains; however, there is a larger proportion of genes not in COGs in *S. rugosus* (46.1 %) compared to *S. rotundus* (41.3 %) (Earl et al. 2011; Sikorski et al. 2010).

Phylogenetic Structure of the Family and its Genus

The 16S rRNA gene sequences of the two type strains of the genus *Segniliparus* differ by only 1.1 % (Sikorski et al. 2010) (*Fig. 40.1*). The next closest relatives of the genus *Segniliparus*, as based on 16S rRNA gene sequences, are the members of the genus *Rhodococcus*, family of *Nocardiaceae*, which share 93.3–94.8 % 16S rRNA genes sequence similarity with strain *Segniliparus rotundus* CDC 1076^T (Ludwig et al. 2012; Sikorski et al. 2010). A BLAST survey against the nucleotide database identified only very few entries at a similarity above 93 %, e.g., as obtained from wastewater (Del Casale et al. 2011), suggesting a rather limited ecological distribution of the genus *Segniliparus*.

Phenotypic Analyses

Segniliparus Butler et al. 2005

Segniliparus (Seg.ni.li.pa'rus. L. adj. segnis slow; Gr. adj. liparos fat/fatty; N.L. masc. n. *Segniliparus* the slow fatty one, the one

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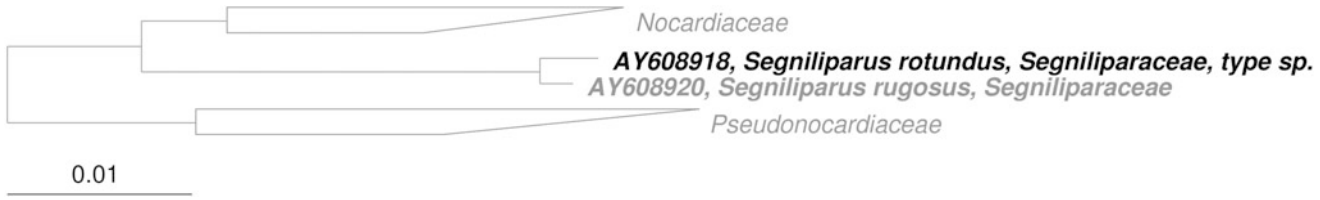

Fig. 40.1

Phylogenetic reconstruction of the family *Segniliparaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Table 40.1

Phenotypic and chemotaxonomic characteristics of the type strains of *Segniliparus rotundus* and *S. rugosus*. Both strains are nonmotile. The range and optimum of NaCl and pH for growth, the presence of oxidase and polar lipids, the assimilation of aliphatic hydrocarbons, the resistance to radiation, and the peptidoglycan type were not determined

Properties	*Segniliparus rotundus* CDC 1076^{T} [a, b]	*Segniliparus rugosus* CDC 945^{T} [a, c]
Morphology	Similar-sized short rods	Irregular-sized rods
Pigmentation	Nonpigmented	Nonpigmented
Gram stain	nd, most probably negative	nd, most probably negative
Temperature for growth (°C)		
Range	28–37	22–42
Optimum	33	33
Metabolism	Aerobic	Aerobic
Reduction of nitrate	–	Variable
Presence of		
Catalase	+	+
Diagnostic peptidoglycan amino acids[d]	*meso*-dpm	*meso*-dpm
Major fatty acids	Saturated straight chain, tuberculostearic acid	Saturated straight chain, tuberculostearic acid
Major respiratory lipoquinone[e]	MK-8(H_4)/MK-8(H_2), few MK-8(H_6), traces of MK-9(H_2)	nd
G+C content (mol%)	66.8	68.1

Symbols: + positive; – negative; *nd* not determined
[a]Butler et al. 2005
[b]Sikorski et al. 2010
[c]Earl et al. 2011
[d]*meso*-dpm, *meso*-diaminopimelic acid
[e]MK, menaquinone

with slow fats, to indicate the possession of slowly reacting fatty acids, i.e., late-eluting mycolic acids detected with HPLC). The genus comprises two species, *Segniliparus rotundus* and *S. rugosus*.

The main features of the sole genus in the family *Segniliparaceae* are listed in *Table 40.1*. *Segniliparus* species form nonmotile rod-shaped cells with 0.4–0.9 μm width and 1.0–4.5 μm length. The cells are aerobic and acid–alcohol fast. Spores or aerial mycelium have not been observed, though the cells are occasionally V-shaped but not with true branching (Butler et al. 2005). The cells grow in 3–4 days on days on Löwenstein–Jensen (LJ), Middlebrook 7H10, and Middlebrook 7H11 agar at an optimal temperature of 33 °C and yield nonpigmented, non-photochromogenic colonies that do not produce a diagnostic odor. The growth on heart infusion (HI) agar is poor. The cells show arylsulfatase activity but are negative for niacin production. A semiquantitative catalase test produces bubbles of >45 mm (Butler et al. 2005). A definite range of salinity tolerance is not known, but growth tolerance with

sodium chloride on LJ and American Trudeau Society (ATS) media in 14 days is described to be positive (Butler et al. 2005). Urea is hydrolyzed but acetamide, adenine, casein, citrate, esculin, hypoxanthine, tyrosine, and xanthine are not. D-glucose, maltose, and trehalose are used as carbon sources and produce acid, whereas adonitol, L-arabinose, cellobiose, dulcitol, i-erythritol, galactose, i-myoinositol, lactose, mannose, melibiose, raffinose, L-rhamnose, salicin, and sodium citrate are not (Butler et al. 2005). Utilization of D-fructose, glycerol, D-mannitol, D-sorbitol, and sucrose is variable. The API CORYNE test kit numerical profile is 2040000 and revealed that the two species are positive for β-glucosidase and pyrazinamidase activities but negative for alkaline phosphatase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and pyrrolidonyl arylamidase activity at 33 °C. The antimicrobial susceptibility patterns have been determined from several strains of both species using serial twofold broth microdilution assays and are listed in detail in *Table 40.2*. The species have the same fatty acid composition, with prominent fatty acids of $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, and tuberculostearic acid (Butler et al. 2005). The quinones are mainly MK-8(H_4) and MK-8(H_2) with some MK-8(H_6) and traces of MK9(H_2) (Sikorski et al. 2010). The species do not produce a *Rhodococcus equi*-specific ChoE virulence factor (Butler et al. 2005). The gas–liquid chromatography thermal cleavage product of the mycolic acids is a $C_{24:0}$ acid–methyl ester. The high-performance liquid chromatography mycolic acid pattern consists of three late-emerging groups of peaks with the final peak co-eluting with the high-molecular-mass internal standard. Thin-layer chromatography demonstrates three nonpolar α^+ (C_{84}-C_{100}) -, α (C_{73}-C_{83}) -, and α' (C_{60}-C_{66}) – mycolic acid chemical functional groups. These ultralong non-oxygenated α-mycolates with high levels of *cis* unsaturation are a special and sole phenotypic characteristic of *Segniliparus* species. Overall 65 homologous mycolic acids have been observed in *Segniliparus*. The overall length of the mycolic acids, which is among the longest lipids known in cell biology, is distinctly atypical of rapid growing mycolata (Hong et al. 2012; Lanéelle et al. 2013).

Isolation, Enrichment, and Maintenance Procedures

The type strain of *S. rotundus* was isolated from human sputum in a public health laboratory in Tennessee, USA, in 2005 or before (Butler et al. 2005). The type strain of *S. rugosus* was isolated from human sputum in a public health laboratory in Alabama, USA, in 1998 (Earl et al. 2011). Information of case histories on the type strains is not available. Several other strains have been isolated from humans, for some of them also case histories have been published (Butler et al. 2007; Hansen et al. 2009; Koh et al. 2011).

Strains of the genus *Segniliparus* do not require special procedures for maintenance and long-term storage. Can be stored frozen at –24 °C in appropriate medium or water containing 43 % glycerol and in liquid nitrogen in appropriate medium or water containing 5 % dimethylsulfoxide without loss of viability. Long-term preservation is by lyophilization with 20 % skin milk.

Ecology

Habitat

The type strains of the genus *Segniliparus* have been isolated from human sputum in 2005 or before (Butler et al. 2005; Earl et al. 2011). Further strains have been isolated from patients with cystic fibrosis (*S. rugosus*, most probably USA, but also in Australia) and bronchiectasis (*S. rotundus*, South Korea), from sputum, bronchus, or nasal samples (Butler et al. 2007; Hansen et al. 2009; Koh et al. 2011). The presence of *S. rugosus* in *Ixodes ricinus* ticks was identified by denaturating gradient gel electrophoresis (DGGE) of 16S rRNA gene amplicons and subsequent sequencing of the DGGE band. The ticks were collected in Sunnmøre, Norway, in May/June/September 2010 both as host-seeking ticks and feeding ticks picked from cats and dogs (Tveten and Sjåstad 2011). This suggests that transmission of *S. rugosus* between mammalian hosts can take place via ticks (Tveten and Sjåstad 2011). Further isolates of *S. rugosus* have been obtained from a subadult female California sea lion (*Zalophus californianus*) stranded on the beach of San Onofre, California, USA, in April 2010 (Evans 2011). Though in environmental databases hardly any 16S sequences related to *Segniliparus* are present, this finding addresses the question of whether *S. rugosus* could be free-living in the oceans or part of the flora of any number of ocean-dwelling vertebrates or invertebrates (Evans 2011; Sikorski et al. 2010).

Pathogenicity, Clinical Relevance

Although both *S. rugosus* and *S. rotundus* are officially classified to belong to risk group 1 (TRBA 2010), occasionally members of the species are suspected to behave as opportunistic pathogens in immunocompromised humans. This appears to be specifically true for humans suffering from cystic fibrosis (CF) (Butler et al. 2007; Hansen et al. 2009) and lung diseases such as tuberculosis and bronchiectasis (Koh et al. 2011). Hence, it is supposed that *Segniliparus* species can cause pneumonia in patients with bronchiectasis (Koh et al. 2011). Clinically, the CF cases exhibited a marked and rapid decline in lung function and radiologic studies over a short period of time which was not characteristic of CF or infections usually associated with this disease (Butler et al. 2007). However, the public health significance of *Segniliparus* species is still unclear. Also other mammalians such as sea lions may be affected by *Segniliparus* species (Evans 2011). Potentially, members of *Segniliparus* may be transmitted via ticks (Tveten and Sjåstad 2011), but may also originate from an environmental source (Butler et al. 2007).

Table 40.2

Antimicrobial susceptibility patterns of *Segniliparus rotundus* and *S. rugosus* isolates. The numbers are minimal inhibitory concentrations in μg/ml determined by using a microbroth dilution assay

	S. rotundus ATCC BAA-972Ta (human sputum, USA)	*S. rotundus* CIP 108380$^{T\ b}$ (human sputum, USA)	*S. rotundus* ATCC BAA-973[a] (human nasal, USA)	*S. rotundus*[b] (human sputum, Korea)	*S. rugosus* ATCC BAA-974Ta (human sputum, USA)	*S. rugosus* CIP 108378^{Tb} (human sputum, USA)	*S. rugosus* ATCC BAA-975[a] (human bronchus, USA)	*S. rugosus* MO 1714[a] (human sputum, USA)	*S. rugosus* MB 549[a] (human BAL, USA)	*S. rugosus* AS 513[a] (human BAL, USA)	*S. rugosus*[c] (human sputum, Australia)
Amikacin (AMK)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Amoxicillin clavulanic acid (AMC)	2/1	nd	4/2	nd	64/32	nd	>64/32	>64/32	> 64/32	32/16	64/32
Ceftriaxone (CEF)	4	nd	16	nd	64	nd	>64	>64	>64	64	>64
Ciprofloxacin (CIP)	≤0.25	≤0.125	≤0.12	1	16	>16	4	8	8	2	4
Clofazimine (CLO)	0.5	nd	1	nd	2	nd	2	>2	>2	>2	nd
Clarithromycin (CLR)	4	4	8	2	32	64	64	>64	>64	>64	16
Ethambutol (EMB)	>16	>32	>16	>32	>16	>32	>16	>16	>16	>16	nd
Cefoxitin (FOX)	16	2	32	>256	64	8	256	>256	>256	128	64
Gatifloxacin (GAT)	2	nd	0.5	nd	8	nd	8	2	8	1	0.5
Imipenem (IMP)	2	≤0.5	2	16	2	4	4	2	2	1	1
Linezolid (LZD)	2	nd	2	nd	64	nd	>64	64	32	32	64
Minocycline (MIN)	≤0.5	nd	≤0.5	nd	>32	nd	>32	>32	>32	>32	>32
Moxifloxacin (MOX)	≤0.12	≤0.125	≤0.5	1	0.5	≤0.125	8	8	8	2	nd
Rifabutin (RFB)	0.25	nd	1	nd	0.5	nd	1	2	2	1	nd
Rifampin (RIF)	≤0.25	≤0.125	0.5	>16	>16	>16	>16	>16	>16	>16	nd
Streptomycin (STR)	8	nd	16	nd	>128	nd	>128	128	>128	>128	nd
Sulfamethoxazole (SMX)	4	16	4	>128	4	>128	8	8	32	8	nd
Tigecycline (TIG)	nd	nd	1	nd	>2	nd	>2	>2	>2	>2	nd
Trimethoprim-sulfamethoxazole (SXT)	≤0.25/4.8	nd	≤0.25/4.8	nd	≤0.25/4.8	nd	0.5/9.5	0.5/9.5	2/38	≤0.25/4.8	2/38
Tobramycin (TOB)	>64	>32	64	>32	>64	>32	>64	>64	>64	>64	>64

nd not determined, *BAL* bronchoalveolar lavage

[a]Butler et al. 2007

[b]Koh et al. 2011

[c]Hansen et al. 2009

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