26 The Family *Xanthobacteraceae*

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Abstract
The family *Xanthobacteraceae*, established in 2005 on the basis of 16S rRNA phylogeny, is affiliated with the *Alphaproteobacteria*. Currently (May 2012) it encompasses 7 genera (*Xanthobacter* [type genus], *Ancylobacter*, *Azorhizobium*, *Labrys*, *Pseudolabrys*, *Pseudoxanthobacter*, and *Starkeya*) and 28 species. All members grow as aerobic chemoheterotrophs, but facultative chemolithoautotrophy with hydrogen and/or reduced sulfur compounds is found in many species. Nitrogen fixation is widespread; the genus *Azorhizobium* entirely consists of N_2-fixing symbionts that live in association with leguminous plants. Some species can grow on unusual substrates such as alkenes, halogenated aliphatic and aromatic compounds, terpenes, thiophenes, or polyaromatic compounds. Representatives of the family can be found worldwide in freshwater lakes and streams, soils, wetlands, and in polluted sites.

Taxonomy, Historical and Current

Family *Xanthobacteraceae* Lee, Liu, Anzai, Kim, Aono, and Oyaizu 2005, 1916[VP]

Xan.tho.bac.te.ra'ce.ae. N.L. masc. n. *Xanthobacter*, type genus of the family; suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Xanthobacteraceae*, the *Xanthobacter* family.

Gram-negative, rod-shaped chemoorganotrophic or facultatively chemolithoautotrophic bacteria, motile or nonmotile. Do not form spores. Some species fix N_2. The major isoprenoid quinone is Q-10. The family *Xanthobacteraceae* was circumscribed on the basis of phylogenetic analysis of 16S rRNA sequences. The family is phenotypically, metabolically, and ecologically diverse.

Type genus: *Xanthobacter*.

The mol% G+C of the DNA varies between 61 and 69.

The family *Xanthobacteraceae* was created in 2005, based on 16S rRNA sequence comparisons (Lee et al. 2005). At the time of writing (May 2012), the family contained 7 genera with 28 species (Tables 26.1–26.7): *Xanthobacter* [type genus] (8 species, the names of 2 of which were thus far effectively but not validly published), *Ancylobacter* (7 species, including one whose name was effectively but not yet validly published), *Azorhizobium* (2 species), *Labrys* (7 species), *Pseudolabrys* (1 species), *Pseudoxanthobacter* (1 species), and *Starkeya* (2 species).

The genus *Xanthobacter* was established by Wiegel et al. (1978) based on numerical taxonomy comparisons of organisms assigned at the time to the genus *Corynebacterium*. The species *Corynebacterium autotrophicum* (Baumgarten et al. 1974) was renamed *Xanthobacter autotrophicus*, and proposed as the type species of the new genus. A comparative study showed that the

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Table 26.1
The genera classified within the family *Xanthobacteraceae*, as of May 2012

Genus	Number of species	Type species	General properties
Xanthobacter [type genus]	8[a]	*Xanthobacter autotrophicus*	Rod-shaped, sometimes twisted or pleomorphic cells. Refractile (polyphosphate) and lipid (poly-β-hydroxybutyrate) bodies are evenly distributed in the cells. Aerobic, with a strictly respiratory type of metabolism. Colonies are opaque and generally slimy, yellow due to the presence of zeaxanthin dirhamnoside. Nearly all strains can grow chemolithoautotrophically in mineral media under an atmosphere of H_2, O_2, and CO_2, as well as chemoorganoheterotrophically on methanol, ethanol, n-propanol, n-butanol, and various organic acids as carbon sources. The carbohydrate utilization spectrum is limited. Some strains can use substituted thiophenes as sole carbon, energy, and sulfur sources. N_2 is fixed under a decreased O_2 pressure
Ancylobacter	7[a]	*Ancylobacter aquaticus*	Curved rods. Rings are occasionally formed prior to cell separation. Cells are encapsulated. Some strains produce gas vesicles. Generally nonmotile or motile by means of a single polar flagellum. Obligately aerobic with a strictly respiratory type of metabolism. Colonies are white to cream colored. Chemoorganotrophic, using a variety of sugars or salts of organic acids as carbon sources. Chemolithotrophic growth has been reported on molecular hydrogen. Some strains are facultatively methylotrophic, using methanol and formate
Azorhizobium	2	*Azorhizobium caulinodans*	Motile, short, rod-shaped cells, showing peritrichous flagella on solid medium and one or more lateral flagella in liquid medium. Obligately aerobic. N_2 is fixed under microaerobic conditions. Among sugars, only glucose is oxidized; the favorite carbon substrates are organic acids such as lactate or succinate. Grow also on malonate and on proline. Starch is not hydrolyzed. Denitrification is not observed. Nodulate the stems and roots of leguminous plants of the genus *Sesbania*
Labrys	7	*Labrys monachus*	Motile or nonmotile cells; may or may not possess triangular radial symmetry. They may have two to three short prosthecae, can be aerobes or facultative anaerobes. Cells divide by budding. Most strains are obligately aerobic, chemoorganotrophic. Use carbohydrates and some organic acids as sole carbon and energy sources. May be facultative methylotrophs
Pseudolabrys	1	*Pseudolabrys taiwanensis*	Non-motile, short, rod-shaped cells that multiply by division and not by budding. Methanol, methylamine, formaldehyde, and formamide are not used as sole carbon sources
Pseudoxanthobacter	1	*Pseudoxanthobacter soli*	Motile, short, rod-shaped cells that accumulate poly-β-hydroxybutyrate as polar inclusion bodies
Starkeya	2	*Starkeya novella*	Nonmotile, short, rod-shaped cells. Strictly aerobic facultative chemolithoautotrophs that grow on thiosulfate and tetrathionate but not on elemental sulfur or thiocyanate. Neutrophilic and mesophilic. Growth is also observed with formate. Some strains may degrade methylated sulfides

[a]Including species whose names have been effectively but not yet validly published

nitrogen-fixing facultative chemolithotrophic isolate known as *Mycobacterium flavum* strain 301 (Federov and Kalininskaya 1961) can be classified within the new genus as *Xanthobacter flavus* (Malik and Claus 1979). Later 16S rRNA sequence comparison confirmed the phylogenetic relationship. Based on 16S rRNA phylogeny, the species *Blastobacter viscosus* 7d (Loginova and Trotsenko 1980) and *Blastobacter aminooxidans* 14a (Doronina et al. 1984) were moved from the genus *Blastobacter* (*Bradyrhizobiaceae*) to the genus *Xanthobacter* as *X. viscosus* and *X. aminoxidans* (Doronina and Trotsenko 2003).

The genus name *Ancylobacter* was proposed by Raj (1983) as a substitute for the genus name *Microcyclus* (Ørskov 1928). The name *Microcyclus* was earlier used for a fungus in 1904, and therefore the name was considered illegitimate, even though it appeared in the Approved Lists of Bacterial Names of 1980. The strain described by Ørskov as the type of *Microcyclus aquaticus* has been lost. Subsequently, new similar strains were isolated by Ørskov, and one of these strains (ATCC 25396) was proposed as the neotype strain of *M. aquaticus*, now *A. aquaticus* comb. nov. (Ørskov 1957; Larkin and Borrall 1979; Staley et al. 2005).

Table 26.2

Comparison of selected characteristics of the members of the genus *Xanthobacter*

Character	*X. autotrophicus* [a, b]	*X. agilis* [b, c]	*X. aminoxidans* [d]	*X. flavus* [b, e]	*X. polyaromaticivorans* [f]	*X. tagetidis* [g]	*X. viscosus* [d]	*X. xylophilus* [a,h]
Basonym	*Corynebacterium autotrophicum*		*Blastobacter aminooxidans*	Formerly assigned to *Mycobacterium flavum* (a name not found in the approved lists of names of 1980)	(A name effectively but not yet validly published)		*Blastobacter viscosus*	(A name effectively but not yet validly published)
Type strain	ATCC 35674	ATCC 43847	ATCC BAA-299	DSM 338	NR	DSM 11105	ATCC BAA-298	VKM B-2535
Cell size (μm)	0.4 × 1.0	0.7 × 1.1–3.6	0.8–1.0 × 1.5–3.5	0.5–0.7 × 1.0–2.5	NR	0.5 × 1.0	0.5–0.9 × 1.0–3.2	0.4 × 0.7
Motility	–	+	–	–	–	+	–	–
Growth at 15 °C	w	+	+	w	–	+	+	+
Growth at 37 °C	w	–	–	w	+	+	–	–
Growth at 45 °C	d, w	–	–	–	–	–	–	–
pH range for growth	5.0–9.0	NR	6.5–8.5	NR	NR	6.8–8.7	6.5–8.5	4.8–6.8
Growth with H_2 as energy source	+	+	+	+	+	+	+	–
Growth with thiosulfate as energy source	+	+	NR	+	NR	+	NR	–
N_2 fixation	+	+	+	+	+	+	+	–
Growth on formate, acetate, propionate, butyrate, pyruvate, succinate, fumarate	+	+	NR	+	+	+	Formate, butyrate, pyruvate: +	NR
α-ketoglutarate	+	+	NR	+	NR	+	+	NR
Methylamines	+	+	+	+	NR	NR	–	NR
Citrate	+	–	–	d	NR	+	–	+
Lactate	+	–	+	+	NR	NR	NR	NR
Malonate	d	–	NR	+	NR	NR	NR	NR
Lactose, sorbose, raffinose, rhamnose	+	–	NR	–	–	–	NR	–
Fructose	+	–	NR	d	–	+	+	–
Glucose	+	–	+	d	–	+	+	–
Mannose	d	–	NR	d	–	+	NR	–
Sucrose	d	–	NR	–	–	+	NR	–

Table 26.2 (continued)

Character	*X. autotrophicus* [a, b]	*X. agilis* [b, c]	*X. aminoxidans* [d]	*X. flavus* [b, e]	*X. polyaromaticivorans* [f]	*X. tagetidis* [g]	*X. viscosus* [d]	*X. xylophilus* [a,h]
Nitrate reduced to nitrite	+	–	+	+	+	W	+	NR
Urease	d	NR	–	–	–	NR	–	NR
G+C content of DNA (mol%)	65–70	68–69	69.1	68–69	65	68–69	66.3	63.6
Sample source and site	Soil, mud, water	Lake water, Switzerland	Sewage purification system of a paper mill, Russia	Turf podzol soil, USSR	Crude oil tank sludge	Roots of *Tagetes* plants	Activated sludge of a paper mill, Russia	Acidic water with decaying spruce wood

Data taken from: [a]Baumgarten et al. (1974); Wiegel et al. (1978)
[b]Wiegel (2005); Wiegel (2006)
[c]Jenni and Aragno (1987)
[d]Doronina and Trotsenko (2003)
[e]Malik and Claus (1979)
[f]Hirano et al. (2004)
[g]Padden et al. (1997)
[h]Zaichikova et al. (2010a)
NR not reported, *w* weak, *d* 11–89 % of the strains are positive
Additional data on growth substrates are given by Wiegel (2005, 2006) and are given in the original species descriptions

Table 26.3

Comparison of selected characteristics of the members of the genus *Ancylobacter*

Character	*A. aquaticus* [a]	*A. abiegnus* [b]	*A. dichloromethanicus* [c]	*A. oerskovii* [d]	*A. polymorphus* [e]	*A. rudongensis* [f]	*A. vacuolatus* [e, g]
Basonym	*Micrococcus aquaticus*	(A name effectively but not yet validly published)					Previously described as *'Renobacter vacuolatum'*
Type strain	ATCC 25396	VKM B-2563	DSM 21507	DSM 18746	DSM 2457	JCM 11671	DSM 1277
Cell size (μm)	0.3–1.0 × 1.0–3.0	0.65–0.9 × 1.35–1.50	0.4–0.5 × 0.7–0.9	0.5–0.6 × 0.9–1.7	0.8–1.0	0.6–0.8	0.8–1.0
Rod morphology	Curved	Cocci	Curved	Pleomorphic	Curved	Curved	Curved
Maximum growth temperature on slant	34	25	37	40	42	40	37
Growth at 3 % NaCl	–	–	–	+	+	+	–
Nitrate reduction	+	NR	–	–	+	–	+
Urease	+	NR	+	+	+	+	+
Autotrophic growth with H_2	+	–	+	–	+	–	+
Utilization of L-fucose	–	–	+	+	–	–	+
Arabinose	–	–	+	+	+	+	+
D-mannose	–	–	NR	+	–	–	–
L-rhamnose	–	–	–	+	–	–	–
D-malate	+	+	–	+	+	W	–
Malonate	+	NR	NR	+	–	+	+
Gluconate	+	+	NR	+	–	–	+
Citrate	+	+	–	–	–	+	–
Oxalate	+	+	NR	+	+	+	+
Dichloromethane	–	NR	+	NR	–	–	–
Gelatin hydrolysis	–	NR	–	–	+	+	+
G+C content of DNA (mol%)	66–69	66.8	64.5	68.0	65.5	68.2	65.5
Sample source and site	Woodlake waters, freshwater ponds	Acidic water with decaying spruce wood	Contaminated soil, chemical plant, Russia	Soil	River mud	Roots of *Spartina anglica*, China	Soil, Russia

Data taken from: [a]Ørskov (1928); Raj (1983)
[b]Zaichikova et al. (2010b)
[c]Firsova et al. (2009)
[d]Lang et al. (2008)
[e]Xin et al. (2006)
[f]Xin et al. (2004)
[g]Nikitin (1971)
w weakly positive, *NR* not reported
Additional data on growth substrates are given by Staley et al. (2005) and are given in the original species descriptions

Table 26.4
Comparison of selected characteristics of the members of the genus *Azorhizobium*

Character	*A. caulinodans*[a]	*A. doebereinerae*[b]
Type strain	LMG 6465	LMG 9993
Cell size (μm)	0.5–0.6 × 1.5–2.5	0.6–0.9 × 1.5
Motility	Peritrichous flagella on solid medium; one lateral flagellum in liquid medium	2–4 lateral flagella
Host	*Sesbania rostrata* (stem)	*Sesbania virgata* (root) can form ineffective root nodules in *S. rostrata*, *Macroptilium atropurpureum* and pseudonodules on *S. rostrata* stems
N_2 fixation	+	+
Growth on glucose	+	–
Sucrose	–	–
Mannitol	–	–
DL-lactate	+	+
L-leucine	+	–
G+C content of DNA (mol%)	66–68	NR
Sample source and site	Root and stem nodules of *Sesbania rostrata*	Root nodules of *Sesbania virgata*, Brazil

Data taken from: [a]Dreyfus et al. (1988)
[b]de Souza Moreira et al. (2006)
NR not reported
Additional data on growth substrates are given by Kuykendall (2005) and are given in the original species descriptions

Ancylobacter vacuolatus (Xin et al. 2006) was earlier described as *Renobacter vacuolatum* (Nikitin 1971).

Taxonomic rearrangement of obligately or facultatively chemolithoautotrophic organisms that oxidize reduced sulfur compounds, earlier classified as members of the genus *Thiobacillus*, led to the removal of *T. novellus* (Starkey 1934) (a member of the *Alphaproteobacteria*) from the genus *Thiobacillus* (*Betaproteobacteria*) and its reclassification as *Starkeya novella* gen. nov., comb. nov. (Kelly et al. 2000).

Phylogenetic Structure of the Family and Its Genera

Figure 26.1 shows a Neighbor Joining tree of the type strains of the 28 species of the family *Xanthobacteraceae*. The family is associated with the *Alphaproteobacteria*, close relatives being the families *Hyphomicrobiaceae*, *Bradyrhizobiaceae*, *Beijerinckiaceae*, *Methylocystaceae*, and *Acetobacteraceae*.

The family *Xanthobacteraceae*, originally with five genera (*Xanthobacter*, *Azorhizobium*, *Ancylobacter*, *Labrys*, and *Starkeya*) was proposed by Lee et al. (2005) based on 16S rRNA comparisons of the members of the *Alphaproteobacteria*. It is interesting to note that in an earlier phylogenetic analysis that did not include *Xanthobacter* spp., *Labrys monachus* did not cluster with *Starkeya novella* (*Thiobacillus novellus*) and with *Ancylobacter aquaticus* (Fritz et al. 2004). The close phylogenetic relationship between *Xanthobacter* and *Azorhizobium* was first reported in 1996 (Rainey and Wiegel 1996; Wiegel 2005). Already then, it was noted that members of *Xanthobacter* and *Azorhizobium* are intermixed in the tree, as seen also in *Fig. 26.1*. A Maximum Likelihood (RAxML) tree constructed (not shown) placed the two species of *Azorhizobium* on a single branch surrounded by members of the genus *Xanthobacter*. Both the Neighbor Joining and the Maximum Likelihood trees place the two *Starkeya* species between the *Ancylobacter* branches.

Genome Analysis

At the time of writing (May 2012), three genome sequences of members of the *Xanthobacteraceae* had been published (*Table 26.8*): the type strain of *Azorhizobium caulinodans* (Lee et al. 2008), the type strain of *Starkeya novella*, and *Xanthobacter autotrophicus* Py2, a strain that can grow on alkenes (van Ginkel and de Bont 1986) and has many interesting physiological and biochemical features as described below. The chromosomes are 4.77–5.37 Mbp in length and contain 4,483–4,847 predicted genes. *X. autotrophicus* Py2 in addition contains a 316-kb plasmid encoding 308 predicted proteins. Of the 4,717 predicted proteins encoded by the 5.37 Mbp genome of *A. caulinodans*, 3.7 % are unique for this organism. Most nodulation functions as well as a putative type-IV secretion system are found in a distinct "symbiosis region" (Lee et al. 2008).

Phages

Wilke and Schlegel (1979) described three phages infecting *Xanthobacter autotrophicus* strain GZ29. Two lytic phages CA1 and CA2 have heads of 61–68 nm diameter and tails of 98–100 and 166–175 nm length, respectively. A third phage designated CA3, with a head diameter of 37–43 nm, a 43–50 nm tail, and a small DNA molecule of 3.3 kDa, did not form plaques and was detected only by its transducing activity and by electron microscopy.

Forty-three plaque-forming phages against the stem-nodulating *Azorhizobium caulinodans* were isolated from rhizosphere soil of different leguminous plant species. They all had a head and short (14–18 nm) non-contractible and non-flexible tails and were assigned to the *Podoviridae* (Sharma et al. 2008).

Table 26.5

Comparison of selected characteristics of the members of the genus *Labrys*

Character	*L. monachus*[a]	*L. methylaminiphilus*[b]	*L. miyagiensis*[c]	*L. neptuniae*[d]	*L. okinawensis*[c]	*L. portucalensis*[e]	*L. wisconsinensis*[f]
Type strain	ATCC 43932	ATCC BAA-1080	NBRC 101365	LMG 23578	DSM 18385	LMG 23412	DSM 19619
Cell size (μm)	1.1–1.5	0.7–1.0 × 1.0–1.2	NR	0.7–0.9 × 1.2–1.5	NR	0.5–1.0 × 0.8–1.0	1.0–1.5 × 2.0-3.0
Motility	+	–	+	–	+	–	+
Temperature range (°C)	5–40	10–35	15–32	15–35	15–32	16–37	10–40
NaCl growth range (%)	0–0.7	NR	0–0.3	NR	0–0.3	NR	0–1.2
Capsule formation	–	+	+	+	+	+	+
Anaerobic growth	–	–	–	–	–	–	+
Oxidase	+	+	NR	–	+	+	+
Catalase	w	+	w	w	w	+	w
Growth on D-ribose	+	NR	NR	NR	NR	NR	+
D-xylose	+	NR	+	NR	+	NR	+
Sucrose	+	+	+	–	+	NR	+
Arabinose	+	NR	+	+	+	+	+
Cellobiose	+	NR	+	NR	+	NR	+
Fructose	+	+	+	+	+	NR	+
Lactose	–	+	NR	–	NR	+	–
G+C content of DNA (mol%)	65–68	65.7	61.0–61.4	62.7	62.3	62.9	NR
Sample source and site	Silt, Lake Mustjarv, Estonia	Sediment, Lake Washington, WA, USA	Grassland soil, Japan	Freshwater pond, Taiwan	Root nodule of *Entada phaseoloides*, Japan	Polluted sediment, Portugal	Water of Lake Michigan, WI, USA

Data taken from: [a]Vasilyeva and Semenov (1984)
[b]Miller et al. (2005)
[c]Islam et al. (2007)
[d]Chou et al. (2007)
[e]Carvalho et al. (2008)
[f]Albert et al. (2010)
w weakly positive, *NR* not reported
Additional data on growth substrates are given by Vasilyeva (2005) and are given in the original species descriptions

Table 26.6
Comparison of selected characteristics of the members of the monospecific genera *Pseudolabrys* and *Pseudoxanthobacter*

Character	*Pseudolabrys taiwanensis* [a]	*Pseudoxanthobacter soli* [b]
Type strain	CCUG 51779	DSM 19599
Cell size (μm)	NR	0.2 × 2.0
Motility	–	+
Mode of reproduction	Division	Budding
N_2 fixation	NR	+
Temperature range (°C)	15–37	10–37 (opt. 37)
pH range	4.2–8.5	5.5–10.0 (opt. 7.0)
Growth on methanol or methylamine	–	–
Formaldehyde, formamide	–	
D-ribose	NR	+
Fumarate	+	+
L-malate	w	+
DL-lactate	+	NR
3-hydroxybenzoate	+	–
Predominant quinones	Q-10	Q-10, Q-9, and Q-8
G+C content of DNA (mol%)	67	68.4
Sample source and site	Soil, Taiwan	Soil, Taiwan

Data taken from: [a]Kämpfer et al. (2006)
[b]Arun et al. (2008)
w weakly positive, *NR* not reported
Additional data on growth substrates are given in the original species descriptions

Table 26.7
Comparison of selected characteristics of the members of the genus *Starkeya*

Character	*S. novella* [a]	*S. koreensis* [b]
Basonym	*Thiobacillus novellus*	
Type strain	ATCC 8093	KCTC 12212
Cell size (μm)	0.4–0.8 × 0.8–2.0	0.4–0.8 × 0.8–2.0
Cell morphology	Short rods, coccoidal, or ellipsoidal	Highly curved rods, rings
Motility	–	–
Temperature optimum (°C)	25–30	28–30
pH optimum	7.0–8.0	6.5–8.0
Growth with 3 % NaCl	w	–
Growth on thiosulfate or tetrathionate	+	+
Formate	+	+
Methanol	+	+
Methylamine	–	–
Sucrose	–	–
Maltose	–	–
Citrate	–	+
Malate	–	–
Succinate	–	–
G+C content of DNA (mol%)	67.9	69
Sample source and site	Soil	Rice straw, S. Korea

Data taken from: [a]Starkey (1934), Kelly et al. (2000)
[b]Im et al. (2006)
w weakly positive, *NR* not reported
Additional data on growth substrates are given by Kelly and Wood (2005) and are given in the original species descriptions

Phenotypic Analyses

The Properties of the Genera and Species of *Xanthobacteraceae*

Phenotypically the members of the family *Xanthobacteraceae* are quite diverse. With the other members of the *Alphaproteobacteria*, they share a Gram-negative type of cell wall, presence of ubiquinone Q-10 as the major respiratory quinone (with Q-9, Q-8, and Q-11 sometimes found in minor amounts), and other chemotaxonomic traits such as the types of fatty acids present. Predominant polyamines are putrescine and *sym*-homospermidine, as characterized in *Xanthobacter autotrophicus* 7c and CB2, *Azorhizobium caulinodans*, and *Labrys wisconsinensis* (Hamana et al. 1990; Wiegel 2006; Albert et al. 2010). They are all aerobes, although *Labrys wisconsinensis* was described as a facultative anaerobe as growth was obtained anaerobically on plate count broth supplemented with 0.075 % agar (Albert et al. 2010). Chemolithoautotrophic growth is widespread within the family, with hydrogen and/or reduced sulfur compounds serving as electron donor and energy source. Another property found in many of the genera is the ability to fix N_2.

Genus *Xanthobacter* Wiegel, Wilke, Baumgarten, Opitz, and Schlegel 1978, 580[AL]

Xan.tho.bac'ter Gr. adj. *xanthos*, yellow; N.L. masc. n. *bacter*, rod, staff; N.L. masc. n. *Xanthobacter*, yellow rod.

Rod-shaped, sometimes twisted cells, 0.4–1.0 × 0.8–6.0 μm, nonmotile or motile by peritrichous flagella. Gram-negative type of cell wall. Pleomorphic cells are sometimes produced on media containing succinate and other tricarboxylic acid cycle intermediates, whereas coccoid cells as well as cells up to 10 μm long are produced on media containing an alcohol as the sole

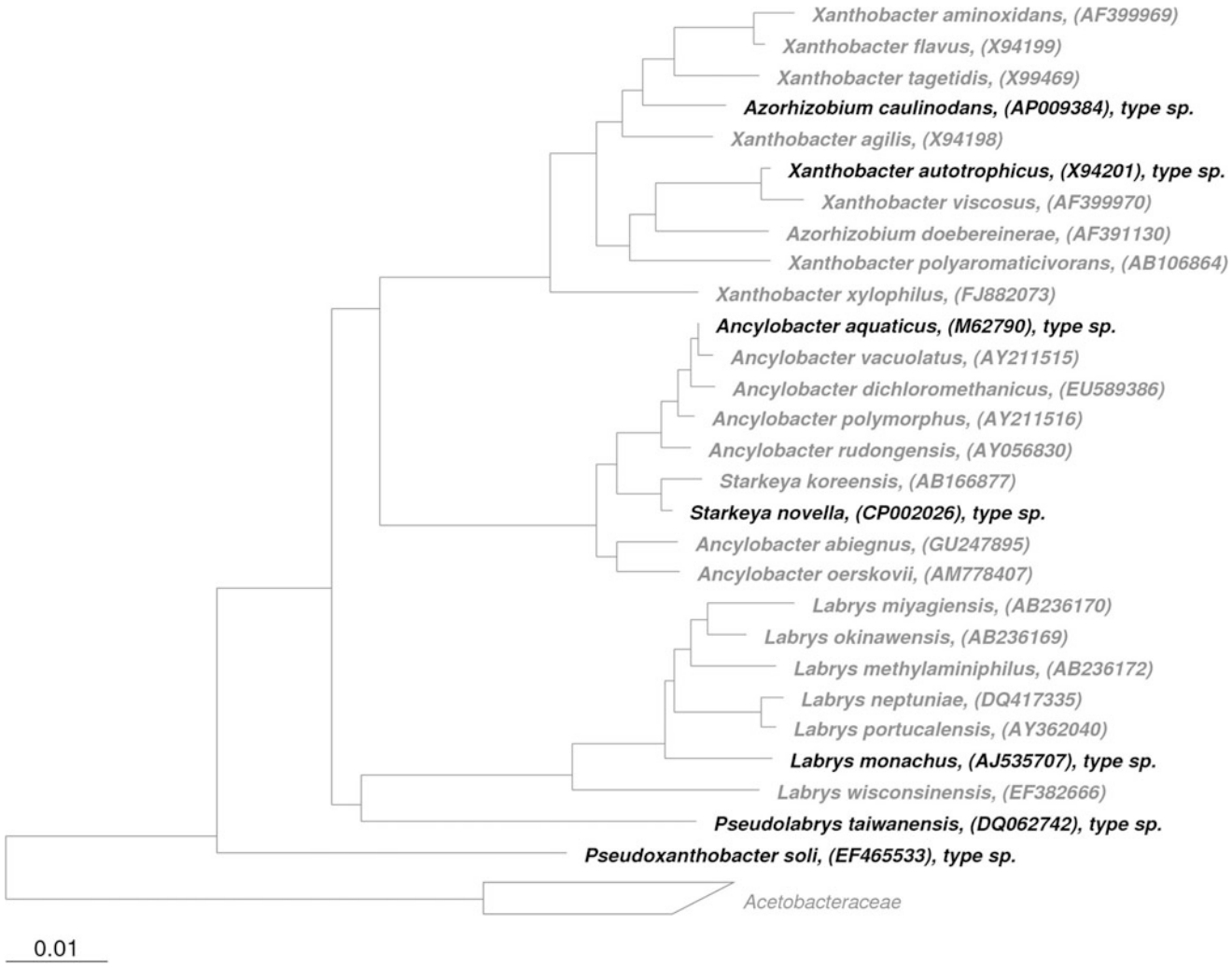

Fig. 26.1

Phylogenetic reconstruction of the family *Xanthobacteraceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment 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

carbon source. Refractile (polyphosphate) and lipid (poly-β-hydroxybutyrate) bodies are evenly distributed in the cells. Due to polyphosphate granules, the Gram reaction frequently appears falsely to be positive or variable. Aerobic, with a strictly respiratory type of metabolism. Neutrophilic and mesophilic. Colonies are opaque and generally slimy, yellow due to the presence of zeaxanthin dirhamnoside. Most strains can grow chemolithoautotrophically in mineral media under an atmosphere of H_2, O_2, and CO_2, as well as chemoorganoheterotrophically on methanol, ethanol, n-propanol, n-butanol, and various organic acids as carbon sources. The carbohydrate utilization spectrum is limited. Some strains can use substituted thiophenes as sole carbon, energy, and sulfur sources. When degrading aliphatic epoxides, tested strains contain coenzyme M, which otherwise is a typical coenzyme of the obligate anaerobic methanogenic archaea. N_2 is fixed under a decreased O_2 pressure.

The mol% G+C of the DNA is 65–70.

Type species: *Xanthobacter autotrophicus*.

The genus *Xanthobacter* currently contains eight species: *X. autotrophicus*, *X. agilis*, *X. aminoxidans*, *X. flavus*, *X. polyaromativorans* (a name effectively but not yet validly published), *X. tagetidis*, *X. viscosus*, and *X. xylophilus* (a name effectively but not yet validly published). The main features of the members of the genus *Xanthobacter* are summarized in *Table 26.2*.

Table 26.8
Properties of the sequenced genomes of members of the *Xanthobacteraceae* (as of May 2012)

Property	*Xanthobacter* sp. Py2[a]	*Azorhizobium caulinodans* ORS571^{T} [b]	*Starkeya novella* DSM 506^{T}
Accession number	NC_009720 (chromosome) NC_009717 (plasmid)	AP009384	NC_014217 CP002026
Genome length (bp)	5,308,934 (chromosome)	5,369,772	4,765,023
G+C content	67.5 (chromosome)	67	67.9
Extrachromosomal elements	Plasmid pXAUT01 (316,164 bp; G+C content 65.3)	0	NR
% Coding bases	NR	89	NR
Number of predicted genes	4,847 (chromosome) 308 (plasmid)	~4,779	4,483
Predicted protein-coding genes	NR	4,717	4,431
% of proteins with putative function	NR	3,588	NR
Number of 16S rRNA genes	2	3	1

[a]For the description of the isolate, see van Ginkel and de Bont (1986)
[b]Data taken from Lee et al. (2008)
NR not reported

Additional comments:

- Irregular twisted cells (*X. tagetidis*) and branched cells (*X. autotrophicus*, *X. flavus*) are commonly found during growth on tricarboxylic acid cycle intermediates. Branching cells do not show septa at the branching points (Wiegel 2005).
- Most members of the genus multiply by symmetric division, but *X. viscosus* and *X. aminoxidans* reproduce by budding (Doronina and Trotsenko 2003). *X. polyaromaticivorans*, a species that degrades polycyclic and heterocyclic aromatic compounds, is atypical as it shows no autotrophic growth (Hirano et al. 2004).
- *X. flavus* was originally described as nonmotile. However, reexamination of the type strain showed peritrichous flagella in exponential cultures grown on methanol, ethanol, *n*-propanol, isopropanol, butanol, or gluconate, but not in media containing citrate, fumarate, malate, succinate, glutamate, glutamine, yeast extract, or in cells growing autotrophically on $H_2 + CO_2$ (Reding et al. 1992).
- Cellular fatty acids are high in $C_{18:1}$ and include $C_{18:0}$, 3-OH $C_{16:0}$, and (in *X. agilis*) $C_{19:0}$ cyclo ω7*c* (Urakami et al. 1995).
- *X. polyaromaticivorans* is slightly pinkish-orange, probably due to a zeaxanthin with terminal substitutions different from zeaxanthin dirhamnoside (Wiegel 2006).
- *X. xylophilus*, isolated from acidic low-salinity dystrophic water with decaying spruce wood, is moderately acidophilic, and uses organic acids (citrate, oxalate, succinate, gluconate), as well as xylose and xylan (Zaichikova et al. 2010a).
- *X. tagetidis* is associated with the roots of marigold (*Tagetes*) plants, which accumulate high concentrations of thiophenes. It grows on substituted thiophenes, and also is a facultative autotroph that can grow on H_2, thiosulfate, or sulfide as electron donors. It fixes CO_2 using ribulose bisphosphate carboxylase/oxygenase (Padden et al. 1997).
- All strains of *X. autotrophicus* and *X. flavus* produce an α-polyglutamine capsule located between the cell wall and the slime (Wiegel 2005).
- Two methanol degrading isolates 25P and 32P, classified as strains of *X. autotrophicus*, were described by Doronina et al. (1996).

Genus *Ancylobacter* Raj 1983, 397^{VP}

An.cy.lo.bac'ter. Gr. adj. *ankulos*, crooked, curved; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Ancylobacter*, a curved rod.

Curved rods, 0.3–1.0 × 1.0–3.0 μm. Rings (0.9–3.0 μm outer diameter) are occasionally formed prior to cell separation. Cells are encapsulated. Some strains produce gas vesicles. Gram-negative. Generally nonmotile or motile by means of a single polar flagellum. Obligately aerobic with a strictly respiratory type of metabolism. Colonies are white to cream colored. Oxidase and catalase positive. Chemoorganotrophic, using a variety of sugars or salts of organic acids as carbon sources. Chemolithotrophic growth has been reported on molecular hydrogen. Some strains are facultatively methylotrophic, using methanol and formate.

The mol% G+C of the DNA is 66–69.

Type species: *Ancylobacter aquaticus*.

The genus *Ancylobacter* currently contains seven species: *A. aquaticus*, *A. abiegnus* (a name effectively but not yet validly published), *A. dichloromethanicus*, *A. oerskovii*, *A. polymorphus*, *A. rudongensis*, and *A. vacuolatus*. The main features of the members of the genus *Ancylobacter* are summarized in *Table 26.3*.

Additional comments:

- Overviews of the biology of *Ancylobacter* were published by Raj (1989) and by Staley et al. (2005).

- Staley et al. (2005) consider the type strain of *A. aquaticus* to be avacuolate, despite a claim to the contrary (Raj 1977).
- Autotrophic growth of several strains was reported with hydrogen (Namsaraev and Nozhevnikova 1978; Malik and Schlegel 1981) or with thiosulfate as energy source (Stubner et al. 1998). CO_2 fixation is mediated by ribulose bisphosphate carboxylase/oxygenase (Loginova et al. 1978; Firsova et al. 2009).
- *A. dichloromethanicus*, isolated from contaminated soil, uses dichloromethane, methanol, formate, formaldehyde, and a range of larger carbon sources (Firsova et al. 2009).
- The major fatty acid of *A. aquaticus* is $C_{18:1}$; further present $C_{16:0}$, $C_{18:0}$ $C_{19:0}$ cyclo. The major respiratory quinone is Q-10, with minor amounts of Q-9 and Q-11 (Urakami and Komagata 1986).

Genus *Azorhizobium* Dreyfus, Garcia, and Gillis 1988, 97[VP]

A.zo.rhi.zo'bi.um. N.L. n. *azotum* [from Fr. n. *azote* (from Gr. prep. *a*, not; Gr. n. *zôê*, life; N.Gr. n. *azôê*, not sustaining life)], nitrogen; N.L. pref. *azo-*, pertaining to nitrogen; N.L. neut. n. *Rhizobium*, a bacterial generic name; N.L. neut. n. *Azorhizobium*, a nitrogen (using) *Rhizobium*.

Motile, short, rod-shaped Gram-negative cells, showing peritrichous flagella on solid medium and one or more lateral flagella in liquid medium. Obligately aerobic. N_2 is fixed under microaerobic conditions when nicotinic acid is provided. Oxidase- and catalase-positive; urease-negative. Among sugars, only glucose is oxidized; the favorite carbon substrates are organic acids such as lactate or succinate. Grow also on malonate and on proline. Starch is not hydrolyzed. Denitrification is not observed. Nodulate the stems and roots of leguminous plants of the genus *Sesbania*.

The mol% G+C of the DNA of the type species is 66.

Type species: *Azorhizobium caulinodans*.

The genus *Azorhizobium* currently contains two species: *A. caulinodans* and *A. doebereinerae*. The main features of the members of the genus *Azorhizobium* are summarized in *Table 26.4*.

Additional comments:

- Overviews of the properties of nitrogen-fixing stem nodules of *Sesbania* and the biology of *Azorhizobium* were published by Dreyfus and Dommergues (1981), Dreyfus et al. (1984), Goormachtig et al. (1998), and Kuykendall (2005).
- *A. doebereinerae* was isolated from root nodules of Brazilian woody species *Sesbania virgata*. Its colonies on YMA agar are similar to those of *A. caulinodans*: scant extracellular polysaccharide, fast to intermediate growth rate, and causing alkalinization of the medium. Neither *A. caulinodans* nor *A. doebereinerae* use mannitol or sucrose, compounds used by most *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Bradyrhizobium* spp. However, they can use DL-lactate. *A. doebereinerae* differs from *A. caulinodans* as it does not use either L-leucine or D-glucose (de Souza Moreira et al. 2006).

Genus *Labrys* Vasilyeva and Semenov 1985, 375[VP] (Effective Publication: Vasilyeva and Semenov 1984, p. 92 (Russian edition)); Emended Islam, Kawasaki, Nakagawa, Hattori and Seki, 2007, 556; Emended Albert, Waas, Langer, Pavlons, Feldner, Rosselló-Mora and Busse, 2010, 1757)

La'brys. N.L. masc. n. *Labrys* (from Gr. n. *labrus*), double-headed ax, an organism resembling a double-headed ax by the shape of the cell.

Motile or nonmotile cells; can be rod-shaped, and may or may not possess triangular radial symmetry. They may have two to three short prosthecae and can be aerobes or facultative anaerobes. Cells divide by budding. Buds are produced directly from the mother cell at the tip of the triangle that lacks prosthecae. In this stage, the cell resembles a double-headed ax. Most strains are aerobic, chemoorganotrophic. Use carbohydrates and some organic acids as sole carbon and energy sources. Oxidase and catalase positive. May be facultative methylotrophs. The primary polar lipids are diphosphatidylglycerol, phosphatidylmonomethylethanolamine, and phosphatidylcholine. The predominant fatty acids are $C_{19:0}$ cyclo ω8*c*, $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ω7*c*. The major ubiquinone is Q-10, and the major polyamine is *sym*-homospermidine.

The mol% G+C of the DNA is 61–68.

Type species: *Labrys monachus*.

The genus *Labrys* currently contains seven species: *L. monachus*, *L. methylaminiphilus*, *L. miyagiensis*, *L. neptuniae*, *L. okinawensis*, *L. portucalensis*, and *L. wisconsinensis*. The main features of the members of the genus *Labrys* are summarized in *Table 26.5*.

Additional comments:

- *L. neptuniae* was isolated from root nodules of the pan-tropical aquatic legume *Neptunia oleracea* (Chou et al. 2007).
- *L. portucalensis* is a fluorobenzene-degrading bacterium obtained from industrially contaminated sediment in northern Portugal. Fluorobenzene is used as a solvent in the pharmaceutical industry, as an insecticide, and as a reagent for plastic and resin polymers production (Carvalho et al. 2005, 2008).
- *L. okinawensis* and *L. miyagiensis* are budding bacteria isolated from rhizosphere habitats in Japan (Islam et al. 2007).
- *Labrys methylaminiphilus* was isolated from freshwater Lake Washington sediment following enrichment on

methylamine. It also uses a variety of mono- and disaccharides, organic acids, aromatic compounds, and alcohols. Methanol is not used. It also grows on polymers such as agarose and humic acid (Miller et al. 2005).
– Some species accumulate considerable amounts of poly-β-hydroxybutyrate (Vasilyeva 2005).

Genus *Pseudolabrys* Kämpfer, Young, Arun, Shen, Jäckel, Rosselló-Mora, Lai, and Rekha 2006, 2470[VP]

Pseu.do.la'brys. Gr. adj. *pseudês* false; N.L. masc. n. *Labrys* a bacterial genus name; N.L. masc. n. *Pseudolabrys* the false *Labrys*.

Nonmotile, short, rod-shaped Gram-negative cells that multiply by division and not by budding. Methanol, methylamine, formaldehyde, and formamide are not used as sole carbon sources. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$, and $C_{19:0}$ cyclo ω8*c*.

The mol% G+C of the DNA of the type species and only species described is 67.

Type species: *Pseudolabrys taiwanensis*. The main features of the single member of the genus *Pseudolabrys* are summarized in Table 26.6.

Genus *Pseudoxanthobacter* Arun, Schumann, Chu, Tan, Chen, Lai, Kämpfer, Shen, Rekha, Hung, Chou, and Young 2008, 1573[VP]

Pseu.do.xan.tho.bac'ter. Gr. adj. *pseudês* false; N.L. masc. n. *Xanthobacter* a bacterial genus name; N.L. masc. n. *Pseudoxanthobacter* the false *Xanthobacter*.

Motile, short, rod-shaped Gram-negative or Gram-variable cells that accumulate poly-β-hydroxybutyrate as polar inclusion bodies. The major quinone is Q-10. The characteristic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine, phosphatyidyldimethylethanolamine, and an unknown aminolipid. The predominant fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo.

The mol% G+C of the DNA of the type species and only species described is 68.

Type species: *Pseudoxanthobacter soli*. The main features of the single member of the genus *Pseudoxanthobacter* are summarized in Table 26.6.

Additional comments:

– *P. soli* is a nitrogen-fixing species isolated from soil. Its major respiratory quinone is Q-10, with minor amounts of Q-9 and Q-8. The organism can fix N_2 and multiplies by budding (Arun et al. 2008).

Genus *Starkeya* Kelly, McDonald, and Wood 2000, 1800[VP]

Star.key'a. N.L. fem. n. *Starkeya*, named after Robert L. Starkey, who made important contributions to the study of soil microbiology and sulfur biochemistry.

Nonmotile, short, rod-shaped Gram-negative cells. Colonies grow on thiosulfate agar. Biotin is required. Strictly aerobic and facultative chemolithoautotrophic. Oxidize and grow on thiosulfate and tetrathionate but not on elemental sulfur or thiocyanate. Neutrophilic and mesophilic. Contain ubiquinone Q-10. Major cellular fatty acids are $C_{18:1}$ and $C_{19:0}$ cyclo. Growth is also observed with formate. Some strains may degrade methylated sulfides. Isolated from soils and presumably widely distributed.

The mol% G+C of the DNA is 67–69.

Type species: *Starkeya novella*.

The genus *Starkeya* currently contains two species: *S. novella* and *S. koreensis*. The main features of the members of the genus *Starkeya* are summarized in Table 26.7.

Additional comments:

– For optimal autotrophic development, biotin is required. When grown on formate, high levels of ribulose bisphosphate carboxylase/oxygenase are expressed (Kelly and Wood 2005).
– *S. novella* uses a variety of sugar alcohols, amino acids, carboxylic acids, and fatty acids for heterotrophic growth; reduced sulfur compounds serving as electron donors include thiosulfate, tetrathionate, dimethylsulfide, and dimethylsulfoxide (Kelly et al. 2000).
– A *S. novella* isolate from sewage was shown to oxidize methanethiol, dimethylsulfide, and dimethyldisulfide (Cha et al. 1999).

Isolation, Enrichment, and Maintenance Procedures

Strategies for the enrichment and isolation of *Xanthobacter* species can be based on the ability to fix N_2 under chemolithoautotrophic growth conditions under a gas mixture of H_2 (10 %), N_2 (70–75 %), CO_2 (10 %), and air (5–10 %), and on the specific yellow color and the characteristic "fried-egg" appearance of colonies caused by slime production on media containing 1 % succinate and nutrient broth, and the appearance of branched cells in such colonies (Wiegel 2005, 2006). Some isolates were obtained by chance, such as *X. autotrophicus* strain 7c, which was isolated from an enrichment for propane-oxidizing bacteria, using black mud of a pond in Germany as inoculum; the *Xanthobacter* obtained did not use propane. *X. tagetidis* can be reproducibly enriched from *Tagetes* (marigold) roots using thiophene-2-carboxylate or thiophene-2-acetate as substrates (Padden et al. 1997).

For enrichment and isolation of gas-vacuolated strains of *Ancylobacter*, 100 ml of a freshwater source can be added to a sterile aluminum foil-covered beaker containing 10 mg Bacto peptone (Difco). After 2 weeks incubation, at room temperature the culture is plated onto a hydrolysate medium containing glucose (Van Ert and Staley 1971; Staley et al. 2005). For the isolation of motile variants of gas-vacuolate strains of *Ancylobacter aquaticus*, selection on soft agar plates can be used: Growth radiating from the center of the colony consists of motile cells (Lara and Konopka 1987).

Ancylobacter oerskovii and two strains of *Ancylobacter polymorphus* were isolated from soil after enrichment with oxalate. All known species of the genus appear to use oxalate as the sole source of carbon (Lang et al. 2008), a finding that can probably be used as the basis for selective isolation procedures. Enrichment on dichloromethane as carbon and energy source, amended with a low concentration of yeast autolysate, was successfully applied for the isolation of *A. dichloromethanicus* (Firsova et al. 2009).

Azorhizobium grows well on nitrogen-free agar media, distinguishing the genus from other legume-nodulating bacteria (*Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*) (Dreyfus et al. 1988). Lactate and succinate are preferred substrates for growth (Kuykendall 2005). Selective inhibitors are also useful in studies of *Azorhizobium* and related plant-associated nitrogen-fixing bacteria: *Azorhizobium* is resistant to carbencillin; nalidixic acid inhibits *Azorhizobium* but allows growth of *Rhizobium* (Robertson et al. 1995).

Low nutrient concentrations may favor growth of *Labrys* spp. in enrichment cultures. This, *L. miyagiensis*, isolated from grassland soil in Japan, grows in 1/100 diluted nutrient broth but is inhibited by full-strength nutrient broth medium (Islam et al. 2007). *L. methylaminiphilus*, a facultatively methylotrophic bacterium obtained from sediment of Lake Washington, WA, USA, was enriched using mineral medium with 0.01 % methylamine (Miller et al. 2005). The type strain of *L. monachus* was isolated from silt samples from Lake Mustjarv (Estonia) using horse manure extract, obtained by heating 1 % (w/v) dry manure in distilled water. The sediment is left to settle and a liquid medium is prepared from the supernatant (Vasilyeva 2005). Some other *Labrys* species were obtained on nonselective media. *L. neptuniae* was isolated from root nodules of the tropical aquatic legume *Neptunia oleracea* on yeast extract —mannitol agar (Chou et al. 2007). Finally, *L. wisconsinensis* was isolated from Lake Michigan water as a colony that grew on Difco Plate Count Agar—a nonselective general medium for heterotrophic bacteria (Albert et al. 2010).

The type strains (and thus far only isolates) of the soil bacteria *Pseudoxanthobacter soli* and *Pseudolabrys taiwanensis* were both isolated as colonies on nutrient agar, without any prior enrichment or selection procedure (Arun et al. 2008; Kämpfer et al. 2006).

No selective isolation procedures for *Starkeya* spp. have yet been tested. Vitamins may be important in determining the outcome of enrichment and isolation experiments: For optimal autotrophic growth, *S. novella* requires biotin, and for optimal heterotrophic growth yeast extract, biotin, or other vitamins such as pantothenate may be required, depending on the substrate (Kelly et al. 2000). *S. koreensis* was isolated from rice straw using R2A agar, a nonselective general low-nutrient plating medium (Im et al. 2006).

Maintenance

Cultures can be maintained if they are refrigerated at 4 °C for a number of weeks. For long-term preservation, lyophilization is recommended.

Physiological and Biochemical Features

Physiologically and biochemically there are a number of noteworthy features within in the family *Xanthobacteraceae*: the ability of some strains of *Xanthobacter*, *Ancylobacter*, and *Labrys* to degrade chlorinated and brominated alkanes, alkenes, and aromatic compounds; the degradation of thiophenes by *X. tagetidis*; the ability of *Xanthobacter* spp. to grow on polyaromatic compounds; and the growth of some *X. autotrophicus* strains to grow on ethylene, propylene, and other alkenes.

Xanthobacter autotrophicus GJ10 was isolated on 1,2-dichloroethane as the sole carbon and energy source. It possesses a hydrolytic haloalkane dehydrogenase with a broad substrate specificity that degrades dichloroethane to 2-chloroethanol. This intermediate is further metabolized via chloroacetaldehyde to chloroacetic acid, which is dechlorinated by a second dehalogenase to glycolate. A similar strain GJ11 was isolated from sediment of the River Rhine (Janssen et al. 1984, 1985). *Xanthobacter* sp. strain TM1, isolated from a wastewater treatment plant receiving domestic and pharmaceutical effluent in Portugal, degrades dichloromethane, chloroacetic acid, dichloroethane, 2-chloroethanol, 2-fluorobenzoate, 3-fluorobenzoate, 4-fluorobenzoate, 2-chlorobenzoate, 4-chlorobenzoate, and methanol (Emanuelsson et al. 2009). *Xanthobacter* sp. strain ENV481, isolated from a microcosm model with aquifer soil and groundwater from a landfill, New Jersey, can degrade bis (2-chloroethyl) ether, a compound used as a solvent for fats and greases, a cleaning fluid for textiles, a constituent of paints and varnishes, and an insecticide (McClay et al. 2007). *Xanthobacter flavus* strain 14p1, isolated from river sludge in Germany, degrades 1,4-dichlorobenzene but no other aromatic or chloroaromatic compounds. The degradation pathway starts with dioxygenation, followed by ring opening via *ortho* cleavage of dichlorocatechol to 2,5-dichloro-*cis*,*cis*-muconic acid (Spiess et al. 1995; Spiess and Görisch 1996; Sommer and Gorisch 1997). *X. autotrophicus* strain GJ10 grows on 1,2-dichloroethane,

bromochloromethane, dibromomethane, and 1-bromo-2-chloroethane. A novel pathway of degradation of dihalomethanes to formaldehyde was proposed. Cells growing on 1,2-dichloroethane converted 2-fluoroethanol and 1-chloro-2-fluoroethane to 2-fluoroacetate (Torz et al. 2007). *Labrys portucalensis* can grow on fluorobenzene (Carvalho et al. 2008). Co-metabolism of chlorinated compounds may also occur: *X. autotrophicus* strains GJ10 and Py2 degrade trichloroethylene during growth on propene (Reij et al. 1995; Inguva and Schreve 1999). When grown on propylene, *Xanthobacter* strain Py2 can degrade trichloroethylene, 1-chloroethylene (vinyl chloride), 1,3-dichloropropylene, 2,3-dichloropropylene, and other related compounds. Addition of propylene oxide, propionaldehyde, and glucose enhanced the rate of degradation of chlorinated alkenes (Ensign et al. 1992).

Several isolates of the genus *Ancylobacter* degrade chlorinated aliphatic compounds. *A. dichloromethanicus* uses dichloromethane (Firsova et al. 2009). *A. aquaticus* strain GJ10 (Janssen et al. 1984, 1985) and strains AD25 and AD27, isolated from slurries of brackish water sediment and activated sludge, respectively (van den Wijngaard et al. 1992), degrade 1,2-dichloroethane. Strains AD25 and AD27 also use 2-chloroethylvinylether as sole carbon and energy source. Such chlorinated ethers are synthesized for the production of anesthetics, sedatives, and cellulose ethers (van den Wijngaard et al. 1993). Other chlorinated compounds broken down by *Ancylobacter* strains include 2-chloroethanol, chloroacetate, and 2-chloropropionate (Staley et al. 2005; van den Wijngaard et al. 1992). The organochlorine fungicide pentachloronitrobenzene (PCNB) could be degraded by *Labrys portucalensis* strain pcnb-21, isolated from a PCNB-polluted soil in China (Li et al. 2011). *Labrys* sp. strain Wy1, isolated from soil in a rubber estate in Malaysia, can use the herbicide 2,2-dichloropropionate (2,2-DCP) as sole source of carbon (Wong and Huyop 2011).

Xanthobacter tagetidis was isolated from the roots of marigold (*Tagetes*) plants. *Tagetes* species accumulate thiophenes in the roots at concentrations up to 1 % of the root mass. *X. tagetidis* grows on thiophenes such as thiophene-2-carboxylate, thiophene-3-carboxylate, on analogs of these compounds (pyrrole-2-carboxylate, furan-2-carboxylate), and on the condensed thiophene dibenzothiophene (Padden et al. 1997).

Xanthobacter polyaromaticivorans 127 W grows on a range of polycyclic and heterocyclic aromatic compounds under extremely low oxygen concentrations. Polycyclic aromatic hydrocarbons used include anthracene, fluorene, naphthalene, phenanthrene, dibenzothiophene, dibenzofuran, and biphenyl. It also degrades dibenzothiophene (Hirano et al. 2004). Another *Xanthobacter* strain, isolated from forest soil in the UK by enrichment with cyclohexane vapor as the carbon source, metabolizes cyclohexane via cyclohexanol, cyclohexanone, and 1-oxa-2-oxocycloheptane (ε-caprolactone) to adipic acid (Trower et al. 1985).

Biochemically one of the most interesting processes performed by some *Xanthobacter* isolates is the degradation of alkenes. Alkene-utilizing *Xanthobacter* strains were obtained from enrichment cultures with propene and 1-butene. A monooxygenase was found to be involved, forming 1,2-epoxyalkanes as intermediate (van Ginkel and de Bont 1986). Alkene degradation and the metabolism of the epoxide intermediates was studied in-depth in *X. autotrophicus* strain Py2, which grows on ethylene, propylene, and butylene (Small and Ensign 1997). The involvement of coenzyme M (2-mercaptoethanesulfonate) in the process came as a big surprise. Coenzyme M has been known for many decades as a central component of the biochemical pathway of methanogenesis in archaea, but it was never before found in any other organisms. *Xanthobacter* strain Py2 possesses a linear megaplasmid that encodes enzymes of aliphatic alkene and epoxide metabolism and coenzyme M biosynthesis. Epoxidation of propylene to epoxypropane is followed by a sequence of three reactions resulting in epoxide ring opening and carboxylation to form acetoacetate. Coenzyme M plays a central role in epoxide carboxylation by serving as the nucleophile for epoxy ring opening and as the carrier of the C_3 unit that is finally carboxylated to acetoacetate, releasing the coenzyme (Sluis and Ensign 1997; Krum and Ensign 2001; Krishnakumar et al. 2008; Pandey et al. 2011). Shotgun proteomics revealed proteins specific to growth on propylene, including the enzymes necessary for the biosynthesis of coenzyme M (Broberg and Clark 2010).

Another substrate metabolized via an epoxide intermediate is the terpene limonene, used by *Xanthobacter* sp. C20. This strain was isolated from the River Rhine using cyclohexane as the sole carbon and energy source. It possesses a novel bioconversion pathway in which limonene is converted to limonene-8,9-epoxide in a reaction that involves cytochrome P-450 (van der Werf et al. 2000).

Ecology

Members of the genus *Xanthobacter* may be ubiquitous in wet soil and sediments. They can be found in freshwater (*X. agilis*), wet soil containing decaying organic material (*X. autotrophus*, *X. flavus*), compost of root balls of *Tagetes* (*X. tagetidis*) (Padden et al. 1997), and they are associated with plant roots including wetland rice (Oyaizu-Masuchi and Komagata 1988; Reding et al. 1991; Wiegel 2005, 2006). One *X. flavus* originated from marine sediment (Lidstrom-O'Connor et al. 1983; Meijer et al. 1990). *X. xylophilus* is moderately acidophilic (opt. 5.5, range 4.8–6.8) and was found in acidic low-salinity dystrophic water with decaying spruce wood (Zaichikova et al. 2010a). *Xanthobacter* species may play an important role in the degradation of toxic organic compounds in polluted environments. *X. viscosus* and *X. aminoxidans* were found in activated sludge of a water treatment plant processing paper mill pulp (Loginova and Trotsenko 1980; Doronina et al. 1984; Doronina and Trotsenko 2003). *X. autotrophicus* was suggested to be involved in the biodegradation of toluene in a freshwater stream in Delaware (Tay et al. 1999).

Ancylobacter aquaticus is found in freshwater habitats, including ponds, creeks, and lakes (Van Ert and Staley 1971; Konopka et al. 1976) and in rice paddies and soil environments (Stubner et al. 1998). *A. abiegnus* was isolated from dystrophic

Table 26.9
Sensitivity of species of the family *Xanthobacteraceae* to selected antibiotics

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ampicillin	–	NR	–	+	–	–	+	–	–	+	+	–	+	NR	NR	+
Chloramphenicol	–	+	–	+	–	–	NR	–	–	NR	NR	–	NR	–	NR	NR
Erythromycin	–	NR	–	NR	NR	NR	NR	–	–	–	–	NR	–	–	–	NR
Novobiocin	+	NR	NR	NR	–	–	–	NR	NR	NR	NR	+	NR	NR	NR	NR
Penicillin	+	NR	–	–	NR	NR	NR	NR	–	+	+	–	+	NR	–	NR
Tetracycline	NR	NR	NR	–	NR	NR	NR	NR	–	+	+	+	+	-	+	+
Kanamycin	NR	NR	NR	NR	+	NR	NR	NR	–	+	+	+	+	–	NR	+
Streptomycin	NR	NR	NR	NR	+	+	+	+	+	NR	NR	+	NR	NR	NR	–

Species: *1 Xanthobacter autotrophicus*, *2 X. agilis*, *3 X. flavus*, *4 X.tagetidis*, *5 X. xylophilus*, *6 Ancylobacter abiegnus*, *7 A. dichloromethanicus*, *8 A. oerskovii*, *9 Labrys methylaminiphilus*, *L monachus*, *10 L. myagiensis*, *11 L. neptuniae*, *12 L. okinawensis*, *13 L. portucalensis*, *14 Pseudoxanthobacter soli*, *15 Starkeya koreensis*
+ sensitive, – resistant, *NR* not reported
Data were derived from the species descriptions (see *Table 26.2–26.7*) and from Wiegel (2005, 2006)

waters with decaying spruce wood (Zaichikova et al. 2010b). *Ancylobacter* species may also be involved in the degradation of toxic compounds. *A. dichloromethanicus*, a facultatively methylotrophic bacterium that can grow on dichloromethane, was isolated from contaminated soil in Russia (Firsova et al. 2009). *Ancylobacter* sp. strain XJ-412-1 degrades the herbicide metsulfuron-methyl. It was isolated from an agricultural soil in China, which had been exposed to sulfonylurea herbicides for many years (Lu et al. 2011).

Azorhizobium is associated with stem- or root nodules of *Sesbania* and some other leguminous plants (Dreyfus and Dommergues 1981; Dreyfus et al. 1984, 1988; Kuykendall 2005; de Souza Moreira et al. 2006). A comparative study of rhizosphere and non-rhizosphere soils in four vegetation zones of Senegal showed *Azorhizobium* to be more abundant on leaves and stems than *Rhizobium* in three out of the four vegetation zones. Approximately 90 % of the stem nodules and 39–48 % of the root nodules on *S. rostrata* were formed by *Azorhizobium* (Robertson et al. 1995).

Little is known about the distribution of *Labrys* species in nature. They have been recovered from unpolluted freshwater environments (Vasilyeva 2005; Albert et al. 2010) as well as from polluted sites (e.g., the fluorobenzene-degrading *L. portucalensis* (Carvalho et al. 2008). *L. neptuniae* was isolated from root nodules of the aquatic pan-tropical legume *Neptunia oleracea*, but it probably is not the dominant bacterium there: Over 95 % of the colonies of *Neptunia*-associated bacteria in Taiwan that developed on yeast extract—mannitol agar belonged to *Allorhizobium undicola* (Chou et al. 2007).

Hardly anything is known about the distribution and the ecological role of the genera *Pseudolabrys* and *Pseudoxanthobacter*. It is also known little about the ecological niches where *Starkeya* species may be important. The type strains of *S. novella* and *S. koreensis* were isolated from soil and from rice straw, respectively (Starkey 1934; Im et al. 2006), not from environments rich in sulfide.

Pathogenicity, Clinical Relevance

No pathogenic bacteria are known within the *Xanthobacteraceae*. The plant-associated species of the genus *Azorhizobium* that colonize the stems of *Sesbania* and some other leguminous plants live in symbiosis with their host, and they were never shown to cause harm to their plant hosts (Dreyfus et al. 1984; Kuykendall 2005).

Table 26.9 summarizes data on the sensitivity of members of the *Xanthobacteraceae* to different antibiotics. Such data are not available for all members of the family, and the information present does not show any clear patterns, possibly except for the fact that all species tested were sensitive to erythromycin. Wiegel (2006) commented that the available data do not suggest antibiotic typing as a valid method for identification of *Xanthobacter*. This may be the case for other genera of the family as well. The resistance of *Azorhizobium* to carbencillin was mentioned above and allows the selective isolation with the exclusion of *Rhizobium* (Robertson et al. 1995).

Application

As shown above, many representatives of the genera *Xanthobacter*, *Ancylobacter*, and *Labrys* degrade toxic compounds: chlorinated alkanes, alkenes, polyaromatic compounds, thiophenes, etc., and the bacteria may be involved in the biodegradation of such compounds in polluted environments. Many such stains were isolated from sites polluted with such chemicals. However, no applications based on the use of such bacteria in bioremediation operations are known.

The polysaccharide slime produced by *Xanthobacter* species may have interesting biotechnological applications. Wiegel (2006) mentioned the possible use of these polysaccharides as drag-reducing substances for minimizing friction in turbulent flows in pipelines and water turbines or as viscosifiers in oil fields.

An intriguing application of gas-vacuolated *Anyclobacter aquaticus* was proposed: Mosquitocidal toxin genes from *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* were introduced into *Ancylobacter* by electroporation. The transformed cells exhibited significant toxicity toward mosquito larvae. Such transgenic *Ancylobacter* could be released in water bodies infested with mosquito larvae, and due to the buoyancy of the cells conferred by the gas vesicles the toxin will accumulate at the water surface (Yap et al. 1994). No information could be found whether this interesting idea has ever been developed into field applications.

Starkeya novella, due to its potential to oxidize sulfide and methylated sulfur compounds, and its adaptability to a broad pH range (5–10), could be applied in biofilters for the removal of bad-smelling sulfur compounds, for example, from piggery wastewater (Chung et al. 1997).

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