Antonie van Leeuwenhoek (2015) 107:467–485
DOI 10.1007/s10482-014-0344-8

ORIGINAL PAPER

A phylogenomic and molecular marker based taxonomic framework for the order *Xanthomonadales*: proposal to transfer the families *Algiphilaceae* and *Solimonadaceae* to the order *Nevskiales* ord. nov. and to create a new family within the order *Xanthomonadales*, the family *Rhodanobacteraceae* fam. nov., containing the genus *Rhodanobacter* and its closest relatives

Sohail Naushad · Mobolaji Adeolu · Shirley Wong · Misbah Sohail · Herbert E. Schellhorn · Radhey S. Gupta

Received: 8 September 2014 / Accepted: 28 November 2014 / Published online: 7 December 2014

Abstract The current taxonomy of the order *Xanthomonadales* is highly problematic and no comprehensive phylogenomic studies have been completed that include the most divergent members within the order. In this work, we have completed a phylogenomic analysis of a wide range of genomes, five of which were sequenced for the first time for this work, representing the vast majority of the diversity within the order *Xanthomonadales*. Using comparative genomic techniques, we have identified a large number of conserved signature inserts/deletions (CSIs) that are specifically found in different groups of related organisms, at different taxonomic levels, within the order. Our phylogenetic analyses do not support a monophyletic grouping of the members of the order *Xanthomonadales* and no CSIs were identified which are uniquely shared by all sequenced species within this order. However, our work has identified 10 CSIs which are specific to all members of the family *Xanthomonadaceae* and an additional 10 and 11 CSIs that are specific to one of two phylogenetically well-defined clades within the family *Xanthomonadaceae*. On the basis of the identified CSIs and the results of phylogenomic analyses, we propose a new taxonomic framework for the order *Xanthomonadales*. In this proposal, the families *Algiphilaceae* and *Solimonadaceae* (*Nevskiaceae*), which do not branch with the other members of the order *Xanthomonadales*, are transferred into the order *Nevskiales* ord. nov. The remaining members of the order *Xanthomonadales* are divided into two families: the family *Xanthomonadaceae,* containing the genus *Xanthomonas* and its closest relatives, and a new family, *Rhodanobacteraceae* fam. nov., containing the genus *Rhodanobacter* and its closest relatives. Additionally, we have also emended descriptions of the order *Lysobacterales*, the family *Lysobacteraceae*, and the family *Nevskiaceae* to indicate that they are earlier synonyms of the order *Xanthomonadales*, the family *Xanthomonadaceae*, and the family *Solimonadaceae*, respectively.

Electronic supplementary material The online version of this article (doi:10.1007/s10482-014-0344-8) contains supplementary material, which is available to authorized users.

S. Naushad · M. Adeolu · M. Sohail · R. S. Gupta (✉)
Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON L8N 3Z5, Canada
e-mail: gupta@mcmaster.ca

S. Wong · H. E. Schellhorn
Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada

Keywords *Xanthomonadales* · *Lysobacterales* · *Lysobacteraceae* · *Rhodanobacteraceae* · *Nevskiales* · *Nevskiaceae* · *Salinisphaeraceae* · Phylogenetic trees · Conserved signature indels · Molecular signatures

Introduction

The order *Xanthomonadales* is an early diverging group of bacteria within the class *Gammaproteobacteria* (Cutino-Jimenez et al. 2010; Williams et al. 2010; Naushad and Gupta 2013). The order *Xanthomonadales* currently contains 5 families (viz. *Algiphilaceae, Nevskiaceae, Sinobacteraceae*, *Solimonadaceae*, and *Xanthomonadaceae*) which contain 30 genera encompassing a large number of species that possess a diverse range of phenotypic and biochemical characteristics (Saddler and Bradbury 2005a; Parte 2013). The members of this order include a number of major plant pathogens that have significant economic and agricultural impact. Members of the genera *Xylella* and *Xanthomonas*, in particular, are major phytopathogens which cause a wide variety of serious diseases in more than 400 agriculturally important plants including tomatoes, bananas, citrus plants, rice, and coffee plants (da Silva et al. 2002; Van Sluys et al. 2003; Lee et al. 2005; Chatterjee et al. 2008; Salzberg et al. 2008; Ryan et al. 2011). The order also contains the genus *Stenotrophomonas* which harbours a number of increasingly important multidrug resistant opportunistic pathogens that are responsible for hospital-acquired infections in immunodeficient patients (Crossman et al. 2008; Looney et al. 2009). Despite the important plant and human pathogens present within this order, the taxonomy of this group is highly problematic and no comprehensive phylogenetic studies have been completed that focus specifically on the interrelationships of the different members within the order *Xanthomonadales* (Gao et al. 2009; Cutino-Jimenez et al. 2010; Williams et al. 2010; Naushad and Gupta 2013; Tindall 2014b).

The current taxonomy of the order *Xanthomonadales* is largely based on 16S rRNA sequence analysis (Saddler and Bradbury 2005a; Gutierrez et al. 2012; Losey et al. 2013). However, the 16S rRNA gene sequence has shown limited ability to resolve the branching and relationships of organisms within the order *Xanthomonadales* (Zhou et al. 2008; Cutino-Jimenez et al. 2010; Yilmaz et al. 2013). Phylogenetic trees based on the 16S rRNA gene sequence often do not resolve a monophyletic cluster of all *Xanthomonadales*; the most divergent members of the order often branch separately from the majority of the species within the group (Yilmaz et al. 2013). Apart from the 16S rRNA sequence, no biochemical, morphological or physiological characteristics are known which distinguish the order *Xanthomonadales* from all other bacteria or the families and major phylogenetic clusters within the order from each other (Saddler and Bradbury 2005a; Gutierrez et al. 2012; Losey et al. 2013). Thus, it is of interest to identify shared characteristics that can clearly elucidate the evolutionary relationships within this highly diverse group of organisms and form the basis for a coherent taxonomic framework of the order.

Whole genome sequences for members of the order *Xanthomonadales* provide a rich resource for the discovery of molecular characteristics which are unique to evolutionarily related organisms (Gao et al. 2009; Cutino-Jimenez et al. 2010; Naushad and Gupta 2013). One useful type of shared molecular characteristic that has been a focus of recent research are Conserved Signature Indels (CSIs), which are insertions/deletions uniquely present in protein sequences from a group of evolutionarily related organisms (Gupta 2010; Gao and Gupta 2012b; Gupta and Lali 2013; Gupta et al. 2013; Gupta 2014). Due to the specificity of CSIs for particular groups of bacteria, they represent molecular synapomorphies (markers of common evolutionary decent) which can be used to identify and demarcate specific bacterial groups in clear molecular terms (Gupta 1998, 2010). We have previously carried out comparative genomic analysis of a limited number of members from the order *Xanthomonadales* in which we identified a large number of CSIs in diverse proteins that were uniquely present in all analyzed members of the order or a subgroup of the *Xanthomonadales* (Naushad and Gupta 2013). In this work, we have extended these studies, by carrying out detailed phylogenomic and comparative genomic analyses on a greatly expanded dataset on members of the order *Xanthomonadales* which includes 43 genomes from the NCBI, JGI, and EzBioCloud genome databases and 5 additional *Xanthomonadales* genomes, which we have sequenced *de novo,* representing 2 families, 20 genera, and 42 named species. Our analyses have identified no phylogenetic

support for a monophyletic grouping of all sequenced members the order *Xanthomonadales* and no CSIs were identified which are uniquely shared by all sequenced species within the order *Xanthomonadales* suggesting that the order *Xanthomonadales* does not represent a single monophyletic lineage. Additionally, we have identified 31 CSIs which are either specific to the family *Xanthomonadaceae* or to one of its subgroups which demarcate these groups in molecular terms. On the basis of the identified CSIs and the results of phylogenomic analyses, we propose a new taxonomic framework for the order *Xanthomonadales*. In this proposal, the families *Algiphilaceae* and *Solimonadaceae* (*Nevskiaceae*), which do not branch with the other members of the order *Xanthomonadales*, are transferred into the order *Nevskiales* ord. nov, along with their closest evolutionary neighbour, the family *Salinisphaeraceae* fam. nov. The remaining members of the order *Xanthomonadales* are divided into two families: the family *Xanthomonadaceae,* containing the genus *Xanthomonas* and its closest relatives, and a new family, *Rhodanobacteraceae* fam. nov., containing the genus *Rhodanobacter* and its closest relatives. Lastly, the descriptions of the order *Lysobacterales*, the family *Lysobacteraceae*, and the family *Nevskiaceae* are emended to indicate that they are earlier synonyms of the order *Xanthomonadales*, the family *Xanthomonadaceae*, and the families *Solimonadaceae* and *Sinobacteraceae*, respectively.

Methods

DNA extraction and genome sequencing

Five *Xanthomonadales* isolates were sequenced *de novo* in this study; *Dyella japonica* DSM 16301^T (Genbank accession number JPLA00000000), *Luteibacter rhizovicinus* DSM 16549^T (JPLB00000000), *Thermomonas brevis* DSM 15422^T (JPLC00000000), *Xanthomonas hyacinthi* DSM 19077^T (JPLD00000000), and *Xanthomonas pisi* DSM 18956^T (JPLE00000000). The isolates were obtained from the German Collection of Microorganisms and Cell Cultures (Leibniz-Institut DSMZ). The isolates were grown for 24 h under the growth conditions described in Supplemental Table 1. Genomic DNA was extracted using a CTAB based DNA extraction methodology (Wilson 1987) with specific modifications for *Xanthomonadales* described by Jaufeerally-Fakim and Dookun (2000). The DNA samples were diluted to 0.2 ng/µl and standard Illumina multiplex libraries were generated using the Nextera XT DNA Sample Prep Kit. The fragment size distribution of each library was verified using the Agilent High Sensitivity DNA Kit. Sequencing was performed using an Illumina HiSeq 2000 and 150 bp paired end reads were generated. Genomes were assembled using CLC Genomics Workbench 7.0.4 with default *de novo* assembly parameters and trimmed for contamination using the UniVec vector database (Build 8.0) (Table 1).

Phylogenetic sequence analysis

Phylogenetic analysis was performed on a concatenated sequence alignment of 15 highly conserved housekeeping proteins (viz. dimethyladenosine transferase, alanyl-tRNA synthetase, arginyl-tRNA synthetase, chaperone protein DnaK, signal recognition particle-docking protein FtsY, chaperonin GroL, DNA gyrase subunit A, DNA gyrase subunit B, ATP-dependent DNA helicase UvrD, valyl-tRNA synthetase, Isoleucyl-tRNA synthetase, DNA polymerase I, SecA, RpoB, and RpoC) which have been widely used for phylogenetic analysis (Kyrpides et al. 1999; Charlebois and Doolittle 2004; Ciccarelli et al. 2006). Sequences for these proteins were obtained from the NCBI and JGI-IMG genome databases for strains of all *Xanthomonadales* and a representative selection of outgroup *Gammaproteobacteria* (which included members from the orders *Aeromonadales, Alteromonadales, Cardiobacteriales, Chromatiales,* "Enterobacteriales"*, Legionellales, Methylococcales, Oceanospirillales, Pasteurellales, Pseudomonadales,* "Salinisphaerales"*, Thiotrichales*, and "Vibrionales") and *Betaproteobacteria*. Sequences for these proteins were also obtained from the five *Xanthomonadales* genomes which we have sequenced in this work and the genome of *Riemerella anatipestifer*, which was used to root the tree. Multiple sequence alignments for these proteins were created using Clustal_X 1.83 (Jeanmougin et al. 1998) and concatenated into a single alignment file. Poorly aligned regions from this alignment file were removed using Gblocks 0.92 (Castresana 2000). The resulting alignment, which contained 6995 aligned amino acids, was used for phylogenetic analysis. The maximum-likelihood tree based on 100 bootstrap replicates of this alignment

Table 1 Characteristics of the *Xanthomonadales* genomes used for phylogenetic analysis

Organism	Accession #	Genome size (Mb)	G−C %	Genome source
Arenimonas composti TR7-09	AUFF01	3.16	70.8	DOE-JGI
Arenimonas oryziterrae DSM 21050	ATVD01	3.09	65.6	DOE-JGI
Dyella ginsengisoli LA-4	AMSF01	4.55	67.7	Shanghai Jiao Tong University
Frateuria aurantia DSM 6220	CP003350	3.60	63.4	DOE-JGI
Hydrocarboniphaga effusa AP103	AKGD01	5.19	65.2	Chonbuk National University
Ignatzschineria larvae DSM 13226	AZOD01	2.46	40.4	DOE-JGI
Luteimonas mephitis DSM 12574	AULN01	3.42	68.5	DOE-JGI
Lysobacter antibioticus HS124	CAQP01	5.14	69.0	OARDC
Lysobacter defluvii DSM 18482	AUHT01	2.72	70.3	DOE-JGI
Nevskia ramosa DSM 11499	ATVI01	4.52	64.4	DOE-JGI
Pseudoxanthomonas sp. GW2	ALIP01	3.35	71.4	Shanghai Jiao Tong University
Pseudoxanthomonas spadix BD-a59	CP003093	3.45	67.7	Lee et al. (2012)
Pseudoxanthomonas suwonensis 11-1	CP002446	3.42	70.2	DOE-JGI
Rhodanobacter denitrificans 2APBS1	CP003470	4.23	67.5	Kostka et al. (2012)
Rhodanobacter fulvus Jip2	AJXU01	3.88	65.6	Im et al. (2004)
Rhodanobacter sp. 115	AJXS01	4.24	64.7	Kostka et al. (2012)
Rhodanobacter spathiphylli B39	AJXT01	3.91	66.5	De Clercq et al. (2006)
Rhodanobacter thiooxydans LCS2	AJXW01	4.09	67.2	Lee et al. (2007)
Rudaea cellulosilytica DSM 22992	ARJQ01	4.34	63.6	DOE-JGI
Silanimonas lenta DSM 16282	AUBD01	2.65	71.1	DOE-JGI
Singularimonas variicoloris DSM 15731	ARNM01	4.12	69.1	DOE-JGI
Solimonas flavus DSM 18980	AUFV01	4.46	68.9	DOE-JGI
Stenotrophomonas maltophilia K279a	AM743169	4.85	66.3	JCV
Stenotrophomonas sp. SKA14	ACDV01	5.02	66.4	Crossman et al. (2008)
Wohlfahrtiimonas chitiniclastica DSM 18708	AQXD01	1.99	44.1	DOE-JGI
Xanthomonas albilineans GPE PC73	FP565176	3.85	62.9	Pieretti et al. (2009)
Xanthomonas arboricola MAFF 301420	BAVC01	5.00	65.3	NIFTS
Xanthomonas axonopodis 12-2	AJJO01	5.27	64.4	Kasetsart University
Xanthomonas campestris 8004	CP000050	5.15	65.0	Qian et al. (2005)
Xanthomonas citri Aw12879	CP003778	5.40	64.7	Jalan et al. (2013)
Xanthomonas fragariae LMG 25863	AJRZ01	4.18	62.2	ILVO
Xanthomonas fuscans 4834-R	FO681494	5.09	64.7	Darrasse et al. (2013)
Xanthomonas gardneri ATCC 19865	AEQX01	5.53	63.7	University of Florida
Xanthomonas oryzae KACC 10331	AE013598	4.94	63.7	Lee et al. (2005)
Xanthomonas perforans 91-118	AEQW01	5.26	65.0	University of Florida
Xanthomonas sacchari NCPPB 4393	AGDB01	4.90	69.0	Studholme et al. (2011)
Xanthomonas translucens ART-Xtg29	ANGG01	4.10	68.6	ART
Xanthomonas vasicola NCPPB 1326	AKBK01	4.95	63.3	Studholme et al. (2011)
Xanthomonas vesicatoria ATCC 35937	AEQV01	5.53	64.1	University of Florida
Xylella fastidiosa 9a5c	AE003849	2.73	52.6	Meidanis et al. (2002)
Xylella fastidiosa Ann-1	AAAM04	2.73	52.0	DOE-JGI
Xylella fastidiosa M12	CP000941	2.48	51.9	Chen et al. (2010)
Xylella fastidiosa Temecula 1	AE009442	2.52	51.8	Van Sluys et al. (2003)

Genomic information was collected from: http://www.ncbi.nlm.nih.gov/genomes/

DOE-JGI Genome sequenced by the United States Department of Energy Joint Genome Institute, *OARDC* genome sequenced by the Ohio Agricultural Research and Development Center, *JCV* genome sequenced by the J. Craig Venter Institute, *NIFTS* genome sequenced by the National Agriculture and Food Research Organization Institute of Fruit Tree Science, *ILVO* genome sequenced by the Institute for Agricultural and Fisheries Research, *ART* genome sequenced by the Research Station Agroscope Reckenholz-Tänikon

was constructed using MEGA 5.2 (Tamura et al. 2011) employing the Whelan and Goldman substitution model.

A 16S rRNA gene sequence based phylogenetic tree was also created based on 197 sequences that included representative strains of all cultured *Xanthomonadales* genera. 16S rRNA gene sequences larger than 1,200 bp were obtained for all strains used in our concatenated protein based phylogenetic tree and all type strains classified under the order *Xanthomonadales* in the Ribosomal Database Project (Cole et al. 2014). A maximum-likelihood tree based on these sequences was created using 100 bootstrap replicates of the 16S rRNA sequence alignments in MEGA 5.2 (Tamura et al. 2011) employing the General Time-Reversible (Tavaré 1986) substitution model.

Identification and assessment of specificity of conserved signature indels

Identification of CSIs that are commonly shared by members of the *Xanthomonadaceae* was carried out as described by Naushad and Gupta (2013). Briefly, for the identification of CSIs, BLASTp searches were performed on each protein in the genome of *Rhodanobacter fulvus* Jip2. These searches were performed using the default BLAST parameters against all available sequences in the GenBank non-redundant database. For those proteins for which high scoring homologs (E values $< 1e^{-20}$) were present in other species from the *Xanthomonadales*, multiple sequence alignments were created using the Clustal_X 1.83 program (Jeanmougin et al. 1998). These alignments were visually inspected for the presence of insertions or deletions that were flanked on both sides by at least 5–6 conserved amino acid residues in the neighbouring 30–40 amino acids. Indels that were not flanked by conserved regions were not further considered, as they do not provide useful molecular markers. To assess the specificity of the indels we identified here and to reassess the specificity of the indels identified in our previous work, we carried out detailed BLASTp and tBLASTn searches against both the NCBI and JGI-IMG genome databases using as query short sequence segments containing the indel and the flanking conserved regions (60–100 amino acids long). Local tBLASTn searches were also completed on the indel containing regions for genomes of *Xanthomonadales* organisms missing from the NCBI and JGI-IMG genome databases. To ensure that the identified signatures are only present in *Xanthomonadales* homologues, the 250 BLAST hits with the highest similarity to the query sequence were examined for the presence or absence of these CSIs. Signature files were created and formatted using the programs Sig_Create and Sig_Style (accessible from Gleans.net) as described by Gupta (2014). In this work, we report the results of CSIs that are specific for different groups within the *Xanthomonadales* and where similar CSIs were not observed in any other bacteria in the top 250 BLAST hits. Due to space constraints, the sequence alignment files presented here contain sequence information for a limited number of species within the order *Xanthomonadales* and a representative selection of outgroup species. However, in each case, all members of the order and outgroups exhibited similar sequence characteristics to the representatives.

Results

Phylogenetic analysis

The current understanding of the evolutionary relationships of the *Xanthomonadales* is based largely on analyses of the 16S rRNA gene (Saddler and Bradbury 2005a; Gutierrez et al. 2012; Losey et al. 2013). In past studies, the 16S rRNA gene sequence has shown limited ability to resolve some of the phylogenetic relationships of organisms within the order *Xanthomonadales* (Zhou et al. 2008; Yilmaz et al. 2013). Phylogenetic trees based on multiple conserved genes/proteins have been shown to provide greater resolving power than those based on any single gene or protein (Rokas et al. 2003; Wu et al. 2009). Thus, we have constructed a highly resolved phylogenetic tree of the *Xanthomonadales* based on a concatenated set of 15 housekeeping and ribosomal proteins (Fig. 1). In this concatenated protein based phylogenetic tree a majority of the members of the *Xanthomonadales* formed a well-supported monophyletic clade which branched as an outgroup of the other members of the *Gammaproteobacteria*. The members of the order *Xanthomonadales* formed two distinct and well-supported main monophyletic clades: one clade consisting of members of the family *Xanthomonadaceae* and another clade consisting of the family *Solimonadaceae* (including the genera *Nevskia* and *Hydrocarboniphaga*) and the

species *Salinisphaera shabanensis*, a member of the family "Salinisphaeraceae". The *Xanthomonadaceae* clade contained two smaller clades that were well-supported by bootstrap analysis. The first of these clades, contained the genera *Xanthomonas*, *Xylella*, *Stenotrophomonas*, *Lutimonas*, *Lysobacter* and their relatives (Clade 1) while the second clade contained the genera *Rudaea*, *Dylella*, *Luteibacter*, *Rhodanobacter* and *Frateuria* (Clade 2). Two members of the *Xanthomonadales*, *Wohlfahrtiimonas chitiniclastica* and *Ignatzschineria larvae*, branched separately from the rest of the order, in a well-supported clade with members of the order *Cardiobacteriales*, another early diverging group within the class *Gammaproteobacteria*.

We have also produced a phylogenetic tree based on the 16S rRNA gene which contains representative species of *Xanthomonadales* that encompass all of the currently named genera (Fig. 2). The 16S rRNA based phylogenetic tree exhibited broadly similar branching to our concatenated protein based phylogenetic tree. In the 16S rRNA gene tree, the families *Xanthomonadaceae* and *Solimonadaceae* did not form a monophyletic clade and were separated by a large number of organisms. The family *Xanthomonadaceae* was divided into two well-supported clades which were analogous to the clades found in our concatenated protein based phylogenetic tree (Clades 1 and 2). In the 16S rRNA gene tree, the family *Solimonadaceae* (including the genera *Nevskia*, *Hydrocarboniphaga*, and *Alkanibacter*) branched with the family *Algiphilaceae*, another disparate group within the *Xanthomonadales*, and the genus *Steroidobacter,* which is currently recognized as a member of the *Xanthomonadaceae*. As in our concatenated protein based phylogenetic tree, *Solimonadaceae* and the other disparate members of the *Xanthomonadales* showed an association with the members of the genus *Salinisphaera*, the sole members of the family "Salinisphaeraceae". Additionally, in the 16S rRNA based phylogenetic tree, the genera *Wohlfahrtiimonas* and *Ignatzschineria*, which branched with the order *Cardiobacteriales* in our concatenated protein based phylogenetic tree, formed a weakly supported monophyletic group with the other members of the family *Xanthomonadaceae*. However, *Wohlfahrtiimonas* and *Ignatzschineria* were well separated from the other *Xanthomonadaceae* by a long branch.

Conserved signature indels

CSIs that are restricted to a group of related species are a novel class of molecular marker with high utility for evolutionary studies (Gupta 1998; Rokas and Holland 2000; Gupta 2010; Gao and Gupta 2012a; Gupta 2014). Recently, CSIs have been used to define novel taxonomic groups and to propose important taxonomic changes for various groups of bacteria (viz. Spirochaetes, Aquificae, *Neisseriales*, and *Bacillus*) at different taxonomic ranks (Adeolu and Gupta 2013; Bhandari et al. 2013; Gupta and Lali 2013; Gupta et al. 2013). We have recently reported a comparative genomic analysis on a limited number of members of the order *Xanthomonadales* in which we identified a large number of CSIs in diverse proteins that were uniquely present in all available members of the order or different phylogenetic groups within the order and absent in homologs from all other bacterial groups (Naushad and Gupta 2013). However, the genomes analyzed in our previous study were all from members of one family within the *Xanthomonadales*, the family *Xanthomonadaceae*, and did not include any of the more divergent species within the order whose phylogenetic placement is less clear. In this work, we have reassessed the specificity of these previously identified CSIs for a large number of additional *Xanthomonadales*, including five strains which we have sequenced, *de novo*, covering a vast majority of the diversity within the order and thereby have identified 31 CSIs which are either specific to the family *Xanthomonadaceae* or to one of its subgroups and absent in all other sequenced bacterial groups.

Of the 31 CSIs described in this work, none were present in all members of the order *Xanthomonadales*. All of the CSIs identified in our previous study of the *Xanthomonadales* (Naushad and Gupta 2013) were found to be specific to only the family *Xanthomonadaceae* or one of its subgroups. Of the 31 CSIs identified, 10 were uniquely found in all or most members of the *Xanthomonadaceae*, except *Wohlfahrtiimonas* and *Ignatzschineria*, and absent in organisms from all other sequenced bacterial groups. One example of a CSI uniquely present in members of the *Xanthomonadace* is shown in Fig. 3. In the example, an 18 aa insertion in a conserved region of glutaminyl t-RNA synthetase is uniquely present in all members of the *Xanthomonadace*, except

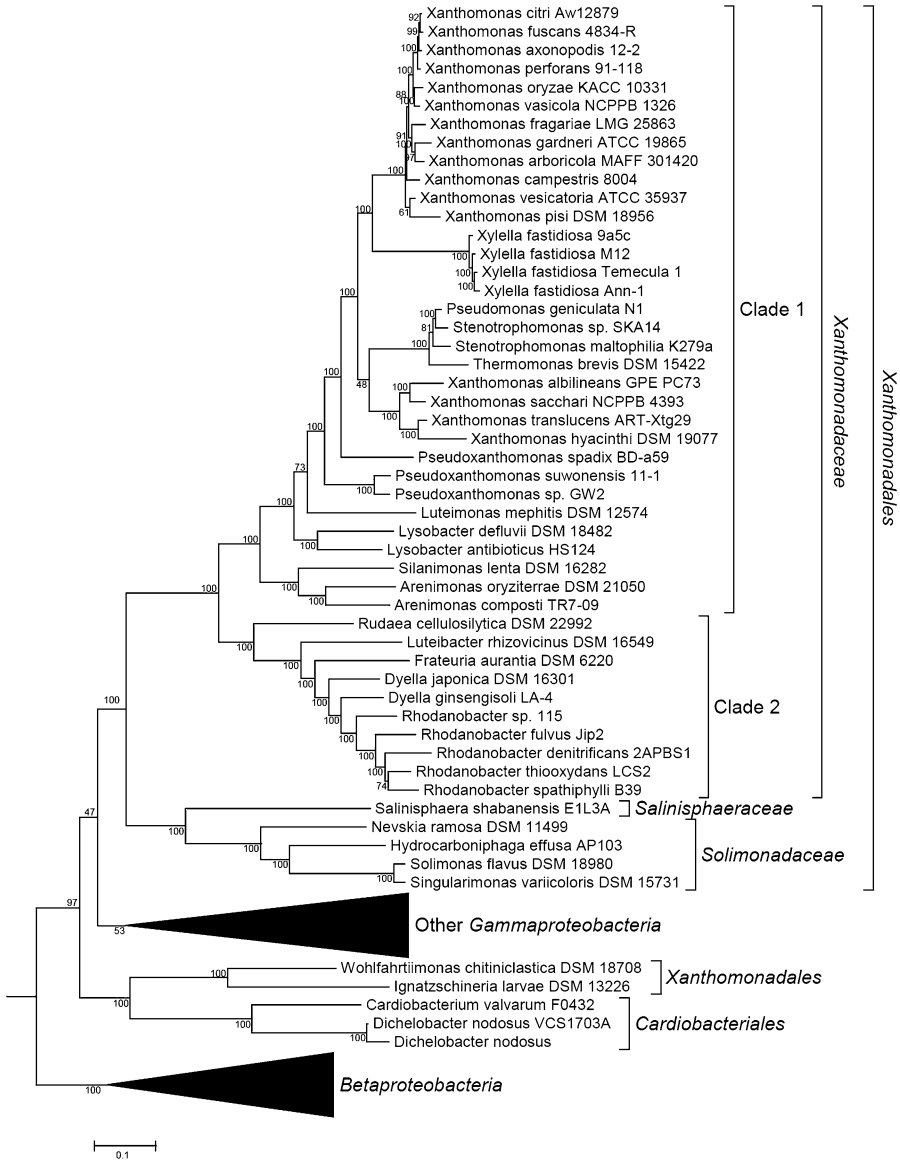


Fig. 1 A maximum-likelihood phylogenetic tree of the order *Xanthomonadales*, other *Gammaproteobacteria*, and *Betaproteobacteria* based on the concatenated amino acid sequences of 25 conserved proteins. Bootstrap values are shown at branch nodes. The major groups within the order *Xanthomonadales* as well as the related taxa, *Salinisphaeraceae* and *Cardiobacteriales*, are indicated

Wohlfahrtiimonas and *Ignatzschineria*, but it is not found in sequences from any other bacterial group. Sequence information for the 9 other CSIs specific for all members of the *Xanthomonadace*, except *Wohlfahrtiimonas* and *Ignatzschineria* are presented in Supplemental Figs. 1–9 and a summary of all 10 *Xanthomonadace* specific CSIs is presented in Table 2A. Our analyses have also identified 10 CSIs which were found to be unique molecular characteristics of most members of Clade 1 of the *Xanthomonadaceae*. Two examples of such CSIs are presented in Fig. 4. One CSI, a 4 aa insert in DNA polymerase III subunit alpha, is present in all members of Clade 1 of the *Xanthomonadaceae* except *Silanimonas lenta* (Fig. 4a), while the other CSI, a 4 aa insert in the protein protoheme IX farnesyltransferase, is uniquely present in all members of Clade 1 of the *Xanthomonadaceae* except the early branching genera *Arenimonas* and *Silanimonas* (Fig. 4b). Sequence information for the other identified CSIs specific to

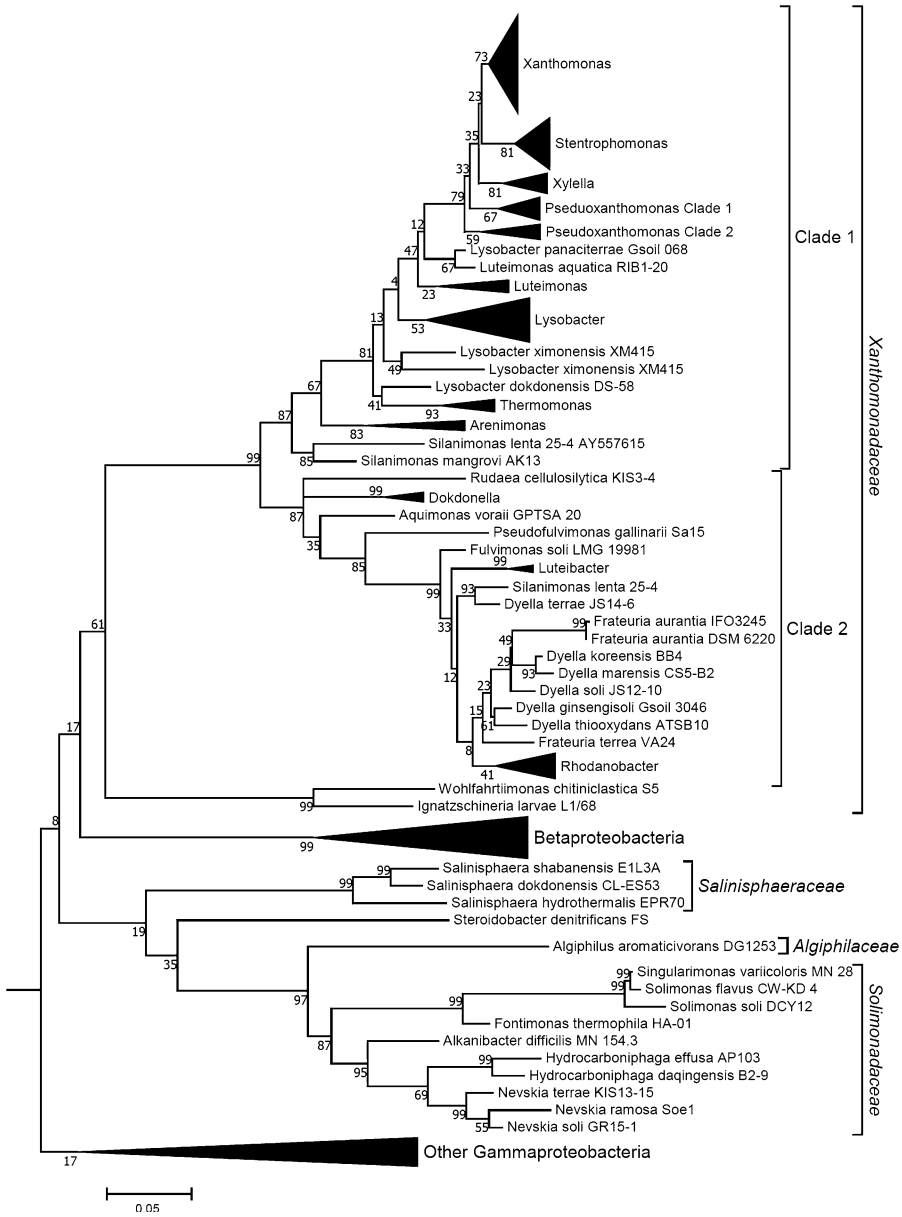


Fig. 2 A maximum-likelihood tree based on the 16S rRNA gene sequences of representative strains of all named *Xanthomonadales* species. Bootstrap values are shown at branch nodes. The major groups within the order *Xanthomonadales* as well as the related taxon, *Salinisphaeraceae*, are indicated

the members of Clade 1 of the *Xanthomonadaceae* are presented in Supplemental Figs. 10–17 and summarized in Table 2B.

Our analyses have also identified 11 CSIs that were specifically found in diverse proteins from members of Clade 2 of the *Xanthomonadaceae*, 7 of which were uniquely found in all members of Clade 2 of the *Xanthomonadaceae* except the early branching genus *Rudaea*. An example of a CSI specifically found in all members of Clade 2 of the *Xanthomonadaceae* is shown in Fig. 5a. In this CSI a 1 aa insert in the protein uridylyltransferase is shown to be found in all members of Clade 2 of the *Xanthomonadaceae* and absent in all other *Xanthomonadales* and all other bacterial groups. Another CSI, a 4 aa insert in the protein CDP-diacylglycerol–glycerol-3-phosphate 3-phosphatidyltransferase, specifically found in all members of Clade 2 of the *Xanthomonadaceae* except *Rudaea cellulosilytica* is shown in Fig. 5b. Sequence information for the other identified CSIs

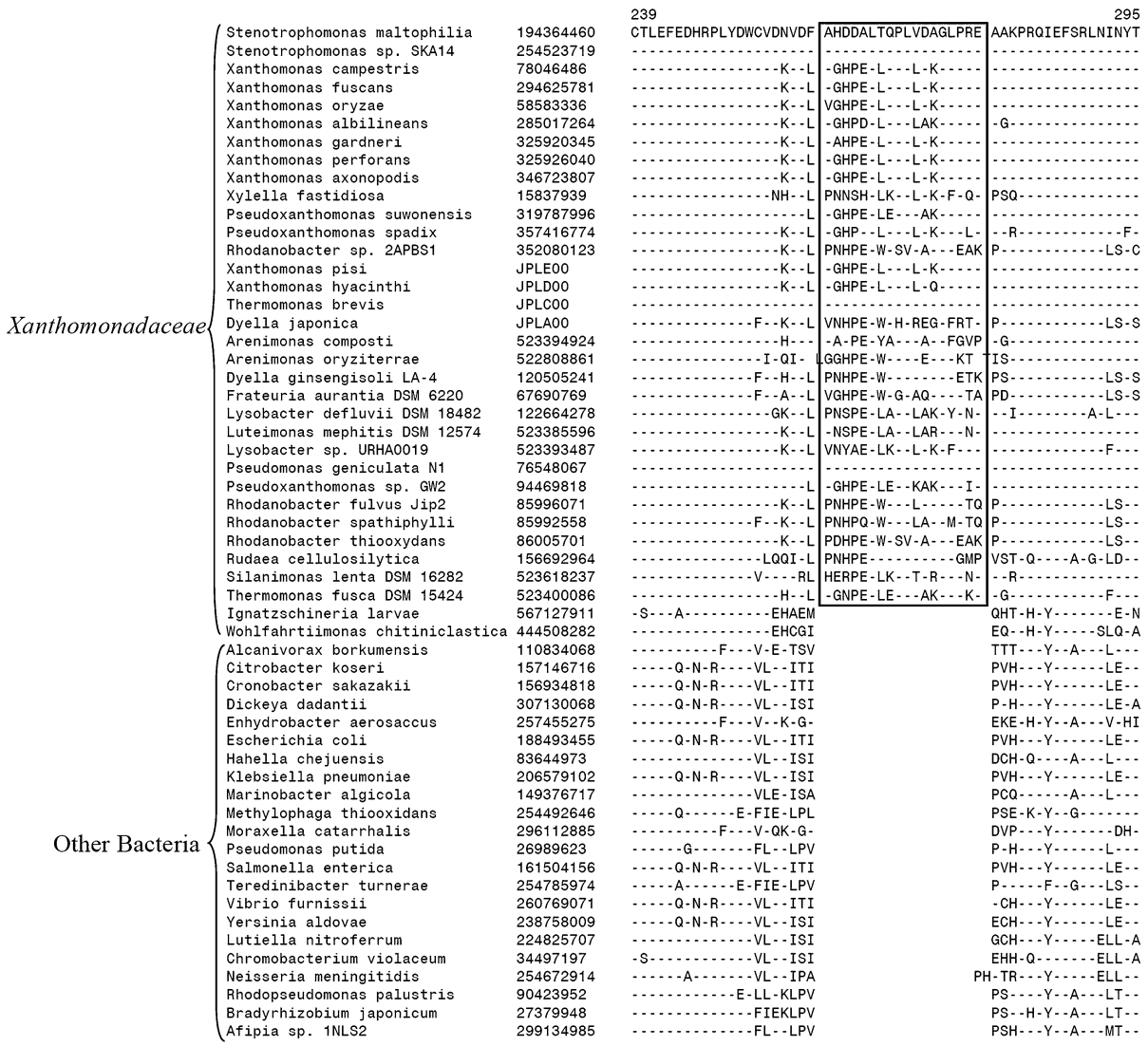


Fig. 3 A partial sequence alignment of the protein Glutaminyl t-RNA synthetase, showing a CSI (*boxed*) that is uniquely present in all members of the order *Xanthomonadales*. Sequence information for only representative *Xanthomonadales* and a limited number other bacteria is shown here. However, unless otherwise indicated, similar CSIs were present in all members of the indicated group and not detected in any other bacterial species in the top 250 BLAST hits. The *dashes* (-) in the alignments indicate identity with the residue in the top sequence. GenBank identification (GI) numbers for each sequence are indicated in the second column. Sequence information for 10 other CSIs that are specific for all sequenced *Xanthomonadales* is provided in Supplemental Figs. 1–9 and Table 2A

specific to the members of Clade 2 of the *Xanthomonadaceae* are presented in Supplemental Figs. 18–26 and summarized in Table 2C and D. Our analyses have not identified any CSIs uniquely found in all of the disparate members of the *Xanthomonadales* or uniquely shared by the genera *Wohlfahrtiimonas* and *Ignatzschineria* and the rest of the *Xanthomonadaceae*.

Discussion

The current phylogeny of the order *Xanthomonadales* is based largely on the analysis of 16S rRNA gene sequences (Saddler and Bradbury 2005a; Gutierrez et al. 2012; Losey et al. 2013). However, the 16S rRNA gene based phylogenies exhibit limited support for a single monophyletic clade consisting of all

Table 2 Conserved signature indels that are specific for different groups of *Xanthomonadales*

Protein name	GI number	Figure number	Indel size	Indel position
A: CSIs Specific for *Xanthomonadales* (*Lysobacterales*)				
Glutaminyl t-RNA synthetase	194364460	Figure 3	18 aa ins	239–295
GTP-binding protein	58580596	Sup. Fig. 1	4 aa ins	303–350
Queuine tRNA-ribosyltransferase	194365393	Sup. Fig. 2	1 aa ins	289–339
Lipoyl synthase	58583575	Sup. Fig. 3	2 aa ins	156–209
Lysyl-tRNA synthetase	194365604	Sup. Fig. 4	3 aa ins	34–85
Dihydroorotate dehydrogenase	71275790	Sup. Fig. 5	7 aa ins	164–213
Carbamoyl phosphate synthase large subunit	166711938	Sup. Fig. 6	1 aa ins	403–457
Aspartate aminotransferase	28197970	Sup. Fig. 7	1 aa del	316–354
DNA polymerase I	194367713	Sup. Fig. 8	1 aa del	28–65
DNA topoisomerase IV subunit B	84624476	Sup. Fig. 9	1 aa del	282–326
B: CSIs Specific for *Xanthomonadaceae* (*Lysobacteriaceae*)				
DNA polymerase III subunit alpha	77747494	Figure 4a	4 aa ins	522–576
Uroporphyrinogen decarboxylase	294625972	Sup. Fig. 10	5 aa ins	295–340
DNA polymerase I	21244827	Sup. Fig. 11	1 aa ins	136–180
Coproporphyrinogen III oxidase	194367710	Sup. Fig. 12	1 aa del	166–215
tRNA isopentenyltransferase	194365248	Sup. Fig. 13	5 aa ins	219–256
Protoheme IX farnesyltransferase	15837961	Figure 4b	4 aa ins	150–192
Ribose-5-phosphate isomerase A	194367055	Sup. Fig. 14	1 aa ins	127–169
Aspartyl-tRNA synthetase	194366904	Sup. Fig. 15	4 aa del	343–391
2-oxoglutarate dehydrogenase E1	194366403	Sup. Fig. 16	1 aa del	782–830
Asparagine synthetase B	194365058	Sup. Fig. 17	2 aa ins	98–132
C: CSIs Specific for *Rhodanobacteriaceae*				
Uridylyltransferase	495713257	Figure 5a	1 aa ins	272–310
Xanthomonadin exporter protein	383315419	Sup. Fig. 18	1 aa del	171–196
Signal peptidase	494142978	Sup. Fig. 19	24 aa ins	111–165
Tryptophan synthase subunit alpha	383316227	Sup. Fig. 20	1 aa del	121–157
D: CSIs Specific for all *Rhodanobacteriaceae* except *Rudaea*				
CDP-diacylglycerol–glycerol-3-phosphate 3-phosphatidyltransferase	469817908	Figure 5b	4 aa ins	63–120
Protease *tldD*	495491439	Sup. Fig. 21	1 aa del	75–126
S-adenosylmethionine decarboxylase	383315616	Sup. Fig. 22	2 aa del	71–123
DEAD/DEAH box helicase	494777343	Sup. Fig. 23	1 aa ins	720–756
F0F1 ATP synthase subunit gamma	495082201	Sup. Fig. 24	17 aa ins	177–230
Proline aminopeptidase P II	469819587	Sup. Fig. 25	1 aa del	135–178
Glycosyl transferase	469816683	Sup. Fig. 26	2 aa del	101–140

members of the *Xanthomonadales* (Yilmaz et al. 2013; Fig. 2). The current taxonomy of the order *Xanthomonadales* is not concordant with 16S rRNA gene based phylogenies of the members of the order and the nomenclature of the order *Xanthomonadales* and a majority of the family names within this order are problematic and not in accordance with the International Code of Nomenclature of Bacteria (Oren 2010; Yilmaz et al. 2013; Tindall 2014a, b). However, apart from the 16S rRNA gene, no reliable morphological, biochemical, or molecular characteristics are known that are specifically shared by all members of this order or its distinct subgroups and can be used for their demarcation and classification (Saddler and

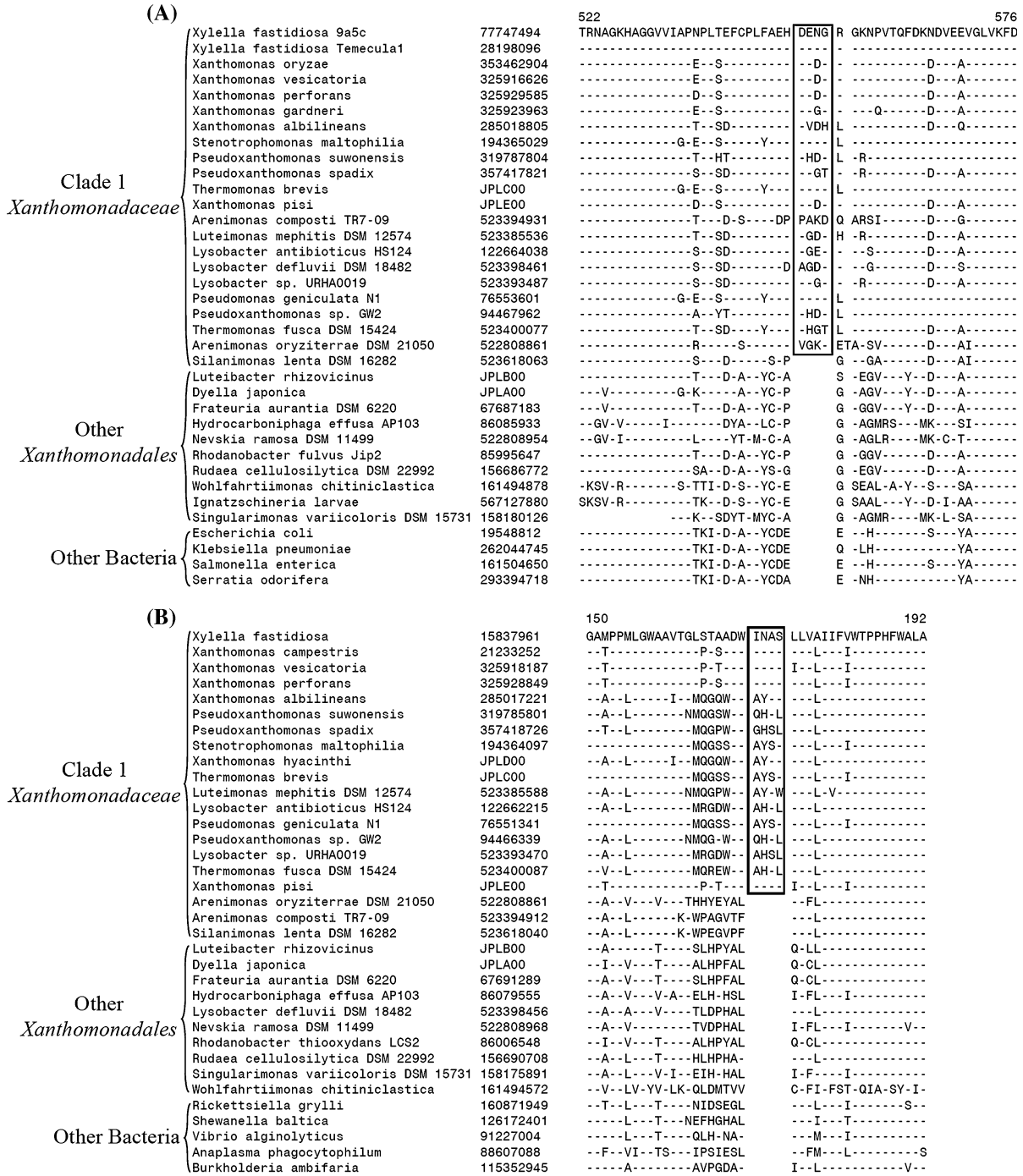
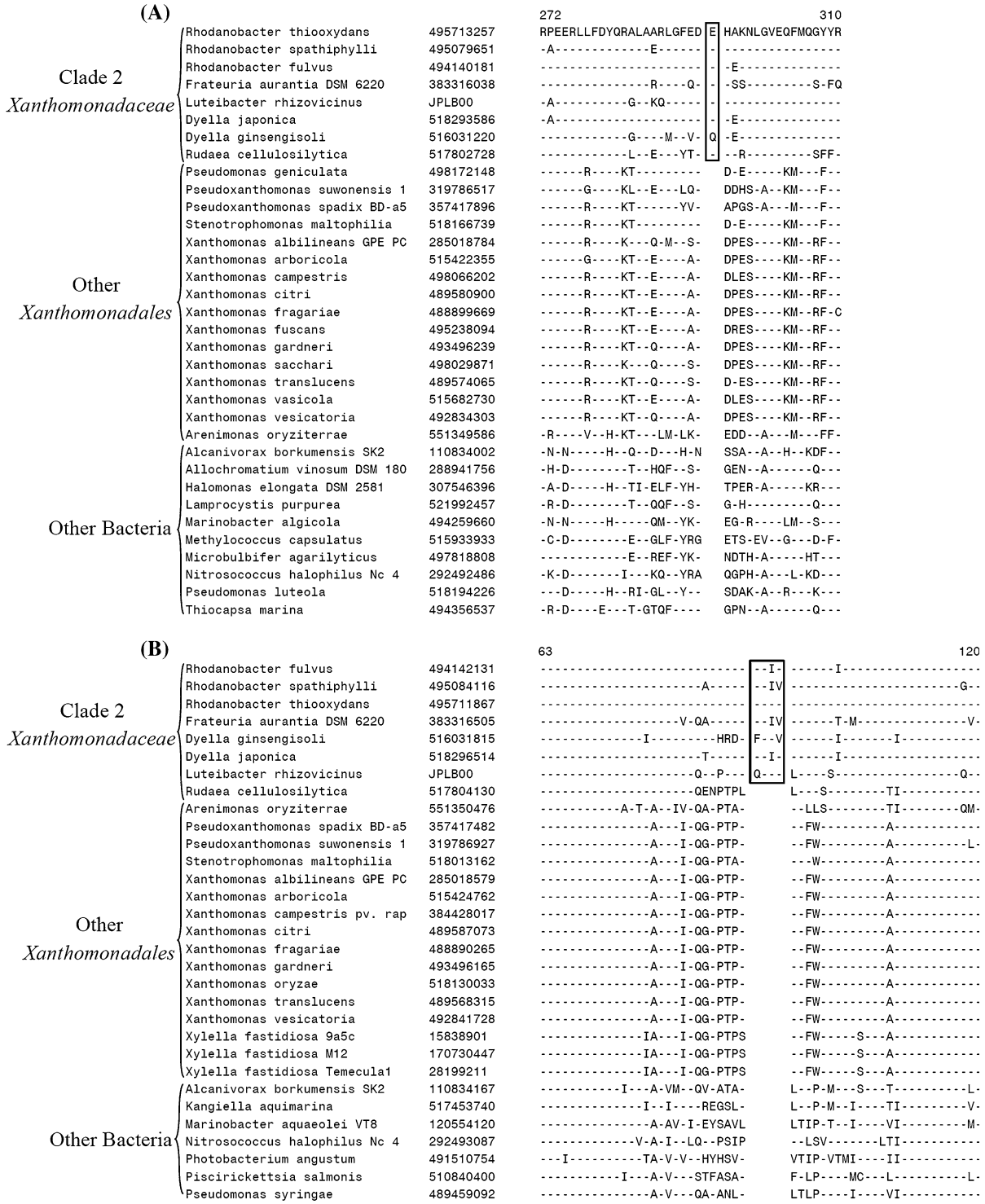


Fig. 4 Partial sequence alignments of **a** DNA polymerase III subunit alpha showing a 4 amino acid insertion (*boxed*) identified in all members of Clade 1 of the *Xanthomonadaceae* except *Silanimonas lenta* **b** the protein Protoheme IX farnesyltransferase showing a 4 amino acid insertion (*boxed*) identified in all members of Clade 1 of the *Xanthomonadaceae* except the genera *Arenimonas* and *Silanimonas*. Due to space constraints, sequence information for only representative *Xanthomonadales* and a limited number other bacteria is shown here, but similar CSIs were present in all members of the indicated group and not detected in any other bacterial species in the top 250 BLAST hits. Sequence information for other CSIs showing similar group specificities are presented in Supplemental Figs. 10–17 and summarized in Tables 2B


(A)
272
310
Clade 2
Xanthomonadaceae
Other
Xanthomonadales
Other Bacteria
Rhodanobacter thiooxydans 495713257 RPEERLLFDYQRALAARLGFED E HAKNLGVEQFMQGYYR
Rhodanobacter spathiphylli 495079651 -A----------E------ - ----------
Rhodanobacter fulvus 494140181 ---------- - -E----------
Frateuria aurantia DSM 6220 383316038 ----------R---Q- - -SS---------S-FQ
Luteibacter rhizovicinus JPLB00 -A---------G--KQ----- - ----------
Dyella japonica 518293586 -A---------- - -E----------
Dyella ginsengisoli 516031220 ----------G---M--V- Q -E----------
Rudaea cellulosilytica 517802728 ----------L--E---YT- - --R---------SFF-
Pseudomonas geniculata 498172148 ------R----KT--------- D-E-----KM---F--
Pseudoxanthomonas suwonensis 1 319786517 ------G----KL--E---LQ- DDHS-A--KM---F--
Pseudoxanthomonas spadix BD-a5 357417896 ------R----KT------YV- APGS-A---M---F--
Stenotrophomonas maltophilia 518166739 ------R----KT--------- D-E-----KM---F--
Xanthomonas albilineans GPE PC 285018784 ------R----K---Q-M--S- DPES----KM--RF--
Xanthomonas arboricola 515422355 ------G----KT--E----A- DPES----KM--RF--
Xanthomonas campestris 498066202 ------R----KT--E----A- DLES----KM--RF--
Xanthomonas citri 489580900 ------R----KT--E----A- DPES----KM--RF--
Xanthomonas fragariae 488899669 ------R----KT--E----A- DPES----KM--RF-C
Xanthomonas fuscans 495238094 ------R----KT--E----A- DRES----KM--RF--
Xanthomonas gardneri 493496239 ------R----KT--Q----A- DPES----KM--RF--
Xanthomonas sacchari 498029871 ------R----K---Q----S- DPES----KM--RF--
Xanthomonas translucens 489574065 ------R----KT--Q----S- D-ES----KM--RF--
Xanthomonas vasicola 515682730 ------R----KT--E----A- DLES----KM--RF--
Xanthomonas vesicatoria 492834303 ------R----KT--Q----A- DPES----KM--RF--
Arenimonas oryziterrae 551349586 -R----V--H-KT---LM-LK- EDD--A---M---FF-
Alcanivorax borkumensis SK2 110834002 -N-N-----H--Q--D---H-N SSA--A--H--KDF--
Allochromatium vinosum DSM 180 288941756 -H-D--------T--HQF--S- GEN--A------Q---
Halomonas elongata DSM 2581 307546396 -A-D-----H--TI-ELF-YH- TPER-A-----KR---
Lamprocystis purpurea 521992457 -R-D--------T--QQF--S- G-H---------Q---
Marinobacter algicola 494259660 -N-N-----H-----QM--YK- EG-R----LM--S---
Methylococcus capsulatus 515933933 -C-D--------E--GLF-YRG ETS-EV--G---D-F-
Microbulbifer agarilyticus 497818808 ------------E--REF-YK- NDTH-A-----HT---
Nitrosococcus halophilus Nc 4 292492486 -K-D-------I---KQ--YRA QGPH-A---L-KD---
Pseudomonas luteola 518194226 ---D-----H--RI-GL--Y-- SDAK-A--R---K---
Thiocapsa marina 494356537 -R-D----E---T-GTQF---- GPN--A------Q---
(B)
63
120
Clade 2
Xanthomonadaceae
Other
Xanthomonadales
Other Bacteria
Rhodanobacter fulvus 494142131 ---------- --I- ------I----------
Rhodanobacter spathiphylli 495084116 ----------A----- --IV ----------G--
Rhodanobacter thiooxydans 495711867 ---------- ---- ----------
Frateuria aurantia DSM 6220 383316505 ----------V-QA----- --IV ------T-M----------V-
Dyella ginsengisoli 516031815 ----------I---------HRD- F--V ------I-------I----------
Dyella japonica 518296514 ----------T----- --I- ------I----------
Luteibacter rhizovicinus JPLB00 ----------Q--P--- Q--- L----S---------Q--
Rudaea cellulosilytica 517804130 ----------QENPTPL L---S--------TI----------
Arenimonas oryziterrae 551350476 ----------A-T-A--IV-QA-PTA- --LLS--------TI--------QM-
Pseudoxanthomonas spadix BD-a5 357417482 ----------A---I-QG-PTP- --FW---------A----------
Pseudoxanthomonas suwonensis 1 319786927 ----------A---I-QG-PTP- --FW---------A---------L-
Stenotrophomonas maltophilia 518013162 ----------A---I-QG-PTA- ---W---------A----------
Xanthomonas albilineans GPE PC 285018579 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas arboricola 515424762 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas campestris pv. rap 384428017 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas citri 489587073 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas fragariae 488890265 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas gardneri 493496165 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas oryzae 518130033 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas translucens 489568315 ----------A---I-QG-PTP- --FW---------A----------
Xanthomonas vesicatoria 492841728 ----------A---I-QG-PTP- --FW---------A----------
Xylella fastidiosa 9a5c 15838901 ----------IA---I-QG-PTPS --FW-----S---A----------
Xylella fastidiosa M12 170730447 ----------IA---I-QG-PTPS --FW-----S---A----------
Xylella fastidiosa Temecula1 28199211 ----------IA---I-QG-PTPS --FW-----S---A----------
Alcanivorax borkumensis SK2 110834167 ----------I---A-VM--QV-ATA- L--P-M---S---T---------L-
Kangiella aquimarina 517453740 ----------I--I----REGSL- L--P-M--I----TI---------V-
Marinobacter aquaeolei VT8 120554120 ----------A-AV-I-EYSAVL LTIP-T--I----VI---------M-
Nitrosococcus halophilus Nc 4 292493087 ----------V-A-I--LQ--PSIP --LSV-------LTI----------
Photobacterium angustum 491510754 ---I----------TA-V-V--HYHSV- VTIP-VTMI----II----------
Piscirickettsia salmonis 510840400 ----------I---A-V---STFASA- F-LP----MC---L---------L-
Pseudomonas syringae 489459092 ----------A-V---QA-ANL- LTLP----I----VI----------

◀**Fig. 5** Partial sequence alignments of **a** the protein Uridylyltransferase showing a 1 amino acid insertion (*boxed*) identified in all members of Clade 2 of the *Xanthomonadaceae* **b** the protein CDP-diacylglycerol–glycerol-3-phosphate 3-phosphatidyltransferase showing a 4 amino acid insertion (*boxed*) identified in all members of Clade 2 of the *Xanthomonadaceae* except the early branching genus *Rudaea*. Sequence information for only representative *Xanthomonadales* and a limited number other bacteria is shown here, but similar CSIs were not detected in any other bacterial species in the top 250 BLAST hits. Sequence information for the other CSIs specific to the Clade 2 *Xanthomonadaceae* are presented in Supplemental Figs. 18–26 and summarized in Table 2C and D

Bradbury 2005a; Gutierrez et al. 2012; Losey et al. 2013). In this work, we have completed a robust phylogenetic analysis of the order *Xanthomonadales* and have utilized comparative genomic techniques to identify large numbers of novel molecular markers of common evolutionary decent (CSIs) shared by subgroups within the *Xanthomonadales*. The CSIs identified in this work both supplement gene based phylogenies and demarcate the groups within the *Xanthomonadales* in more definitive molecular terms. A summary diagram of the identified CSIs and the species in which they are found is shown in Fig. 6.

The results of phylogenetic analyses presented here do not support a monophyletic grouping of the members of the order *Xanthomonadales* and no CSI was identified that is uniquely shared by all members of this order. In phylogenetic trees, members of the order *Xanthomonadales* formed two main clades, one grouping together most of the members from the family *Xanthomonadaceae*, whereas the other clade was comprised of members from the families *Algiphilaceae* (containing the genus *Algiphilus*) and *Solimonadaceae* (containing the genera *Fontimonas*, *Singularimonas* and *Solimonas*) and genera related to these two families (viz. *Alkanibacter*, *Hydrocarboniphaga*, *Nevskia* and *Steroidobacter*). The lack of any identified CSIs or a consistent phylogenetic relationship between these two clades suggests that they may represent distinct evolutionary lineages within the *Gammaproteobacteria*. Additionally, in both our concatenated protein tree and the 16S rRNA gene tree, members of the clade containing *Algiphilaceae* and *Solimonadaceae* families consistently grouped with the members of the genus *Salinisphaera*, the sole members of the family "Salinisphaeraceae", suggesting that the species from these groups may share a common ancestor exclusive of the *Xanthomonadaceae* and other *Gammaproteobacteria*. The genera *Wohlfahrtiimonas* and *Ignatzschineria* branch distinctly from the *Xanthomonadales* in a clade with members of the order *Cardiobacteriales* in our concatenated protein based phylogenetic tree and show limited phylogenetic association with the other members of the *Xanthomonadales* in our 16S rRNA tree. Due to this inconsistent branching, further research will be required to accurately assess the phylogenetic placement of the genera *Wohlfahrtiimonas* and *Ignatzschineria,* but the available data suggests that they do not belong to the order *Xanthomonadales* sensu stricto.

Our work has identified 10 CSIs that support a monophyletic grouping of a majority of the members of the order *Xanthomonadales* that are currently part of the family *Xanthomonadaceae*. These CSIs were initially identified in our earlier comparative genomic study (Naushad and Gupta 2013) and the sequence information for them was updated in the present work for a large number of additional *Xanthomonadales*, including 5 genomes which were sequenced, *de novo*, for this study. Our earlier work identified 13 CSIs which were specific to this group (Naushad and Gupta 2013). Of these, all but 3 CSIs were found to be still specific to the whole group, while the remaining three CSIs were found to be specific for subsets of this large group. The observed specificity of the previously identified CSIs for a distinct bacterial group despite a large increase in the number of analyzed genomes strongly indicate that they constitute reliable molecular characteristics with predictive ability for distinguishing and demarcation of evolutionarily related bacterial groups.

Our work also provides strong molecular and phylogenetic support for the existence of two distinct clades within the *Xanthomonadaceae*: One clade consists of the genera *Xanthomonas*, *Xylella*, *Stenotrophomonas*, *Lutimonas*, *Lysobacter* and their relatives (Clade 1), whereas the other clade groups together members of the genera *Rudaea*, *Dylella*, *Lutibacter*, *Rhodanobacter* and their relatives (Clade 2). The members of these monophyletic clades branch distinctly from each other with strong bootstrap support in both the concatenated protein tree as well as in the 16S rRNA gene trees. Importantly, Clade 1 and Clade 2 are also supported by 10 and 11 identified CSIs, respectively, which serve to clearly distinguish them from each other and every other bacterial group

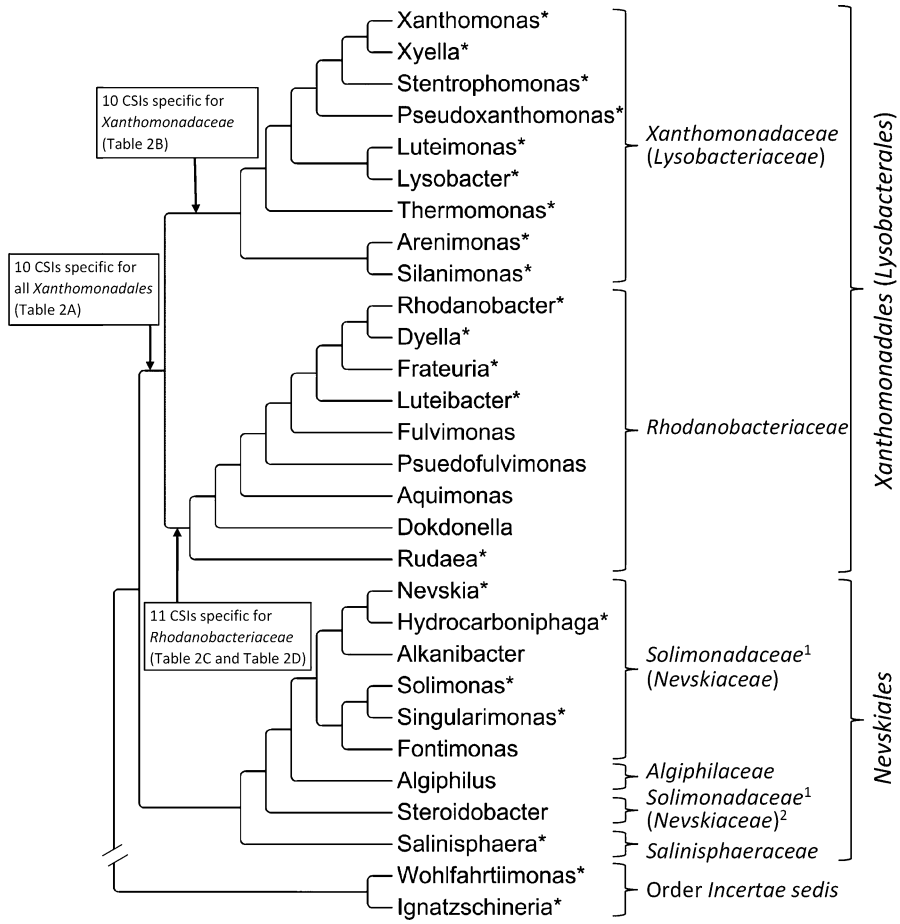


Fig. 6 A summary of the evolutionary relationships of the *Xanthomonadales* genera based upon phylogenetic analyses and the identified CSIs. Genera with genome sequenced members are indicated with *asterisks* (*). The distribution of the identified CSIs and the proposed reclassification of taxonomic groups are indicated. The genera *Wohlfahrtiimonas* and *Ignatzschineria* do not branch with the members of the order *Xanthomonadales* and hence are regarded as order *incertae sedis*. The families *Sinobacteraceae* and *Solimonadaceae* are synonymous; however, only the name of the family *Solimonadaceae* is shown here ([1]). The placement of the genus *Steroidobacter* within the family *Solimonadaceae* (*Nevskiaceae*) is tentative until a more detailed phylogenetic analysis can be completed for this genus ([2])

in molecular terms. This evidence suggests that the Clade 1 and Clade 2 represent two phylogenetically and molecularly distinguishable evolutionary lineages.

Taxonomic implications

Based on the branching of the members of the order *Xanthomonadales* in the concatenated protein and 16S rRNA gene trees and the large number of identified molecular markers (CSIs) that are specific for this group of bacteria, the following main inferences regarding the phylogeny of the *Xanthomonadales* can be derived.

(1) The order presently designated as *Xanthomonadales* contains 2 highly divergent phylogenetic groups, one made up of the members of the family *Xanthomonadaceae* and the other made up of the members of the families *Algiphilaceae*, *Solimonadaceae*, and "Salinisphaeraceae"

(2) The family presently designated as *Xanthomonadaceae*, which harbours a majority of the members from the order *Xanthomonadales*, also contains 2 distinct and distinguishable phylogenetic groups, one consisting of the genera *Xanthomonas*, *Xylella*, *Stenotrophomonas*, *Lutimonas*, *Lysobacter* and their relatives (Clade 1) and another clade consisting of the genera *Rudaea*, *Dylella*, *Lutibacter*, *Rhodanobacter* and their relatives (Clade 2)

Thus, the current taxonomy of the order *Xanthomonadales* does not accurately reflect the evolutionary histories of its members which exhibit enormous genetic diversity. In order to alleviate the taxonomic incongruences within the order *Xanthomonadales*, we

propose that the families *Algiphilaceae* (containing the genus *Algiphilus*) and *Solimonadaceae* (or *Nevskiaceae*) (containing the genera *Fontimonas*, *Singularimonas* and *Solimonas*) and genera related to these two families (viz. *Alkanibacter*, *Hydrocarboniphaga*, *Nevskia* and *Steroidobacter*), which do not branch with the other members of the order *Xanthomonadales*, be placed within a novel order, *Nevskiales* ord. nov., along with their closest evolutionary relatives, the members of the family *Salinisphaeraceae* fam. nov. Further, to recognize the presence of two distinct groups within the family presently designated as *Xanthomonadaceae,* the members of this family should be divided into two families: the family *Xanthomonadaceae* (containing the genera *Arenimonas*, *Luteimonas*, *Lysobacter*, *Metallibacterium*, *Panacagrimonas*, *Pseudoxanthomonas*, *Silanimonas*, *Stenotrophomonas*, *Thermomonas*, *Xanthomonas*, and *Xylella*) and a novel family, *Rhodanobacteraceae* fam. nov. (containing the genera *Aquimonas*, *Chiayiivirga*, *Dokdonella*, *Dyella*, *Frateuria*, *Fulvimonas*, *Luteibacter*, *Pseudofulvimonas*, *Rhodanobacter*, and *Rudaea*). The remaining two genera, *Wohlfahrtiimonas* and *Ignatzschineria*, whose taxonomic affiliation to the above two orders is not supported should be regarded as order *incertae sedis*.

Additionally, the present proposal also serves to help rectify several problems associated with the nomenclature of the order *Xanthomonadales*, the family *Xanthomonadaceae*, and the family *Solimonadaceae* (Tindall 2014b). It has been noted previously (Oren 2010; Tindall 2014a, b) that the names of these taxa are later synonyms for the order *Lysobacterales*, the family *Lysobacteraceae*, and the family *Nevskiaceae*, respectively. In recognition of these nomenclatural concerns, we are providing emended descriptions of the order *Lysobacterales*, the family *Lysobacteraceae*, and the family *Nevskiaceae*, which indicate that they are earlier synonyms of the order *Xanthomonadales*, the family *Xanthomonadaceae*, and the family *Solimonadaceae*, respectively. Descriptions of *Rhodanobacteraceae* fam. nov., *Nevskiales* ord. nov., and *Salinisphaeraceae* fam. nov. and emended descriptions of the order *Lysobacterales* (*Xanthomonadales*), the family *Lysobacteraceae* (*Xanthomonadaceae*), and the family *Nevskiaceae* (*Solimonadaceae*) are provided below.

Emended description of the order *Lysobacterales* Christensen and Cook (1978) (Approved Lists 1980)

Synonym: *Xanthomonadales* Saddler and Bradbury (2005a, b).

The order contains two families, *Lysobacteraceae* and *Rhodanobacteraceae*. Organisms are rods, 0.2–1.8 μm in diameter and 0.8–70 μm in length. Cells are both motile and non-motile. Organisms are aerobic, or facultatively anaerobic. Organisms are chemoorganotrophic and non-spore-forming. Organisms within this order may be either positive or negative in both oxidase and catalase tests. The G + C content of the DNA is 42–75 (mol%).The type genus of the order is *Lysobacter* Christensen and Cook (1978) (Approved Lists 1980) (Skerman et al. 1980) emend. Park et al. (2008).

Organisms from this order are distinguished from all other bacteria examined to date by 10 conserved signature indels in Glutaminyl t-RNA synthetase, GTP-binding protein, Queuine tRNA-ribosyltransferase, Lipoyl synthase, Lysyl-tRNA synthetase, Dihydroorotate dehydrogenase, Carbamoyl phosphate synthase large subunit, Aspartate aminotransferase, DNA polymerase I, and DNA topoisomerase IV subunit B (Tables 2A).

Emended description of the family *Lysobacteraceae* Christensen and Cook (1978) (Approved Lists 1980)

Synonym: *Xanthomonadaceae* Saddler and Bradbury (2005a, b).

The family contains twelve genera, *Arenimonas*, *Luteimonas*, *Lysobacter*, *Metallibacterium*, *Panacagrimonas*, *Pseudoxanthomonas*, *Silanimonas*, *Stenotrophomonas*, *Thermomonas*, *Xanthomonas* and *Xylella*. Organisms are rods, 0.2–1.8 μm in diameter and 0.8–70 μm in length. Cells are both motile and non-motile. Organisms are aerobic, or facultatively anaerobic. Organisms are chemoorganotrophic and non-spore-forming. Organisms within this family may be either positive or negative in both oxidase and catalase tests. The G+C content of the DNA is 42–70 (mol%). The type genus of the family is *Lysobacter*

Christensen and Cook (1978) (Approved Lists 1980) emend. Park et al. (2008).

Organisms from this order are distinguished from all other bacteria examined to date by 10 conserved signature indels in DNA polymerase III subunit alpha, Uroporphyrinogen decarboxylase, DNA polymerase I, Coproporphyrinogen III oxidase, tRNA isopentenyltransferase, Protoheme IX farnesyltransferase, Ribose-5-phosphate isomerase A, Aspartyl-tRNA synthetase, 2-oxoglutarate dehydrogenase E1, and Asparagine synthetase B (Tables 2B).

Description of *Rhodanobacteraceae* fam. nov

Rhodanobacteraceae (Rho.da.no.bac.ter.a,ce'ae N.L. masc. n. *Rhodanobacter* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Rhodanobacteraceae* the family whose nomenclatural type is the genus *Rhodanobacter*).

The family contains nine genera, *Aquimonas*, *Dokdonella*, *Dyella*, *Frateuria*, *Fulvimonas*, *Luteibacter*, *Pseudofulvimonas*, *Rhodanobacter* and *Rudaea*. Organisms are rods, 0.3–0.5 μm in diameter and 1–4.5 μm in length. Cells are both motile and non-motile. Organisms are aerobic, chemoorganotrophic, and non-spore-forming. Organisms within this family may be either positive or negative in both oxidase and catalase tests. The G + C content of the DNA is 62–75 (mol%). The type genus of the family is *Rhodanobacter* Nalin et al. (1999).

Organisms from this order are distinguished from all other bacteria examined to date by 11 conserved signature indels in Uridylyltransferase, a xanthomonadin exporter protein, a signal peptidase, Tryptophan synthase subunit alpha, CDP-diacylglycerol–glycerol-3-phosphate 3-phosphatidyltransferase, Protease *tldD*, S-adenosylmethionine decarboxylase, DEAD/DEAH box helicase, F0F1 ATP synthase subunit gamma, Proline aminopeptidase P II, and Glycosyl transferase (Table 2C, D).

Description of *Nevskiales* ord. nov

Nevskiales (Nev.ski.a'les. N.L. fem. n. *Nevskia* type genus of the order; *-ales* ending to denote an order; N.L. fem. pl. n. *Nevskiales* the order whose nomenclatural type is the genus *Nevskia*).

The order contains three families, *Algiphilaceae, Salinisphaeraceae,* and *Nevskiaceae*. Organisms are rods and cocci, 0.6–1.3 μm in diameter and 0.4–2 μm in length. Cells are non-motile or motile by means of a one or more polar flagella. Organisms are aerobic, or facultatively anaerobic. Organisms are chemoorganotrophic and non-spore-forming. Oxidase and catalase positive. The G+C content of the DNA is 60–68 (mol%). The type genus of the order is *Nevskia* Famintzin 1892 (Approved Lists 1980).

Emended Description of the family *Nevskiaceae* Henrici and Johnson 1935 (Approved Lists 1980)

Synonyms: *Sinobacteraceae* Zhou et al. (2008), *Solimonadaceae* Losey et al. (2013).

The family contains six genera, *Alkanibacter*, *Fontimonas*, *Hydrocarboniphaga*, *Nevskia*, *Solimonas* and *Steroidobacter*.[1] Organisms are rods, 0.6–0.85 μm in diameter and 0.9–2 μm in length. Cells are non-motile or motile by means of a single polar flagellum. Organisms are aerobic, or facultatively anaerobic. Organisms are chemoorganotrophic and non-spore-forming. Oxidase and catalase positive. The G + C content of the DNA is 60–65 (mol%). The type genus of the family is *Nevskia* Famintzin 1892 (Approved Lists 1980).

Description of *Salinisphaeraceae* fam. nov

Salinisphaeraceae (Sa.li.ni.sphae.ra.ce'ae. N.L. fem. n. *Salinisphaera* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Salinisphaeraceae* the family whose nomenclatural type is the genus *Salinisphaera*).

The family contains one genus, *Salinisphaera,* which is also the type genus of the family. The description of the family is the same as that of the

[1] The genus *Steroidobacter* does not branch monophyletically with the other members of the family *Nevskiaceae* in 16S rRNA gene based phylogenies. However, *Steroidobacter* is clearly distinct from the order *Xanthomonadales* and family *Xanthomonadaceae* in which it was previously placed. Its placement within the family *Nevskiaceae* is tentative until more detailed phylogenetic analysis can be completed for this genus.

genus *Salinisphaera* Antunes et al. (2003) emend. Shimane et al. (2013).

Acknowledgments We thank Professor Iain Sutcliffe for valuable comments and suggestions for improvement of this manuscript. This work was supported by a research grant from the Natural Science and Engineering Research Council of Canada to RSG.

References

Adeolu M, Gupta RS (2013) Phylogenomics and molecular signatures for the order *Neisseriales*: proposal for division of the order *Neisseriales* into the emended family *Neisseriaceae* and *Chromobacteriaceae* fam. nov. Anton Leeuw Int J G 104(1):1–24

Antunes A, Eder W, Fareleira P, Santos H, Huber R (2003) *Salinisphaera shabanensis* gen. nov., sp. nov., a novel, moderately halophilic bacterium from the brine–seawater interface of the Shaban Deep, Red Sea. Extremophiles 7(1):29–34

Bhandari V, Ahmod NZ, Shah HN, Gupta RS (2013) Molecular signatures for *Bacillus* species: demarcation of the *Bacillus subtilis* and *Bacillus cereus* clades in molecular terms and proposal to limit the placement of new species into the genus *Bacillus*. Int J Syst Evol Microbiol 63(7):2712–2726

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4):540–552

Charlebois RL, Doolittle WF (2004) Computing prokaryotic gene ubiquity: Rescuing the core from extinction. Genome Res 14(12):2469–2477

Chatterjee S, Almeida RPP, Lindow S (2008) Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. Annu Rev Phytopathol 46:243–271

Chen J, Xie G, Han S, Chertkov O, Sims D, Civerolo EL (2010) Whole genome sequences of two *Xylella fastidiosa* strains (M12 and M23) causing almond leaf scorch disease in California. J Bacteriol 192(17):4534

Christensen P, Cook FD (1978) *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int J Syst Bacteriol 28(3):367–393

Ciccarelli FD, Doerks T, Von Mering C, Creevey CJ, Snel B, Bork P (2006) Toward automatic reconstruction of a highly resolved tree of life. Science 311(5765):1283–1287

Cole J, Wang Q, Fish J, Chai B, McGarrell D, Sun Y, Brown C, Porras-Alfaro A, Kuske C, Tiedje J (2014) Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 42(1):D633

Crossman LC, Gould VC, Dow JM, Vernikos GS, Okazaki A, Sebaihia M, Saunders D, Arrowsmith C, Carver T, Peters N (2008) The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. Genome Biol 9(4):R74

Cutino-Jimenez AM, Martins-Pinheiro M, Lima WC, Martin-Tornet A, Morales OG, Menck CFM (2010) Evolutionary placement of *Xanthomonadales* based on conserved protein signature sequences. Mol Phylogen Evol 54(2):524–534

da Silva AR, Ferro JA, Reinach F, Farah C, Furlan L, Quaggio R, Monteiro-Vitorello C, Van Sluys M, Almeida N, Alves L (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. Nature 417(6887):459–463

Darrasse A, Carrere S, Barbe V, Boureau T, Arrieta-Ortiz ML, Bonneau S, Briand M, Brin C, Cociancich S, Durand K et al (2013) Genome sequence of *Xanthomonas fuscans* subsp. *fuscans* strain 4834-R reveals that flagellar motility is not a general feature of xanthomonads. BMC Genomics 14:761

De Clercq D, Van Trappen S, Cleenwerck I, Ceustermans A, Swings J, Coosemans J, Ryckeboer J (2006) *Rhodanobacter spathiphylli* sp. nov., a gammaproteobacterium isolated from the roots of Spathiphyllum plants grown in a compost-amended potting mix. Int J Syst Evol Microbiol 56(Pt 8):1755–1759

Gao B, Gupta RS (2012a) Microbial systematics in the post-genomics era. Anton Leeuw Int J G 101(1):45–54

Gao B, Gupta RS (2012b) Phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria. Microbiol Mol Biol Rev 76(1):66–112

Gao B, Mohan R, Gupta RS (2009) Phylogenomics and protein signatures elucidating the evolutionary relationships among the *Gammaproteobacteria*. Int J Syst Evol Microbiol 59(2):234–247

Gupta RS (1998) Protein phylogenies and signature sequences: a reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol Mol Biol Rev 62(4):1435

Gupta RS (2010) Applications of conserved indels for understanding microbial phylogeny. In: Oren A, Papke RT (eds) Molecular phylogeny of microorganisms. Caister Academic Press, Norfolk, pp 135–150

Gupta RS (2014) Identification of conserved indels that are useful for classification and evolutionary studies Methods in Microbiology, vol 41. Academic Press: 10.1016/bs.mim.2014.05.003

Gupta RS, Lali R (2013) Molecular signatures for the phylum Aquificae and its different clades: proposal for division of the phylum Aquificae into the emended order *Aquificales*, containing the families *Aquificaceae* and *Hydrogenothermaceae*, and a new order *Desulfurobacteriales* ord. nov., containing the family *Desulfurobacteriaceae*. Anton Leeuw Int J G 104(3):349–368

Gupta RS, Mahmood S, Adeolu M (2013) A phylogenomic and molecular signature based approach for characterization of the phylum Spirochaetes and its major clades: proposal for a taxonomic revision of the phylum. Frontiers in microbiology 4:217

Gutierrez T, Green DH, Whitman WB, Nichols PD, Semple KT, Aitken MD (2012) *Algiphilus aromaticivorans* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium isolated from a culture of the marine dinoflagellate *Lingulodinium polyedrum*, and proposal of *Algiphilaceae* fam. nov. Int J Syst Evol Microbiol 62(11):2743–2749

Henrici AT, Johnson DE (1935) Studies of Freshwater Bacteria: II. Stalked Bacteria, a New Order of Schizomycetes. J Bacteriol 30(1):61–93

Im WT, Lee ST, Yokota A (2004) *Rhodanobacter fulvus* sp. nov., a beta-galactosidase-producing gammaproteobacterium. J Gen Appl Microbiol 50(3):143–147

Jalan N, Kumar D, Yu F, Jones JB, Graham JH, Wang N (2013) Complete genome sequence of *Xanthomonas citri* subsp. citri Strain Aw12879, a restricted-host-range citrus canker-causing bacterium. Genome Announc 1(3):e00235-13

Jaufeerally-Fakim Y, Dookun A (2000) Extraction of high quality DNA from polysaccharides-secreting xanthomonads. Sci Technol Res J Univ Maurit 6:33–40

Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with clustal X. Trends Biochem Sci 23(10):403

Kostka JE, Green SJ, Rishishwar L, Prakash O, Katz LS, Marino-Ramirez L, Jordan IK, Munk C, Ivanova N, Mikhailova N et al (2012) Genome sequences for six *Rhodanobacter* strains, isolated from soils and the terrestrial subsurface, with variable denitrification capabilities. J Bacteriol 194(16):4461–4462

Kyrpides N, Overbeek R, Ouzounis C (1999) Universal protein families and the functional content of the last universal common ancestor. J Mol Evol 49(4):413–423

Lee B-M, Park Y-J, Park D-S, Kang H-W, Kim J-G, Song E-S, Park I-C, Yoon U-H, Hahn J-H, Koo B-S (2005) The genome sequence of *Xanthomonas oryzae* pathovar oryzae KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Res 33(2):577–586

Lee CS, Kim KK, Aslam Z, Lee ST (2007) *Rhodanobacter thiooxydans* sp. nov., isolated from a biofilm on sulfur particles used in an autotrophic denitrification process. Int J Syst Evol Microbiol 57(Pt 8):1775–1779

Lee SH, Jin HM, Lee HJ, Kim JM, Jeon CO (2012) Complete genome sequence of the BTEX-degrading bacterium *Pseudoxanthomonas spadix* BD-a59. J Bacteriol 194(2):544

Looney WJ, Narita M, Mühlemann K (2009) *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. Lancet Infect Dis 9(5):312–323

Losey NA, Stevenson BS, Verbarg S, Rudd S, Moore ER, Lawson PA (2013) *Fontimonas thermophila* gen. nov., sp. nov., a moderately thermophilic bacterium isolated from a freshwater hot spring, and proposal of *Solimonadaceae* fam. nov. to replace *Sinobacteraceae* Zhou et al. 2008. Int J Syst Evol Microbiol 63(1):254–259

Meidanis J, Braga MD, Verjovski-Almeida S (2002) Whole-genome analysis of transporters in the plant pathogen *Xylella fastidiosa*. Microbiol Mol Biol Rev 66(2):272–299

Nalin R, Simonet P, Vogel TM, Normand P (1999) *Rhodanobacter lindaniclasticus* gen. nov., sp. nov., a lindane-degrading bacterium. Int J Syst Bacteriol 49(1):19–23

Naushad HS, Gupta RS (2013) Phylogenomics and molecular signatures for species from the plant pathogen-containing order *Xanthomonadales*. PLoS ONE 8(2):e55216

Oren A (2010) The phyla of prokaryotes—cultured and uncultured. In: Oren A, Papke RT (eds) Molecular phylogeny of microorganisms. Caister Academic Press, Norfolk, pp 85–107

Park JH, Kim R, Aslam Z, Jeon CO, Chung YR (2008) *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. Int J Syst Evol Microbiol 58(2):387–392

Parte AC (2013) LPSN–list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 42:D613–D616

Pieretti I, Royer M, Barbe V, Carrere S, Koebnik R, Cociancich S, Couloux A, Darrasse A, Gouzy J, Jacques MA et al (2009) The complete genome sequence of *Xanthomonas albilineans* provides new insights into the reductive genome evolution of the xylem-limited *Xanthomonadaceae*. BMC Genomics 10:616

Qian W, Jia Y, Ren SX, He YQ, Feng JX, Lu LF, Sun Q, Ying G, Tang DJ, Tang H et al (2005) Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. Genome Res 15(6):757–767

Rokas A, Holland PWH (2000) Rare genomic changes as a tool for phylogenetics. Trends Ecol Evol 15(11):454–459

Rokas A, Williams BL, King N, Carroll SB (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425(6960):798–804

Ryan RP, Vorhölter F-J, Potnis N, Jones JB, Van Sluys M-A, Bogdanove AJ, Dow JM (2011) Pathogenomics of *Xanthomonas*: understanding bacterium–plant interactions. Nat Rev Microbiol 9(5):344–355

Saddler GS, Bradbury JF (2005a) Order III. *Xanthomonadales* ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, Boone, Vos P, Goodfellow M, Rainey FA, Schleifer K-H (eds) Bergey's manual of systematic bacteriology. Springer, Austin, pp 63–122

Saddler GS, Bradbury JF (2005b) *Xanthomonadaceae* fam. nov Validation of publication of new names and new combinations previously effectively published outside the IJSEM, List no 106. Int J Syst Evol Microbiol 55:2235–2238

Salzberg SL, Sommer DD, Schatz MC, Phillippy AM, Rabinowicz PD, Tsuge S, Furutani A, Ochiai H, Delcher AL, Kelley D (2008) Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. BMC Genomics 9(1):204

Shimane Y, Tsuruwaka Y, Miyazaki M, Mori K, Minegishi H, Echigo A, Ohta Y, Maruyama T, Grant WD, Hatada Y (2013) *Salinisphaera japonica* sp. nov., a moderately halophilic bacterium isolated from the surface of a deep-sea fish, *Malacocottus gibber*, and emended description of the genus *Salinisphaera*. Int J Syst Evol Microbiol 63(6):2180–2185

Skerman VBD, McGowan V, Sneath PHA (1980) Approved lists of bacterial names. Int J Syst Bacteriol 30(1):225–420

Studholme DJ, Wasukira A, Paszkiewicz K, Aritua V, Thwaites R, Smith J, Grant M (2011) Draft genome sequences of *Xanthomonas sacchari* and two banana-associated xanthomonads reveal insights into the *Xanthomonas* group 1 clade. Genes 2(4):1050–1065

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura RM (ed) Lectures on mathematics in the life sciences, 17th edn. American Mathematical Society, Providence, pp 57–86

Tindall B (2014a) Names at the rank of class, subclass and order, their typification and current status: supplementary information to Opinion 79. Judicial commission of the international committee on systematics of prokaryotes. Int J Syst Evol Microbiol 64(10):3599–3602

Tindall BJ (2014b) The family name *Solimonadaceae Losey* et al. 2013 is illegitimate, proposals to create the names '*Sinobacter soli*' comb. nov. and '*Sinobacter variicoloris*' contravene the Code, the family name *Xanthomonadaceae* Saddler and Bradbury 2005 and the order name *Xanthomonadales* Saddler and Bradbury 2005 are illegitimate and notes on the application of the family names *Solibacteraceae* Zhou et al. 2008, Nevskiaceae Henrici and Johnson 1935 (Approved Lists 1980) and *Lysobacteraceae* Christensen and Cook 1978 (Approved Lists 1980) and order name *Lysobacteriales* Christensen and Cook 1978 (Approved Lists 1980) with respect to the classification of the corresponding type genera *Solibacter* Zhou et al. 2008 *Nevskia* Famintzin 1892 (Approved Lists 1980) and *Lysobacter* Christensen and Cook 1978 (Approved Lists 1980) and importance of accurately expressing the link between a taxonomic name, its authors and the corresponding description/circumscription/emendation. Int J Syst Evol Microbiol 64(1):293–297

Van Sluys MA, de Oliveira MC, Monteiro-Vitorello CB, Miyaki CY, Furlan LR, Camargo LE, da Silva AC, Moon DH, Takita MA, Lemos EG et al (2003) Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. J Bacteriol 185(3):1018–1026

Williams KP, Gillespie JJ, Sobral BW, Nordberg EK, Snyder EE, Shallom JM, Dickerman AW (2010) Phylogeny of *gammaproteobacteria*. J Bacteriol 192(9):2305–2314

Wilson K (1987) Preparation of genomic DNA from bacteria. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) Current protocols in molecular biology. Wiley, New York, pp 2.4.1–2.4.2

Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ (2009) A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature 462(7276):1056–1060

Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO (2013) The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. doi:10.1093/nar/gkt1209

Zhou Y, Zhang Y-Q, Zhi X-Y, Wang X, Dong J, Chen Y, Lai R, Li W-J (2008) Description of *Sinobacter flavus* gen. nov., sp. nov., and proposal of *Sinobacteraceae* fam. nov. Int J Syst Evol Microbiol 58(1):184–189