Phylogenomic resolution of the bacterial genus *Pantoea* and its relationship with *Erwinia* and *Tatumella*

Marike Palmer[1] *, Emma T. Steenkamp[1], Martin P. A. Coetzee [2], Wai-Yin Chan[1], Elritha van Zyl[1], Pieter De Maayer[3], Teresa A. Coutinho[1], Jochen Blom[4], Theo H. M. Smits[5], Brion Duffy[5], Stephanus N. Venter[1]

[1.] *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*

[2.] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*

[3.] *School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa*

[4.] *Computational Genomics, Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany*

[5.] *Environmental Genomics and Systems Biology Research Group, Institute of Natural Resource Sciences, Zürich University of Applied Sciences (ZHAW), Wädenswil, Switzerland*

* Corresponding author: fanus.venter@up.ac.za

Keywords: phylogenetics, non-phylogenetic signal, MLSA, core genome, *Enterobacteriaceae*

Abstract

Investigation of the evolutionary relationships between related bacterial species and genera with a variety of lifestyles gained popularity in recent years. For analysing the evolution of specific traits, however, a robust phylogeny is essential. In this study we examined the evolutionary relationships among the closely related genera *Erwinia*, *Tatumella* and *Pantoea*, and also attempted to resolve the species relationships within *Pantoea*. To accomplish this, we used the whole genome sequence data for 35 different strains belonging to these three genera, as well as nine outgroup taxa. Multigene datasets consisting of the 1,039 genes shared by these 44 strains were then generated and subjected to maximum likelihood phylogenetic analyses, after which the results were compared to those using conventional multi-locus sequence analysis (MLSA) and ribosomal MLSA (rMLSA) approaches. The robustness of the respective phylogenies was then explored by considering the factors typically responsible for destabilizing phylogenetic trees. We found that the nucleotide datasets employed in the MLSA, rMLSA and 1,039-gene datasets contained significant levels of homoplasy, substitution saturation and differential codon usage, all of which likely gave rise to the observed lineage specific rate heterogeneity. The effects of these factors were much less pronounced in the amino acid dataset for the 1,039 genes, which allowed reconstruction of a fully supported and resolved phylogeny. The robustness of this amino acid tree was also supported by different subsets of the 1,039 genes. In contrast to the smaller datasets (MLSA and rMLSA), the 1,039 amino acid tree was also not as sensitive to long-branch attraction. The robust and well-supported evolutionary hypothesis for the three genera, which confidently resolved their various inter- and intrageneric relationships, represents a valuable resource for future studies. It will form the basis for studies aiming to understand the forces driving the divergence and maintenance of lineages, species and biological traits in this important group of bacteria.

Introduction

In recent years, a number of studies have investigated the evolution of different lifestyles, pathogenicity features and survival strategies within or between bacterial genera based on genomic data (Bennett et al., 2012, Prasanna and Mehra, 2013, Angus et al., 2014, De Maayer et al., 2014, Fouts et al., 2016). The main focus of these studies has often been on understanding how the species or groups of species have evolved and what promoted their biological differentiation. In such a study, an essential first step is to obtain a robust phylogeny for resolving relationships and inferring evolutionary histories (Bennett et al., 2012, Prasanna and Mehra, 2013). These phylogenies are then used for studying the emergence and development of biological traits and for determining the possible causes of divergence within and between genera.

The phylogenetic tree that depicts the relationships among the species of a genus is typically referred to as a "species tree" (Klenk and Göker, 2010, Andam and Gogarten, 2011). For bacteria, species trees have been traditionally inferred using the sequence information from housekeeping genes. These include phylogenetic analysis of 16S ribosomal RNA (rRNA) sequences (Konstantinidis and Tiedje, 2007), multi-locus sequence analysis (MLSA) with 4-7 housekeeping genes (Gevers et al., 2005, Konstantinidis and Tiedje, 2007, Glaeser and Kämpfer, 2015), and more recently, ribosomal MLSA (rMLSA) based on 53 structural ribosomal proteins (Bennett et al., 2012, Jolley et al., 2012). However, the phylogenies generated with these data are often not particularly robust (Konstantinidis and Tiedje, 2007, Brady et al., 2008, Glaeser and Kämpfer, 2015). This is primarily due to a lack of phylogenetic signal in the highly conserved gene datasets used (Fox et al., 1992, Gevers et al., 2005, Konstantinidis and Tiedje, 2005, Staley, 2006, Konstantinidis and Tiedje, 2007, Richter and Rosselló-Móra, 2009). Other issues that may also detract from the overall stability of a species tree pertains to the use of paralogues (i.e., homologues originating from

an intragenomic duplication event) or xenologues (i.e., homologues originating from horizontal gene transfer) (Koonin, 2005). In fact, a number of the markers commonly used in bacterial systematics have been shown to be present in multiple copies in the genomes of certain taxa (Boucher et al., 2004, Conville and Witebsky, 2007) or are even found on plasmid elements (Anda et al., 2015). Some of these genes have also been shown to be acquired horizontally (Rivera et al., 1998, Boucher et al., 2004).

The availability of whole genome sequence (WGS) information has revolutionised the fields of evolutionary biology and bacterial systematics. Despite the fact that horizontal gene transfer (HGT) significantly impacts the evolution of most, if not all, bacterial groups (Woese, 2000, Gogarten et al., 2002, Jain et al., 2002, Boto, 2010, Cohen et al., 2011), it is now possible to infer trees that trace the shared ancestry among all the species of a genus using WGS information. Here the assumption is that the dominant phylogenetic signal in the genome of an individual is reflective of its parental lineage and that this would "overshadow" the signals associated with HGT (Andam and Gogarten, 2011). As a result, the overall evolution of the genus under examination will likely be depicted in the form of a bifurcating tree. For example, robust species trees have been inferred using this approach for *Acinetobacter* (Chan et al., 2012) and *Neisseria* (Bennett et al., 2012).

The WGS-based approach for building species trees involves the use of all (or a large number) of the gene sequences common to the members of the focal genus and its outgroups. This approach is currently regarded as the most reliable approach for inferring species trees (Chan et al., 2012, Lang et al., 2013) because it takes advantage of all of the phylogenetically informative characters included in the genomes of the taxa under investigation (Chan et al., 2012). WGS-based datasets are, therefore, large and their use for inferring species trees outperforms those consisting of single gene or small sets of housekeeping gene sequences (Daubin et al., 2002, Coenye et al., 2005, Galtier and Daubin, 2008, Bennett et al., 2012,

Chan et al., 2012). Compared to smaller datasets, the phylogenetic signal associated with vertical descent in WGS-based datasets far outweighs the noise (Andam and Gogarten, 2011, Chan et al., 2012, Lang et al., 2013). In other words, even if paralogues, xenologues or highly conserved sequences are mistakenly included in the WGS-based dataset, the phylogenetic signal associated with their aberrant evolutionary histories will be diluted by the total signal of vertical descent embedded in these large datasets. This is not the case for the smaller datasets that are conventionally used for inferring bacterial species trees (Rivera et al., 1998, Boucher et al., 2004).

Another benefit of using the shared gene content for inferring species trees is that most of the sequences included in the dataset form part of the so-called core genomes (Daubin et al., 2002, Coenye et al., 2005) of the taxa under investigation. The core genome consists of the genetic material common to the taxon and includes those genes present in nearly all of its members (Lan and Reeves, 2000, Coenye et al., 2005). Accordingly, the core genome usually represents only a small subset of the taxon's pan genome (Makarova et al., 2006, Lukjancenko et al., 2012) and its genes are considered to be essential for survival and often encode products involved in crucial cellular processes (Hacker et al., 2012). The latter, combined with the mainly vertical inheritance of the core genome component (Daubin et al., 2002, Coenye et al., 2005), therefore, highlights the value of using core gene datasets for studying evolutionary trajectories that have shaped the biology and ecology of the taxa under investigation (Daubin et al., 2002, Coenye et al., 2005).

In this study we were interested in reconstructing the species tree for the genus *Pantoea*. This genus currently comprises 23 species and subspecies (Gavini et al., 1989b, Mergaert et al., 1993, Brady et al., 2007, Brady et al., 2008, Brady et al., 2009, Brady et al., 2010a, Popp et al., 2010, Brady et al., 2011, Brady et al., 2012, Gueule et al., 2015, Prakash et al., 2015, Tanaka et al., 2015), with a further two species (*P. pleuroti* and *P. hericii*) recently described

but not yet validated (Ma et al., 2016, Rong et al., 2016). Members of this taxon exhibit a diverse range of phenotypic characteristics, especially in terms of physiological attributes and niche occupation (Brady et al., 2008, Walterson and Stavrinides, 2015). For example, *Pantoea* includes various human and plant pathogens (De Baere et al., 2004, Cruz et al., 2007, Brady et al., 2010a), as well as species with plant growth promoting abilities (Smits et al., 2011, Kim et al., 2012), and species associated with insects (Palmer et al., 2016) and fungi (Ma et al., 2016, Rong et al., 2016) to name but a few. Although *Pantoea* is usually recovered as a monophyletic group in phylogenetic trees, interspecific relationships are not well resolved (Rezzonico et al., 2009, Brady et al., 2012, Tambong et al., 2014, Gueule et al., 2015). Furthermore, the overall position of *Pantoea* within the *Enterobacteriaceae* has not been conclusively established. The genus *Tatumella* is commonly regarded as its sister taxon (Brady et al., 2008, Brady et al., 2010a, Brady et al., 2012), although other intergeneric relationships have also been reported (Brady et al., 2008, Brady et al., 2010b, Brady et al., 2012, Kamber et al., 2012, Smits et al., 2013, Glaeser and Kämpfer, 2015, Gueule et al., 2015). We hypothesize that these inconsistent inter- and intrageneric relationships are mainly due to the small datasets often being used for phylogenetic inference (Brady et al., 2008, Brady et al., 2010b, Glaeser and Kämpfer, 2015). Another contributing factor pertains to incomplete taxon selection where datasets often exclude one or more of the relevant taxa from analyses (Naum et al., 2008, Tambong et al., 2014, Zhang and Qiu, 2015).

The overall goal of this study was therefore to use a WGS-based approach to determine the generic relationships of *Pantoea* within the *Enterobacteriaceae* and then to infer a species tree for *Pantoea.* To achieve these goals, our aims were four-fold. Firstly, to allow for meaningful inter- and intrageneric comparisons, the WGSs of twelve *Pantoea* species were determined, which complemented those of fourteen strains already available in the public domain (Table 1). Secondly, a maximum likelihood phylogeny depicting the relationships

among *Pantoea* species, as well as among *Pantoea* and other genera, were inferred using the aligned shared gene sequences extracted from the WGS data. Thirdly, the robustness of this tree was evaluated by considering the various factors known to negatively affect phylogenetic analyses (Xia and Xie, 2001, Zwickl and Hillis, 2002, Jeffroy et al., 2006, Heath et al., 2008, Philippe et al., 2011). Finally, to determine the possible causes for the incongruent intra- and intergeneric relationships previously reported for *Pantoea* and its relatives (Brady et al., 2010a, Glaeser and Kämpfer, 2015, Gueule et al., 2015), we evaluated the conventional methods (i.e., MLSA and rMLSA) for investigating relatedness amongst taxa by making use of datasets containing representatives of all relevant genera. A robust *Pantoea* species tree will form an essential foundation for future studies focusing on the evolution of characteristics and traits related to the different survival strategies within the genus. This study will also provide the basis for taxonomic clarity in terms of available genome data and the phylogenetic position of *Pantoea* relative to its sister genera within the *Enterobacteriaceae*.

Materials and Methods

Genome sequencing of twelve Pantoea species

The genome sequences of twelve *Pantoea* species (*P. allii*, *P. brenneri*, *P. calida*, *P. conspicua*, *P. cypripedii*, *P. deleyi*, *P. eucrina*, *P. gaviniae*, *P. rodasii*, *P. rwandensis*, *P. septica* and *P. wallisii*) (Table 1, Supplementary Table S1) were determined in this study. For this purpose, the type strains of these twelve species were grown on nutrient agar for 48 hours at 28°C. High quality DNA was extracted using a CTAB method (Cleenwerck et al., 2002). The genomic DNA was then subjected to whole genome shotgun sequencing using the Ion Torrent™ Personal Genome Machine® (PGM) System (ThermoFisher Scientific) at the University of Pretoria Sequencing Facility or the Roche 454 GS-Junior sequencer at

Agroscope Research Station in Wädenswil, Switzerland. The raw sequence reads were trimmed and filtered using FASTX Tools (Gordon and Hannon, 2010), where those with sequence quality scores < 20 were discarded. The trimmed and filtered data were assembled with the Roche Newbler 2.6 or 2.7 programs (Margulies et al., 2005).

Taxon selection

The taxa included in our WGS-based datasets were chosen to span the known diversity of the genus *Pantoea* (hence the generation of additional WGS data here). We also endeavoured to utilize a wide selection of species (with available WGSs) within each of *Erwinia* and *Tatumella*, which are known to be closely related to *Pantoea* (Brady et al., 2008, Brady et al., 2010a, Glaeser and Kämpfer, 2015). These included formally described species as well as potentially novel species, based on average nucleotide identity (ANI) values (Gevers et al., 2005, Konstantinidis and Tiedje, 2005, Richter and Rosselló-Móra, 2009). This was done by obtaining all the relevant WGSs from the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/; accessed 7/5/2015) and then subjecting them to ANI analyses in JSpecies 1.2.1 (Richter and Rosselló-Móra, 2009). These analyses involved pair-wise comparisons of shared regions of the genomes to obtain a similarity value across the genome (Gevers et al., 2005, Konstantinidis and Tiedje, 2005, Richter and Rosselló-Móra, 2009). Where multiple genome sequences for a species were available, the WGS data of its type strain or a suitable conspecific isolate (based on similarity of available housekeeping genes) was used.

Identification of shared genes and construction of datasets

Sets of shared genes were determined with the EDGAR (Efficient Database framework for comparative Genome Analyses using BLAST score Ratios) server (https://edgar.computational.bio.uni-giessen.de) (Blom et al., 2016). The combined fasta files

obtained from EDGAR were split into individual gene files from which five multigene datasets were constructed. The *Erwinia+Pantoea+Tatumella+*Outgroups dataset consisted of the genes shared among all of the species used in this study (Table 1), while the *Erwinia+Pantoea+Tatumella* dataset included those shared among the examined species of the three genera. For the nucleotide substitution and codon bias analyses (see below) a third smaller multigene dataset, *Erwinia+Pantoea+Tatumella_*reduced was constructed which included 11 taxa, specifically selected to represent the diversity within *Erwinia*, *Pantoea* and *Tatumella*. The last two multigene datasets were the conventional MLSA dataset consisting of four genes (*atpD*, *gyrB*, *infB* and *rpoB*) previously used to investigate relationships in these genera (Brady et al., 2008, Glaeser and Kämpfer, 2015), and the rMLSA dataset that consists of 52 of the known 53 genes encoding the structural ribosomal proteins (Bennett et al., 2012, Jolley et al., 2012).

For the *Erwinia+Pantoea+Tatumella+*Outgroups dataset, five subsets were constructed by grouping the genes included in this dataset into broad functional groupings. This was done by subjecting the genes to functional annotation using the Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al., 2008). Five subsets ('Cellular functioning', 'Metabolism', 'Informational', 'External factors' and 'Unclassified') containing the amino acid sequences were generated based on the subsystem classification of the genes.

For the *Erwinia+Pantoea+Tatumella+*Outgroups dataset, three subsets were constructed based on the type of selection experienced by the genes. For this purpose, individual gene alignments (see below) were subjected to selection analysis using HyPhy (Pond and Muse, 2005) as implemented in MEGA 6.06 (Tamura et al., 2013). Gene-wide dN/dS values were determined for each individual gene. These values were then plotted as a line graph in Microsoft Excel 2013. Of the 1039 shared genes, those with a dN/dS below 1 were regarded as being under purifying selection, while those with dN/dS higher than 1 were considered as

being under diversifying selection. Genes with dN/dS values ranging from 0.9 to 1.1 were viewed as potentially experiencing neutral or nearly neutral evolution. Three datasets containing the amino acids sequences of the genes under different selection pressure were thus constructed and referred to as 'Purifying' (dN/dS < 1), 'Diversifying' (dN/dS > 1) and 'Neutral' (0.9 < dN/dS > 1.1).

Sequence alignments

Except for the *Erwinia+Pantoea+Tatumella*_reduced dataset, which required codon-based alignment, all datasets were treated as follows. Individual gene files for all datasets were batch-aligned using MUSCLE (Edgar, 2004) as part of the CLC Main Workbench 7.6 package (CLC Bio). The alignments were then subjected to GBLOCKS 0.91 b (Castresana, 2000) to discard any parts of alignments with missing data. For all multigene datasets, the relevant aligned gene sequences were concatenated and partitioned using FASconCAT-G v. 1.02 (Kuck and Longo, 2014). In all cases, amino acid alignments were generated in addition to the nucleotide datasets. Amino acid datasets were partitioned with the appropriate amino acid model determined by ProtTest 3.4 (Abascal et al., 2005) as implemented in FASconCAT-G. All nucleotide multigene datasets were also concatenated with the third codon positions excluded from the datasets.

Both the nucleotide and amino acid sequences for the *Erwinia+Pantoea+Tatumella*_reduced datasets were treated in the same manner. The *Erwinia+Pantoea+Tatumella*_reduced dataset was batch-aligned with MUSCLE. We then manually curated the individual nucleotide gene files in BioEdit (Hall, 2011) to ensure that all gene alignments were in the correct reading frame, as well as to discard any regions with a large amount of missing data. These aligned sequences were then concatenated with FASconCAT-G to obtain a supermatrix for both the

nucleotide and amino acid sequences, as well as a data matrix with the third codon positions excluded from the nucleotide datasets.

Phylogenetic analyses

For phylogenetic analysis of the *Erwinia*+*Pantoea*+*Tatumella*+Outgroups, *Erwinia*+*Pantoea*+*Tatumella*, MLSA and rMLSA amino acid and nucleotide datasets, RAxML 8.2.1 (Stamatakis, 2014) was used to construct maximum likelihood (ML) trees. Suitable partitioning files for use in this software were produced by FASconCAT-G (Kuck and Longo, 2014). For the amino acid dataset, each gene utilized the best-fit substitution model as indicated by ProtTest 3.4 (Abascal et al., 2005) with independent model parameters. For the nucleotide dataset, each gene utilized the General Time Reversible (GTR) model of substitution (Tavaré, 1986) with independent model parameters. In the analyses, parameters for the GTR model were independently estimated and optimized for each of the respective gene sequences. Thus, the appropriate substitution model (based on the substitution rates and α shape parameter) was inferred for each gene in the dataset, which allowed for ML analyses to be conducted with the model parameters that best fit each gene. Because of computational demands, RAxML was only used to obtain trees with the best likelihood, and branch support was estimated separately. This involved approximate likelihood analyses of the unpartitioned datasets using FastTree 2.1.8 (Price et al., 2010) from which non-parametric, Shimodaira-Hasegawa-like branch support values (Guindon et al., 2010) were estimated. We also used Seqboot (Felsenstein, 2005) to construct 1,000 bootstrap replicate data matrices for the datasets, which were then analysed with FastTree, from which bootstrap support values were estimated using the publicly available perl script CompareToBootstrap.pl (http://www.microbesonline.org/fasttree/treecmp.html).

Analysis of the five functional data subsets (i.e., 'Cellular functioning', 'Metabolism', 'Informational', 'External factors' and 'Unclassified'), as well as the selection datasets (i.e., 'Purifying', 'Diversifying' and 'Neutral') were performed with FastTree 2.1.8 (Price et al., 2010) to obtain approximate likelihood phylograms. Non-parametric, Shimodaira- Hasegawa-like branch support values (Guindon et al., 2010), as well as bootstrap support obtained using Seqboot (Felsenstein, 2005) and CompareToBootstrap.pl (Price et al., 2010), were also estimated for the topologies obtained.

Homoplasy index

The possible impact of homoplasy (convergent mutations or similarities among taxa that are not due to common ancestry and that can affect tree reconstruction) (Philippe et al 2011; West-Eberhard, 2003) on the *Erwinia+Pantoea+Tatumella+*Outgroups, MLSA and rMLSA datasets (both amino acid and nucleotide data in all three cases and, in the case of the nucleotide datasets, both with and without the third codon positions), was estimated. This was done by calculating the homoplasy index (HI) for each dataset using PAUP* 4.0 (Swofford, 2002). The HI was determined for all parsimony informative sites by making use of the amino acid-based ML topology obtained for the 1,039 shared genes.

Nucleotide substitution saturation analysis

Detailed nucleotide substitution patterns were determined for the *Erwinia+Pantoea+Tatumella*_reduced dataset and the MLSA dataset. This was done by correlating the actual substitutions in the dataset with those inferred under an appropriate model of nucleotide substitution (Jeffroy et al., 2006, Philippe et al., 2011). For this purpose, we used pair-wise uncorrected p-distances (i.e., the proportion, p, of nucleotide sites at which the two sequences being compared are different) and pair-wise nucleotide-based distances under the General Time Reversible (GTR) model (Tavaré, 1986) with the minimum evolution

distance algorithm (Desper and Gascuel, 2002) for the nucleotide sequences. These two estimates were both determined in DAMBE 6.0.1 (Xia and Xie, 2001) and were calculated for the first, second and third codon positions. The same was done for the amino acid *Erwinia+Pantoea+Tatumella+*Outgroups dataset by using pair-wise amino acid-based distances under the Jones-Taylor-Thornton (JTT) model (Jones et al., 1992) using MEGA 6.0.6 (Tamura et al., 2013). Microsoft Excel 2013 was then used to graphically plot the respective distances and to perform linear regression analyses for determining the slope of the regression line fitting the data.

Codon usage bias

The relative synonymous codon usage (RSCU) for *Erwinia*, *Pantoea* and *Tatumella* was determined from the *Erwinia+Pantoea+Tatumella+*Outgroups dataset using DAMBE 6.0.1 (Xia and Xie, 2001). The data obtained for all species of each genus analysed, were used to calculate the mean for the genus, with the minimum and maximum within the group serving as the negative error value and the positive error value. These values for each genus were then plotted per codon and sorted by amino acids in Microsoft Excel 2013. Two-tailed, unpaired t-tests were performed in Microsoft Excel 2013 in a pair-wise manner, to determine whether mean values between genera differed significantly (H_0: $\overline{\text{Genus1}} = \overline{\text{Genus2}}$; $\alpha = 0.05$).

Lineage specific rate heterogeneity

To determine the presence of lineage specific rate heterogeneity, Tajima's relative rate tests (Tajima, 1993) were performed in MEGA 6.0.6 (Tamura et al., 2013). Molecular sequences of three taxa were tested at a time. The amino acid *Erwinia+Pantoea+Tatumella* dataset was used for rate tests. The null hypothesis tested was equal rates across all taxa.

Long branch attraction

The possible involvement of *P. calida*, *P. gaviniae* and *Tatumella* in long branch attraction (LBA) was investigated in the *Erwinia*+*Pantoea*+*Tatumella*+Outgroups amino acid and nucleotide datasets. To determine the effect of the inclusion of these taxa, phylogenetic trees were constructed (as described previously) from the respective datasets *Erwinia*+*Pantoea*+*Tatumella*+Outgroups with the respective inclusion and exclusion of these taxa (Bergsten, 2005).

The same process was then applied to the rMLSA and MLSA datasets, as well as the *Erwinia*+*Pantoea*+*Tatumella*+Outgroups amino acid dataset, with focus on the outgroup taxa included. This involved including various combinations as well as single outgroups for rooting of the trees. These phylogenetic analyses utilized FastTree 2.1.8 (Price et al., 2010) for inferring the tree with SH-support values for branch support.

Results

Genome sequences of twelve Pantoea *species*

The genome assemblies of the twelve species consisted of 3.9-5.8 million bases at sequencing depths ranging from 13x to 155x (Supplementary Table S1). The overall assembly statistics for these new WGSs were comparable to those for most previously reported *Pantoea* species (Smits et al., 2010, Wang et al., 2011, Brown et al., 2012, Hong et al., 2012, Conlan et al., 2014, De Maayer et al., 2014, Lim et al., 2014, Tian and Jing, 2014, Wan et al., 2015). All twelve assemblies have been deposited in the relevant nucleotide database at NCBI (see Table 1 for accession numbers).

Table 1. Isolates with available genome sequences and those determined in this study*****

Species	Strain	Origin	Accession number
Erwinia amylovora	LA 636	Apple, Mexico	CBVT00000000.1
Erwinia billingiae	NCPPB 661 T	Pear, UK	FP236843.1,FP236826.1, FP236830.1
Erwinia mallotivora	BT-MARDI	Papaya, Malaysia	JFHN00000000.1
Erwinia pyrifoliae	DSM 12163 T	Asian pear, Korea	FN392235.1, FN392236.1, FN392237.1
Erwinia tasmaniensis	Et 1-99 T	Apple flowers, Australia	CU468135.1, CU468128.1, CU468130.1, CU468131.1, CU468132.1, CU468133.1
Erwinia toletana	DAPP-PG 735	Olive knot, Italy	AOCZ00000000.1
Erwinia tracheiphila	PSU-1	Wild gourd, USA	APJK00000000.1
Erwinia sp.	9145	Information missing	JQNE00000000.1
Erwinia sp.	Ejp 617	Asian pear, Japan	CP002124.1, CP002125.1, CP002126.1
Pantoea agglomerans	R 190	Apple, Korea	JNGC00000000.1
Pantoea allii *	LMG 24248 T	Onion seed, South Africa	MLFE00000000.1
Pantoea ananatis	LMG 2665 T	Pineapple, Brazil	JFZU00000000.1
Pantoea anthophila	11-2	Hypersaline lake, Hawaii	JXXL00000000.1
Pantoea brenneri *	LMG 5343 T	Human, USA	MIEI00000000.1
Pantoea calida *	LMG 25383 T	Infant formula, -	MLFO00000000.1
Pantoea conspicua *	LMG 24534 T	Human, France	MLFN00000000.1
Pantoea cypripedii *	LMG 2657 T	Orchid, USA	MLJI00000000.1
Pantoea deleyi *	LMG 24200 T	Eucalyptus, Uganda	MIPO00000000.1
Pantoea dispersa	EGD-AAK13	Soil, India	AVSS00000000.1
Pantoea eucalypti	aB	Bark beetle, USA	AEDL00000000.1
Pantoea eucrina *	LMG 2781 T	Human, USA	MIPP00000000.1
Pantoea gaviniae *	LMG 25382 T	Infant formula, -	MLFQ00000000.1
Pantoea rodasii *	LMG 26273T	Eucalyptus, Colombia	MLFP00000000.1
Pantoea rwandensis *	LMG 26275 T	Eucalyptus, Rwanda	MLFR00000000.1
Pantoea septica *	LMG 5345 T	Human, USA	MLJJ00000000.1
Pantoea stewartii ssp. *stewartii*	DC 283	Maize, USA	AHIE00000000.1
Pantoea stewartii ssp. *indologenes*	LMG 2632 T	Fox millet, India	JPKO00000000.1
Pantoea vagans	C9-1	Apple, USA	CP001894.1, CP001893.1, CP001894.1
Pantoea wallisii *	LMG 26277 T	Eucalyptus, South Africa	MLFS00000000.1
Pantoea sp.	At-9b	Leaf cutter ant, USA	CP002433.1, CP002434.1, CP002435.1, CP002436.1, CP002437.1, CP002438.1
Pantoea sp.	A4	Rafflesia flower, Malaysia	ALXE00000000.1
Pantoea sp.	IMH	Soil, Mongolia	JFGT00000000.1
Pantoea sp.	GM01	Poplar, USA	AKUI00000000.1
Pantoea sp.	PSNIH1	Shelf, USA	CP009880.2, CP009881.1, CP010325.1, CP0009882.1, CP010326.1, CP009883.1, CP009884.1
Pantoea sp.	PSNIH2	Hand rail, USA	CP009866.1, CP009867.1, CP009868.1, CP009869.1, CP009870.1, CP009871.1
Tatumella morbirosei	LMG 23360 T	Pineapple, Philippines	CM003276.1
Tatumella ptyseos	ATCC 33301 T	Human, USA	ATMJ00000000.1
Tatumella saanichensis	NML 06-3099 T	Human, Canada	ATMI00000000.1
Tatumella sp.	UCD-D suzukii	Fruit fly, USA	JFJX00000000.1
Brenneria goodwinii	OBR 1	Information missing	CGIG00000000.1
Cronobacter sakazakii	ATCC 29544 T	Human, USA	CP011047.1, CP011048.1, CP011049.1, CP011050.1
Enterobacter cloacae spp. *cloacae*	ATCC 13047 T	Human, USA	CP001918.1, CP001919.1, CP001920.1
Franconibacter helveticus	LMG 23732 T	Fruit powder, Switzerland	AWFX00000000.1
Klebsiella pneumoniae ssp. *pneumoniae*	ATCC 13883 T	Human, -	JOOW00000000.1
Kluyvera ascorbata	ATCC 33433 T	Human, USA	JMPL00000000.1
Pectobacterium carotovorum ssp. *carotovorum*	NCPPB 312 T	Potato, Denmark	JQHJ00000000.1
Serratia marcescens ssp. marcescens	ATCC 13880 T	Pond water, -	JMPQ00000000.1
Yokenella regensburgei	ATCC 49455 T	Insect gut, Germany	JMPS00000000.1

ANI-based taxon selection

Adequate taxon sampling is crucial for the accuracy of phylogenetic analyses by allowing better model and parameter estimation (Zwickl and Hillis, 2002, Heath et al., 2008, Nabhan and Sarkar, 2012) and avoiding artefacts associated with divergent taxa (Kim, 1996, Hillis, 1998, Mitchell et al., 2000, Philippe et al., 2011). We therefore evaluated and improved the taxon selection for this study using the whole genome similarity metric ANI (Gevers et al., 2005, Konstantinidis and Tiedje, 2005, Richter and Rosselló-Móra, 2009). This tool was used to ensure broad and appropriate sampling within each of the three genera, where it enabled identification of undescribed isolates of species for which WGSs are available. These included *Pantoea* sp. A4, At-9b, GM01, PSNIH1, PSNIH2, IMH, *Erwinia* sp. Ejp617 and 9145 and *Tatumella* sp. UCD-D suzukii. This approach also allowed the exclusion from our datasets of what can be considered conspecifics (i.e., those with ANI > 96%) (Gevers et al., 2005, Konstantinidis and Tiedje, 2007, Richter and Rosselló-Móra, 2009) for which WGS information is available (*Pantoea* sp. PSNIH1, PSNIH2, *Erwinia* sp. Ejp617 and *Tatumella* sp. UCD-D suzukii).

ANI analysis was also used to investigate the similarity of the different taxa within these genera. The members of the respective genera all had ANI values *ca*. >75% (Figure 1 and Supplementary Tale S2). However, despite having an ANI value of *ca*. >88% between the pair, *P. gaviniae* LMG 25382^{T} and *P. calida* LMG 25383^{T} showed much lower ANI values with other *Pantoea* species at 73.44-78.48%. For the comparisons of these two species with *Erwinia* and *Tatumella*, ANI values of 73.59-77.54% and 70.9-72.43%, respectively, were obtained. Also, the isolate labelled as "*Pantoea* sp. IMH" likely represents a member of *Erwinia* (Rezzonico et al., 2016) due to the high ANI values it shares with other strains belonging to this genus. The final dataset thus consisted of 44 taxa, which included 21 strains of *Pantoea*, three species of *Tatumella*, nine strains of *Erwinia* (including *Pantoea* sp. IMH),

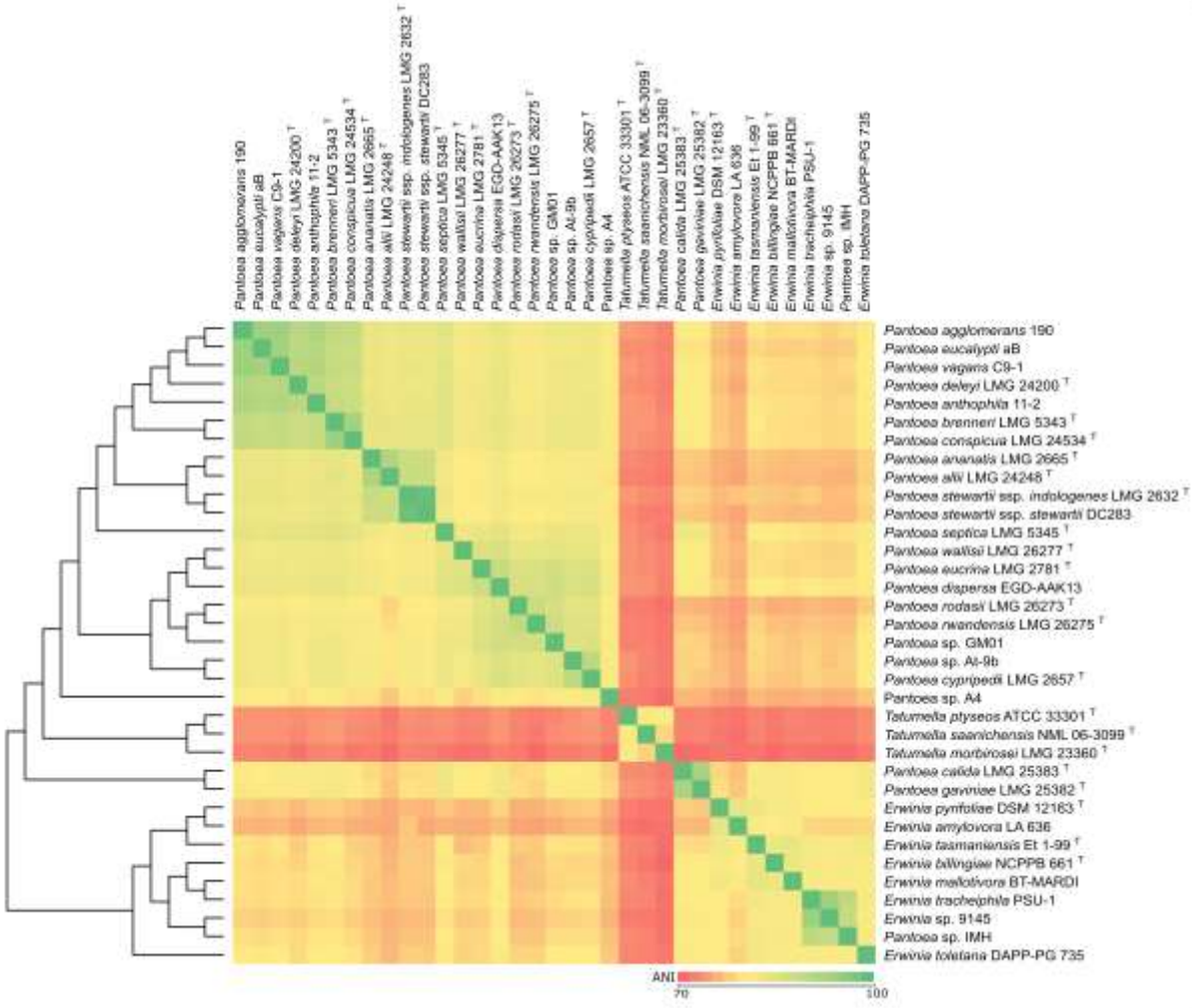


Figure 1 A cladogram inferred from the amino acid topology for the genes shared by *Erwinia*, *Pantoea* and *Tatumella*. Pairwise Average Nucleotide Identities (ANIb) calculated using BLAST in JSpecies (Richter and Rosselló-Móra, 2009) are indicated as a heat map.

in addition to the unusual taxa *P. gaviniae* and *P. calida* (Table 1). The dataset also contained nine taxa from other genera in the *Enterobacteriaceae* that were included to serve as outgroup taxa (Table 1).

WGS-based phylogeny for Pantoea *and its relatives* Erwinia *and* Tatumella

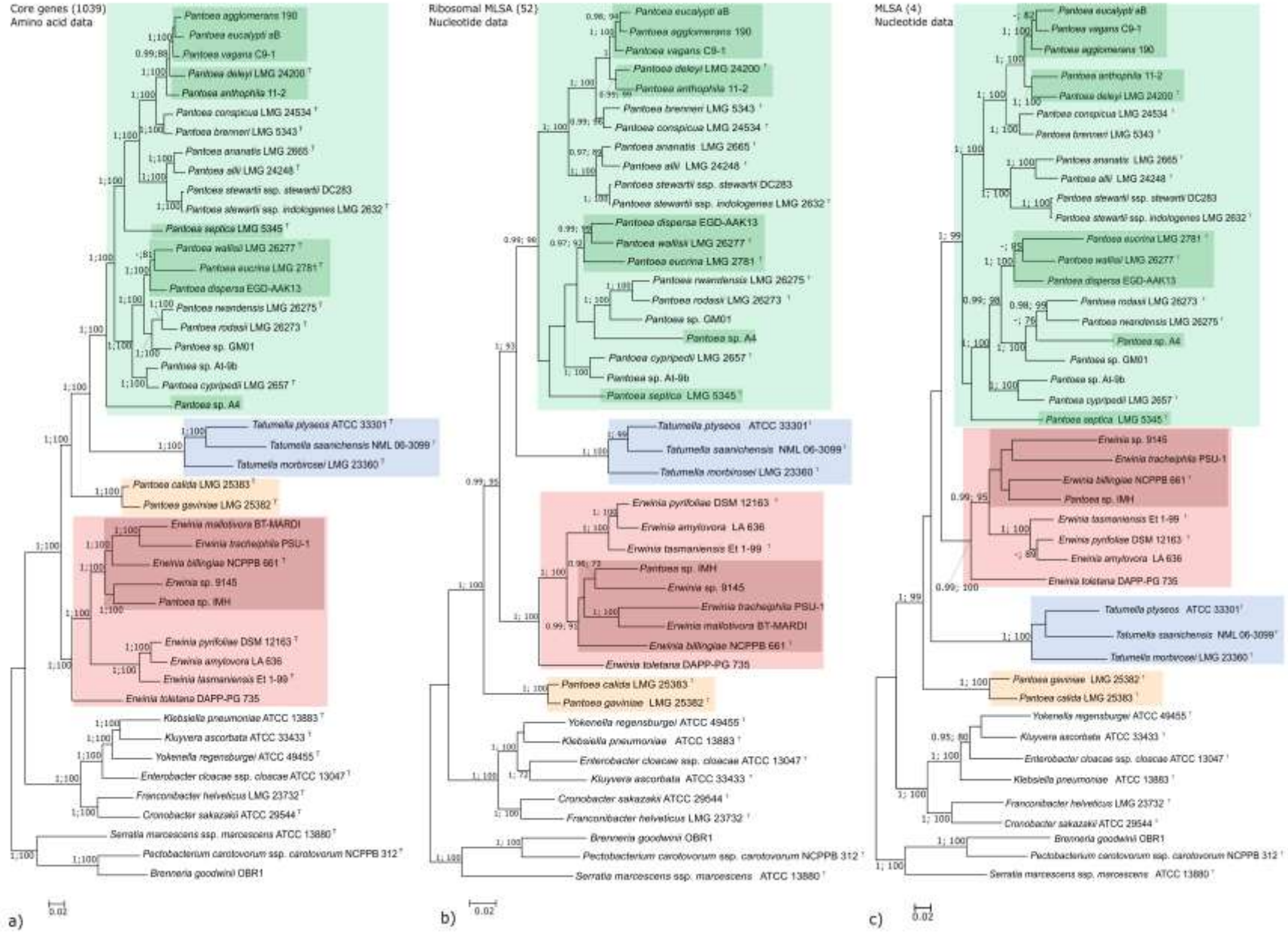
Although the various *Pantoea*, *Erwinia*, and *Tatumella* genomes examined had 1,112 genes in common, the dataset including nine outgroup taxa (i.e., *Erwinia*+*Pantoea*+*Tatumella*+Outgroups) consisted of 44 taxa and 1,039 genes. These genes were identified using the strict orthology estimation implemented in EDGAR (Blom et al., 2016), resulting in a mean % identity of ~69% (median ~73%) and a mean Expect-value of 1e-09 (median 1e-118) for accepted BLAST hits. The nucleotide alignment for the *Erwinia*+*Pantoea*+*Tatumella*+Outgroups dataset contained 679,685 characters, while the amino acid alignment consisted of 224,707 characters. The overall ML topologies obtained for these datasets were similar in terms of relationships among the ingroup taxa. The only differences between the trees related to *Pantoea* sp. A4 and the clade containing *Pantoea eucalypti* (De Maayer et al., 2012), *P. vagans* and *P. agglomerans* (Supplementary Figure S1). However, the results of SH-like tests implemented in RAxML (Figure 2) showed that the amino acid topology does not score significantly worse in terms of likelihood than that of the nucleotide topology for the nucleotide data matrix, while the nucleotide topology scored significantly worse than the amino acid topology for the amino acid data matrix. Based on this information and the various estimates regarding its robustness (see below), the tree inferred from the amino acid *Erwinia*+*Pantoea*+*Tatumella*+Outgroups dataset was regarded as the more accurate hypothesis for describing the inter- and intrageneric relationships among the ingroup taxa.

Topologies	Data matrix: Core AA	Core nt	Ribosomal nt	MLSA nt	Informational AA	Cellular functioning AA	External factors AA	Diversifying AA	Neutral AA	Purifying AA
Core AA	Best	No	Yes	Yes	No	No	No	Yes	No	No
Core nt	Yes	Best	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ribosomal nt	Yes	Yes	Best	No	Yes	Yes	Yes	Yes	Yes	Yes
MLSA nt	Yes	Yes	Yes	Best	Yes	Yes	Yes	Yes	Yes	Yes
Informational AA	No	No	Yes	Yes	Best	No	No	Yes	No	No
Cellular functioning AA	No	No	Yes	Yes	Yes	Best	No	Yes	No	Yes
External factors AA	Yes	Yes	Yes	Yes	Yes	Yes	Best	Yes	No	Yes
Diversifying AA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Best	No	Yes
Neutral AA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Best	Yes
Purifying AA	No	No	Yes	Yes	No	No	No	Yes	No	Best

* Best = Topology obtained with original maximum likelihood analysis
Yes = Likelihood value significantly lower at a confidence level of 1%
No = Likelihood value not significantly lower at a confidence level of 1%
AA = Amino acid sequence based topology/ data matrix
nt = Nucleotide sequence based topology/ data matrix

Figure 2 Shimodaira-Hasegawa (SH) topology tests (Stamatakis, 2014) performed with all topologies showing differences in the relationships among ingroup taxa compared to the topology obtained from the protein sequences of all shared genes. The data matrices are indicated at the top of the figure, with the corresponding topology obtained indicated on the left. The type of data used (nucleotide - nt or amino acid - aa) are indicated for each data matrix. Alternate topologies were scored as either significantly worse or not significantly worse at a confidence interval of 1% based on the likelihood scores obtained for the topologies given the data matrix.

Figure 3 Maximum-Likelihood (ML) phylogenies of a) the amino acid *Erwinia+Pantoea+Tatumella*+Outgroups dataset, b) ribosomal MLSA and c) the conventional MLSA (*atpD, gyrB, infB* and *rpoB*). For the rMLSA dataset a "Swiss-cheese" dataset was constructed due to the absence of mostly single genes in a number of taxa being potentially due to sequencing quality and assembly of genomes. *E. mallotivora* was also excluded from the MLSA dataset as one of the MLSA genes (*gyrB)* was absent from this genome, possibly also due to sequencing quality. All ML trees were constructed from partitioned datasets using RAxML (Stamatakis, 2014) with branch support inferred from FastTree (Price et al, 2010) with SH-support and bootstrap values inferred from 1,000 replicates indicated at nodes. Darkened blocks indicate differences in the relationships among ingroup taxa across the three topologies.



The three genera were recovered as monophyletic groups with high support (Figure 3a). These analyses also showed that the species *P. calida* and *P. gaviniae* probably represent a distinct genus potentially including some newly described species of these genera, while *Pantoea* sp. IMH represents a member of the genus *Erwinia*. Overall, *Pantoea* and *Tatumella* grouped as sister to each other, followed by the *P. calida* and *P. gaviniae* group (potentially a novel genus), with *Erwinia* grouping basal to the other two genera. *Pantoea* was separated into four distinct lineages, where one (represented by a clade containing *P. agglomerans*, *P. allii*, *P. ananatis*, *P. anthophila*, *P. brenneri*, *P. conspicua*, *P. deleyi*, *P. eucalypti*, *P. stewartii* ssp. *indologenes*, *P. stewartii* ssp. *stewartii* and *P. vagans*) was sister to *P. septica*, which together formed the sister group of the third lineage (represented by the clade containing *P. cypripedii*, *P. dispersa*, *P. eucrina*, *P. rodasii*, *P. rwandensis*, *P. wallisii*, *Pantoea* sp. At-9b and GM01). The fourth lineage, represented by *Pantoea* sp. A4, was sister to these three lineages.

Robustness of the* Pantoea *phylogeny

The robustness of the phylogenies obtained from the nucleotide and amino acid *Erwinia*+*Pantoea*+*Tatumella*+Outgroups datasets were evaluated in terms of factors known to cause so-called "non-phylogenetic signal" (Jeffroy et al., 2006, Philippe et al., 2011), as well as potential biases introduced due to the choice of genes analysed (Rivera et al., 1998, Jain et al., 1999, Cohen et al., 2011). The causes of non-phylogenetic signal investigated were homoplasy, substitution saturation, codon usage bias, LBA and lineage specific rate heterogeneity. For identifying inherent biases due to the selected genes, different subsets of the shared genes were constructed. The subsets were either based on the type of selection experienced by the genes, or the functional classes to which the genes belong.

Non-phylogenetic signal – homoplasy

The contribution of homoplasious characters to the *Erwinia+Pantoea+Tatumella+*Outgroups datasets was estimated using PAUP*. These analyses yielded HI values of 0.76, 0.74 and 0.53, respectively, for the dataset with all nucleotides included, the nucleotide dataset with the third codon positions excluded, and the amino acid dataset. Compared to the two nucleotide datasets, the amino acid dataset thus contained substantially fewer homoplasious characters over the tree topology (Bremer, 1994) that could contribute to the non-phylogenetic signal (Philippe et al., 2011). The amino acid dataset is thus superior in that it contains fewer characters competing with the true phylogenetic signal during tree inference (Philippe et al., 2011).

Non-phylogenetic signal –substitution saturation

Substitution saturation (like homoplasy) contributes to the non-phylogenetic signal that competes with the true signal, which detracts from the robustness and accuracy of the inferred tree (Philippe and Forterre, 1999, Xia et al., 2003, Jeffroy et al., 2006, Philippe et al., 2011). To estimate the level of saturation in our WGS-based datasets, the correlation between actual substitutions in the data (represented by p-distances) and substitutions inferred using an appropriate evolutionary model (represented by modelled distances) was inferred (Jeffroy et al., 2006, Philippe et al., 2011). For the three *Erwinia+Pantoea+Tatumella_*reduced datasets consisting, respectively, of the first codon positions, second codon positions, and first plus second codon positions, there is an almost one to one correlation between p-distances and the distances that compensate for potential saturation (Figure 4e, f, h). This was also true for the *Erwinia+Pantoea+Tatumella+*Outgroups amino acid dataset (Figure 4d). These results thus suggest a limited effect of substitution saturation on the amino acid data

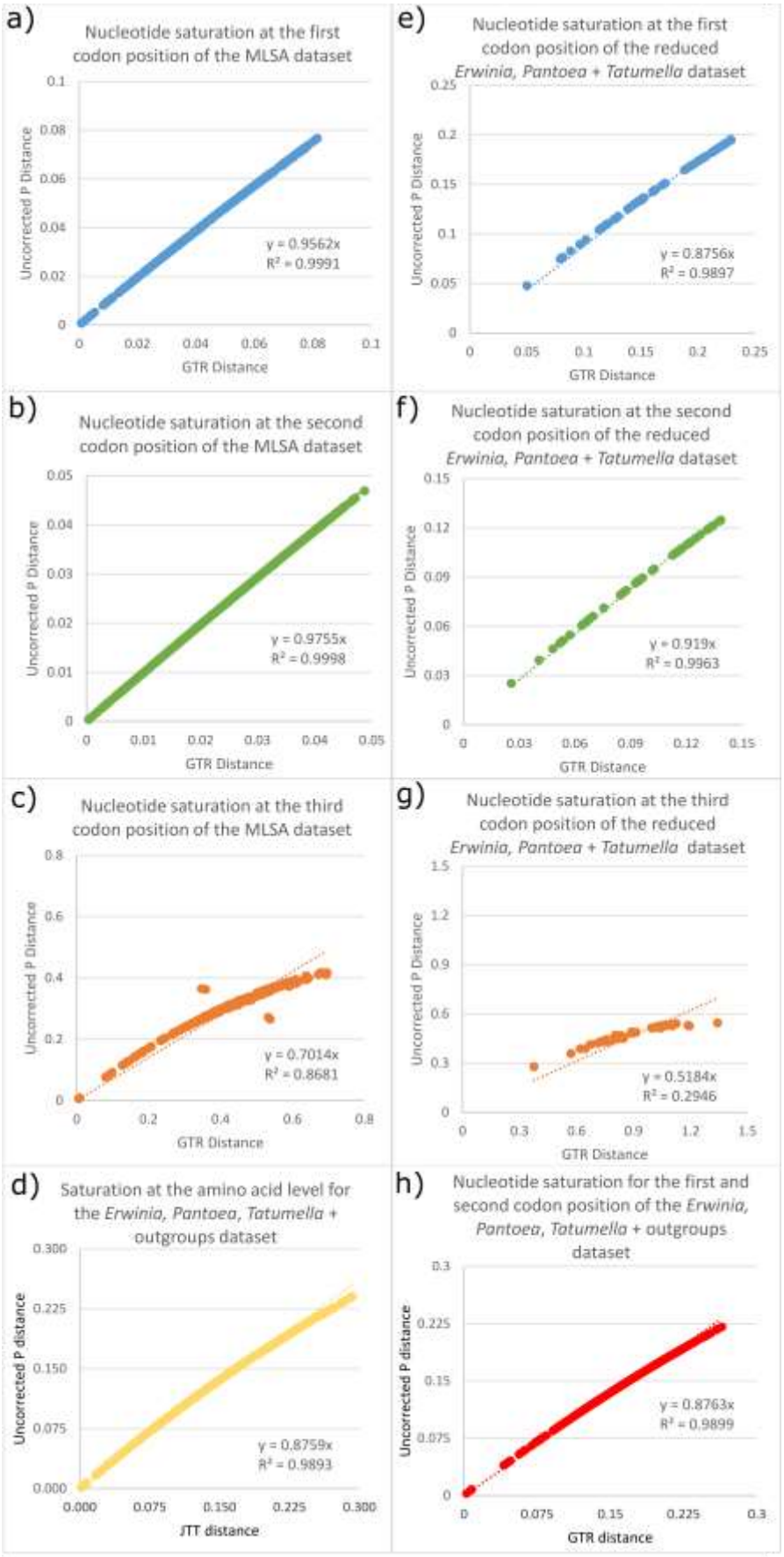


Figure 4 Substitution saturation plots of the first (blue; a), second (green; b) and third (orange; c) codon positions of the MLSA dataset. Uncorrected p-distances are indicated on the y-axis and GTR distances are indicated on the x-axis. The slopes of the linear regression lines for this dataset are 0.9562, 0.9755 and 0.7014 with R^2-values of 0.9991, 0.9998 and 0.8681, respectively. d) Substitution saturation plot of the *Erwinia+Pantoea+Tatumella*+Outgroups amino acid dataset. Uncorrected p-distances are indicated on the y-axis and JTT distances are indicated on the x-axis. The slope of the linear regression line is 0.8759 with an R^2-value of 0.9893. Substitution saturation plots of the first (blue; e), second (green; f) and third (orange; g) codon positions of the *Erwinia+Pantoea+Tatumella* reduced dataset. Uncorrected p-distances are indicated on the y-axis and GTR distances are indicated on the x-axis. The slopes of the linear regression lines for this dataset are 0.8756, 0.919 and 0.5184 with R2-values of 0.9897, 0.9963 and 0.2946, respectively. h) Substitution saturation plot of the combined first and second codon positions for the *Erwinia+Pantoea+Tatumella* reduced dataset. Uncorrected p-distances are indicated the y-axis and GTR distances are indicated on the x-axis. The slope of the linear regression line is 0.8763 with an R^2-value of 0.9899.

and the nucleotide datasets including the first and second codon positions (Jeffroy et al., 2006, Philippe et al., 2011).

The uncorrected and modelled distances were, however, poorly correlated in the nucleotide dataset containing the third codon positions only (Figure 4g). The latter dataset thus contains many more characters that have undergone multiple mutations over evolutionary time, which could explain why one of the nodes in the backbone of the tree inferred from this dataset lacked statistical support (Supplementary Figure S1).

Non-phylogenetic signal – codon composition bias

As an indication of codon usage biases, the RSCU of species in the three genera, *Erwinia*, *Pantoea* and *Tatumella*, were analysed. Upon comparison of either *Erwinia* (28 codons) or *Pantoea* (37 codons) to *Tatumella* (Table 2, Supplementary Figure S2), it was clear that *Tatumella* utilizes a large number of codons for certain amino acids that are different from those used by *Erwinia* and *Pantoea*. This might reflect a bias toward certain nucleotides in *Tatumella* (Nei and Kumar, 2000), particularly at third codon positions (Jeffroy et al., 2006). Like homoplasy and substitution saturation, such biases also contribute the non-phylogenetic signal that might overshadow the true signal during tree reconstruction (Galtier and Gouy, 1995, Jeffroy et al., 2006). The apparent codon composition bias in *Tatumella* is therefore the likely cause of the somewhat longer branch for this genus in our various WGS-based phylogenies.

Non-phylogenetic signal – LBA

LBA is a tree reconstruction artefact which indicates a closer relationship between certain taxa, due to the divergent nature of these taxa compared to the rest of the taxa in the analysis (Bergsten, 2005). The effect of LBA on the tree inferred from the

Table 2. Differences in relative synonymous codon usage between *Erwinia*, *Pantoea* and *Tatumella*

Differences between taxa	**Total significant differences***	**Amino Acids (Codons)**
Pantoea and *Erwinia*	14	**A** (GCG, GCC), **D**(GAU, GAC), **G** (GGU), **I** (AUU), **K** (AAA, AAG), **L** (CUC, UUA, UUG), **S** (UCC, UCG), **V** (GUU)
Pantoea and *Tatumella*	37	**A** (GCU, GCG, GCA), **C** (UGU, UGC), **D** (GAU, GAC), **E** (GAG, GAA), **F** (UUU, UUC), **G** (GGU, GGG, GGC), **H** (CAC, CAU), **K** (AAA, AAG), **L** (CUU), **N** (AAC, AAU), **P** (CCU), **Q** (CAA, CAG), **R** (CGA, CGC, CGU), **S** (AGC, AGU, UCG, UCU), **T** (ACG, ACU), **V** (GUG, GUA), **Y** (UAC, UAU)
Erwinia and *Tatumella*	28	**A** (GCG, GCA), **C** (UGU, UGC), **D** (GAU, GAC), **G** (GGU, GGC), **H** (CAC, CAU), **K** (AAA, AAG), **N** (AAC, AAU), **P** (CCU), **R** (CGC, CGU), **S** (AGC, AGU, UCG, UCU), **T** (ACA, ACG, ACU), **V** (GUG, GUC), **Y** (UAC, UAU)
(*Pantoea*, *Erwinia*) and *Tatumella*	19	**A** (GCA), **C** (UGU, UGC), **G** (GGC), **H** (CAC, CAU), **N** (AAC, AAU), **P** (CCU), **R** (CGC, CGU), **S** (AGC, AGU, UCU), **T** (ACG, ACU), **V** (GUG), **Y** (UAC, UAU)

* Statistical significance as determined with pairwise two-tailed unpaired t-tests ($p < 0.05$)

Erwinia+*Pantoea*+*Tatumella*+Outgroups amino acid dataset (Figure 3a) was evaluated by removing and adding different combinations of taxa with long branches (Bergsten, 2005). These taxa were the *Tatumella* group and the group containing *P. calida* and *P. gaviniae*. Exclusion of these groups (singly or combined) in the amino acid dataset did not alter the position of any of the remaining taxa (ingroup or outgroup), including the basal position of *Pantoea* sp. A4 within *Pantoea* (Supplementary Figure S3). However, differential exclusion of these taxa appeared to alter the topology of the tree inferred using nucleotide data (Supplementary Figure S4), where the presence of these two groups, but particularly the *P. calida* and *P. gaviniae* group, appears to influence the position of *Pantoea* sp. A4. Upon the inclusion of the *P. calida* and *P. gaviniae* group, the basal position of *Pantoea* sp. A4 changes to what is observed in the nucleotide topologies, whereas inclusion of *Tatumella* does not alter this basal position of *Pantoea* sp. A4. These data thus suggest that, despite attempts to counter LBA (i.e., the use of appropriate taxon selection and evolutionary models) (Zwickl and Hillis, 2002, Heath et al., 2008, Nabhan and Sarkar, 2012), the inclusion of certain taxa (specifically *P. calida* and *P. gaviniae*) in the nucleotide dataset has a significant effect on the accuracy of the phylogeny reconstructed from it.

We also tested the possible LBA-effect of outgroup selection on the ML tree inferred from the *Erwinia*+*Pantoea*+*Tatumella*+Outgroups amino acid dataset. The results of the nine separate analyses (which each included the 35 ingroup taxa and one of the nine outgroup taxa) showed that outgroup selection had a limited effect on the robustness of the tree inferred from the *Erwinia*+*Pantoea*+*Tatumella*+Outgroups amino acid dataset (Supplementary Figure S10). For all intrageneric relationships, the only variation observed involved the relationships (generally lacking statistical support) among the closely related *P. agglomerans, P. eucalypti* and *P. vagans*. The only outgroup that affected the intergeneric relationships was *Cronobacter*, which caused *P. calida* and *P. gaviniae* to group sister to the

Pantoea+Tatumella+Erwinia clade. In the remaining eight analyses, these two species formed the sister taxon of the *Pantoea+Tatumella* clade similar to what is observed in the trees inferred from the 44-taxon *Erwinia+Pantoea+Tatumella+*Outgroups amino acid and nucleotide datasets. This suggests that the use of phylogenetic signal associated with the other outgroup taxa sufficiently compensated for the non-phylogenetic signal associated in the *Cronobacter* sequence (Philippe et al., 2011).

Non-phylogenetic signal – lineage specific rate heterogeneity

The first relative rate test utilized the amino acid sequences of a *Pantoea* isolate (*P. agglomerans*), an *Erwinia* isolate (*E. amylovora*) and an outgroup taxon (*S. marcescens*) (Supplementary Table S3). A p-value of 0.62013 was obtained indicating that the null hypothesis of equal rates across the taxa could not be rejected. The second relative rate test utilized the amino acid sequence of a *Pantoea* isolate (*P. agglomerans*), a *Tatumella* isolate (*T. morbirosei*) and an *Erwinia* isolate (*E. amylovora*) as the more distantly related taxon (as is observed from the phylograms; Supplementary Table S4). A p-value of 0 was obtained, thus leading to the rejection of the null hypothesis of equal rates across taxa, indicating lineage specific rate heterogeneity. Various combinations of different representatives of the different genera generally resulted in similar results. These data are thus congruent with the results of the RSCU analysis and suggest that *Tatumella* evolves at a faster evolutionary rate compared to either *Erwinia* or *Pantoea*.

Comparison of data subsets – 'Purifying', 'Diversifying' and 'Neutral' selection

Analysis with HyPhy showed that most of the 1,039 genes included in the *Erwinia+Pantoea+Tatumella+*Outgroups dataset likely experience purifying selection, which is consistent with what has been proposed for housekeeping or core genes involved in essential functions (Koonin, 2005, Koonin and Wolf, 2006, Alvarez-Ponce et al., 2016).

Among the 1,039 shared genes, 218 genes had dN/dS values higher than 1 (diversifying selection) and 820 genes had values lower than 1 (purifying selection) (Supplementary Figure S5), while one gene were too truncated in some taxa to include in the analysis. Of the set of 1,038 gene included in the analyses, only 13 formed part of the neutral or nearly neutral category. These three categories of genes could be expected to evolve at different rates (Alvarez-Ponce et al., 2016), as was clear from the trees inferred using the amino acid datasets (Supplementary Figure S6). However, the tree obtained from the 'purifying' amino acid dataset was fully congruent with the one obtained from the dataset including the amino acids for all 1,039 genes (compare Figure 3a and Supplementary Figure S6). This suggests that the majority of the core genome is under purifying selection and contributes to the overall phylogenetic signal in the combined dataset. The incongruence between the 'neutral' and 'purifying' amino acid trees is likely due, in part, to a lack of phylogenetic signal in the 'neutral' dataset that includes only thirteen genes. The unusual relationships inferred from the 'diversifying' amino acid dataset is probably due to the limited constraints in terms of how these genes evolve, which allows increased fixation of non-synonymous substitutions in these genes. Although differing topologies were observed for these datasets, the likelihood of the tree topology obtained for the amino acid *Erwinia*+*Pantoea*+*Tatumella*+Outgroups dataset measured against the neutral and purifying amino acid datasets could not be rejected based on the SH tests (Figure 2).

Comparison of data subsets – 'Cellular functioning', 'Metabolism', 'Informational', 'External factors' and 'Unclassified' functional categories

To maintain functionality, the genes involved in a specific cellular process (particularly those characterized by high levels of complexity) often evolve in concert and may follow similar evolutionary trajectories (Rivera et al., 1998, Jain et al., 1999, Daubin et al., 2002). The genes involved in certain processes are also more prone to HGT than others, despite representing

part of the core genome component (Rivera et al., 1998, Jain et al., 1999). Therefore, to assess the possible influence that the functional categories might have had on our species tree, the 1,039 genes were separated into their functional categories and subjected to phylogenetic analyses (Supplementary Figure S7). The data subsets comprised of between 80 ('External factors') and 336 genes ('Unclassified'), with the 'Cellular functioning', 'Metabolism' and 'Informational' functional categories incorporating 240, 236 and 281 genes, respectively, with some genes being involved in multiple functional categories. The overall relationships among the ingroup taxa of all subset trees supported the full core genome protein sequence topology, with minor differences within *Erwinia* ('Cellular functioning' and 'External factors' tree topologies) and *Pantoea* ('Informational' tree topology). This suggests that the topology obtained from the concatenation of all shared protein sequences is not influenced by the functional constraints of the chosen genes or large-scale HGT, potentially leading to false phylogenies. Despite these minor topological differences, the species tree obtained from the amino acid *Erwinia*+*Pantoea*+*Tatumella*+Outgroups dataset did also not score significantly worse based on the SH test in terms of likelihood compared to the trees obtained from the data subsets (Figure 2).

Problems with the MLSA and rMLSA trees

ML trees generated from the MLSA dataset (consisting of four protein-coding gene sequences, Supplementary Figure S8) and the rMLSA dataset (consisting of gene sequences for 52 ribosomal proteins, Supplementary Figure S9) all differed markedly from the tree inferred using the amino acid dataset for the 1,039 shared genes (Figures 3a with 3b and 3c). Upon comparison to the WGS-based phylogenies, it could be seen that the alternate topologies tested had significantly lower likelihood values based on the SH test compared to the trees obtained for each dataset during the respective ML analyses (Figure 2). This

indicates drastically different topologies for these datasets that are not reconcilable between these datasets.

In contrast to the 1,039-shared gene tree, both the MLSA and rMLSA trees further included numerous branches lacking statistical support. To some extent this is due to the limited sizes of these datasets, which would accordingly also lack sufficient phylogenetic signal especially at the nucleotide level. This was particularly evident in the MLSA dataset, as has been suggested previously (Gevers et al., 2005). As a measure of phylogenetic noise, HI was 0.745 (all nucleotides), 0.603 (third nucleotide excluded) and 0.526 (amino acid), respectively. HI values for the ribosomal dataset were 0.700 (all nucleotides), 0.672 (third nucleotide excluded) and 0.588 (amino acid). Similar to the 1,039-shared gene dataset, more homoplasious characters were thus present in the MLSA and rMLSA nucleotide datasets than their corresponding amino acid datasets.

As with the 1,039-shared gene dataset, limited substitution saturation was detected in the first and second codon positions of genes included in the smaller MLSA (Figure 4 a, b and c) and rMLSA datasets (results not shown). The phylogenies inferred from the nucleotide MLSA and rMLSA datasets containing only first and second codon positions were overall congruent with those inferred from the respective amino acid datasets (Supplementary Figures S8 and S9). However, inclusion of the third codon positions in the analyses produced trees that were clearly different from the amino acid-based trees of the corresponding dataset (Supplementary Figures S8 and S9).

The MLSA and rMLSA datasets further appeared to be particularly sensitive to LBA. The use of different outgroup-ingroup combinations generated distinct topologies, both in terms of inter- and intrageneric relationships amongst the ingroup taxa, especially in the MLSA dataset (Supplementary Figure S10). Among the nine combinations tested, none were

congruent with the tree topology inferred from the amino acid sequence of the 1,039 shared genes.

Discussion

Among the genomes for twenty-three *Pantoea* strains (twelve of which were determined in this study), three *Tatumella* species, nine *Erwinia* strains and their nine outgroup taxa, a set of 1,039 single-copy shared genes were identified. These genes formed part of the core genomes of the species harbouring them and were most likely inherited in a vertical fashion (Hacker and Carniel, 2001, Daubin et al., 2002). This core genomic component is also thought to be essential for survival as most of these genes are involved in complex processes requiring the interaction of these genes with one another, leading to concerted evolutionary paths (Rivera et al., 1998, Jain et al., 1999, Daubin et al., 2002, Cohen et al., 2011). Shared evolutionary trajectories are thus expected for groups of genes that are functionally constrained due to their intergenic interactions. Thus, the overall similarities of the phylogenies obtained for the different functional subsets were expected, as this overall core component should be evolutionarily relatively cohesive providing congruent phylogenetic hypotheses (Daubin et al., 2002).

The amino acid dataset for the 1,039 genes used in this study contained much less non-phylogenetic signal than the corresponding nucleotide dataset. The term non-phylogenetic signal refers to the combined effects of different kinds of structured phylogenetic noise (Jeffroy et al., 2006, Philippe et al., 2011). Similar to what has been shown previously, the nucleotide dataset contained higher levels of substitution saturation, particularly at third codon positions (Xia et al., 2003, Jeffroy et al., 2006). The nucleotide dataset was also more homoplasious, potentially because the accumulation of convergent mutations in data with four character states is more pronounced than in amino acid data with 20 character states (Xia

et al., 2003, Jeffroy et al., 2006). However, despite being less "noisy", the amino acid dataset remained affected by non-phylogenetic signal. In addition to containing low levels of homoplasy and substitution saturation, the codon usage bias detected in the nucleotide dataset likely gave rise to the lineage-specific rate heterogeneity observed in the amino acid dataset. The non-phylogenetic signals inherent to the amino acid dataset could, therefore, be problematic during tree reconstruction.

In this study, we attempted to limit the negative effects of non-phylogenetic signal during tree inference in three ways (Philippe et al., 2011). Firstly, we utilized strict criteria for identifying the genes included in the analyses, i.e., BLAST bit score ratios adjusted automatically depending on the data analysed (Blom et al., 2016). Although this might have led to the exclusion of less conserved genes, it allowed for the construction of a concatenated dataset consisting mainly of orthologous sequences (related via speciation or vertical descent) (Koonin, 2005). Secondly, to avoid the artificial introduction of "noise", an iteration-based method, which takes into account relatedness during iterative pair-wise alignment, was used to generate optimal sequence alignments (Edgar, 2004, Philippe et al., 2011). Thirdly, phylogenies were inferred using a probabilistic method (i.e., Maximum Likelihood) with appropriate models to approximate the evolution of individual genes making up the dataset (Philippe et al., 2011). Our findings clearly showed that this approach was highly effective for analysing the amino acid dataset, as the non-phylogenetic signal it included did not seem to influence the topology of the final tree. For example, LBA is one of the best-understood outcomes of non-phylogenetic signal (Philippe et al., 2011), yet the tree inferred from the amino acids of 1,039 genes appeared to be relatively unaffected by this phenomenon.

To further interrogate the robustness of the tree inferred from the aligned amino acid sequences of 1,039 genes, different subsets of these data were evaluated phylogenetically. The first set of analyses involved subsets based on selection, where almost 80% of the genes

seemed to experience purifying selection due to high levels of functional conservation (Jain et al., 1999, Lan and Reeves, 2000, Coenye et al., 2005). Not surprisingly, the phylogeny inferred from the amino acids for these genes matched the phylogeny inferred from the 1,039 gene dataset (Sarkar and Guttman, 2004, He et al., 2010). The tree inferred from the 13 neutrally evolving genes lacked resolution, probably due to inadequate phylogenetic signal, similar to what has been observed for other small datasets (Daubin et al., 2002, Coenye et al., 2005, Galtier and Daubin, 2008, Bennett et al., 2012, Chan et al., 2012). The tree inferred from the 218 genes under diversifying selection also lacked resolution, but in this case it is likely due to the accumulation of non-phylogenetic signal introduced during diversifying evolution (Xia et al., 2003, Jeffroy et al., 2006). Overall, however, these results suggest that the majority of the core genome evolved in a cohesive manner due to the purifying selection acting on this genomic compartment.

The second set of analyses concentrated on five subsets of the 1,039 shared genes involved in the different functional categories of the products encoded by individual genes as well as unclassified genes. The trees inferred from all of these five amino acid datasets tested, generally matched the one inferred from the 1,039 amino acid dataset. There were, however, small differences within the topologies obtained for the genes involved in 'Cellular functioning' and the 'Informational' genes, although the sister-groupings observed were without statistical support. Such subtle incongruences in topologies inferred from different functional subsets are not uncommon (Wolf et al., 2001, Lerat et al., 2003, Dutilh et al., 2004, Ma and Zeng, 2004). In fact, much greater discordance is often seen for the phylogenies inferred from different functional subsets when distantly related bacteria are considered (Dutilh et al., 2004, Ma and Zeng, 2004). Thus, despite minor differences observed from the different datasets, possibly due to "noise", the robust amino acid based phylogeny obtained for the full set of shared genes were reflected in all functional subset tree topologies.

Taken together, our findings suggest that the tree inferred from the amino acid data for the 1,039 shared genes represents the best hypothesis of explaining the inter- and intrageneric relationships examined in this study. None of the various factors typically responsible for destabilizing phylogenetic trees (Philippe et al., 2011) appeared to significantly affect it. In other words, despite containing detectable levels of non-phylogenetic signal, the use of amino acid data (Glaeser and Kämpfer, 2015), together with a suitable set of outgroup taxa and the application of appropriate evolutionary models fitted against each gene partition (Jeffroy et al., 2006, Philippe et al., 2011), provided the most robust phylogenetic hypothesis for describing the relationships within *Pantoea* and its relationships with *Tatumella* and *Erwinia*.

Pantoea, *Tatumella* and *Erwinia* were generally recovered as monophyletic groups. In accordance with what has been found previously (Brady et al., 2010b, Brady et al., 2012, Glaeser and Kämpfer, 2015), it was also consistently observed that *Pantoea* and *Tatumella* group as sister to each other. In all phylogenetic analyses (amino acid and nucleotide), the two species *P. calida* and *P. gaviniae* appeared to form a unique and separate cluster. These two species thus represent a novel genus due to the distinctness of these taxa when compared to the closest related taxa (Gavini et al., 1989a, Konstantinidis and Tiedje, 2005). Future description of a novel genus will be required to accommodate these and potentially other atypical *Pantoea* and *Erwinia* species not included in the study.

The robust species tree obtained in this study also allowed elucidating intrageneric relationships as comparison of the different phylogenies produced consistent species groupings within the respective genera. *Pantoea* sp. A4 was consistently recovered as part of *Pantoea* as suggested before (Hong et al., 2012), where it forms a basal lineage within the genus. Contrary to what was expected (Wu et al., 2013, Tian and Jing, 2014), *Pantoea* sp. IMH consistently grouped within *Erwinia*, as the 16S rRNA gene initially used for identification purposes is known to lack resolution within the *Enterobacteriaceae* (Rezzonico

et al., 2009, Glaeser and Kämpfer, 2015) which could have led to the misidentification of this taxon. The description of *Pantoea* sp. IMH as an *Erwinia* species is thus required as it may also represent a novel species. Further study and comparison to other *Erwinia* species is however required to determine whether this isolate forms part of a novel exclusive and cohesive cluster within *Erwinia*.

The results of our study also showed that conventional MLSA and rMLSA are inadequate for inferring inter- and intrageneric relationships due to the limited number of loci used in the analyses. MLSA and rMLSA phylogenies yield inconsistent groupings that lack statistical support. Our analyses showed that this could mostly be attributed to a general lack of true phylogenetic signal from which to reconstruct trees. The little true signal present in the data was likely outcompeted by non-phylogenetic signal during tree building. Accordingly, the MLSA and rMLSA phylogenies were both exceedingly sensitive to LBA where outgroup selection severely affected the topology of the ingroup. Although improved taxon sampling could counter the effects of LBA (Hillis, 1998, Zwickl and Hillis, 2002, Heath et al., 2008), our results showed that this phenomenon remains a problem in smaller datasets. Therefore, apart from the intended use for species delineation, where these approaches have been applied successfully (Brady et al., 2008, Brady et al., 2010a, Brady et al., 2010b, Brady et al., 2012, Glaeser and Kämpfer, 2015), these trees lack robustness for investigating relationships at higher taxonomic levels.

Conclusions

The use of shared gene sets for phylogenomic analyses has proven to be a useful tool for obtaining species trees of bacteria (Daubin et al., 2002, Dutilh et al., 2008, Galtier and Daubin, 2008, Segata and Huttenhower, 2011) and provides better supported and robust phylogenies compared to the commonly employed molecular markers. It has, however, been

suggested that the use of only a few genes with strong phylogenetic signal may be more feasible (Konstantinidis and Tiedje, 2006, Salichos and Rokas, 2013), but their identification will be difficult without the use of a robust phylogeny for comparison. The results presented here indicate that the choice of shared genes for analysis, as well as whether datasets are nucleotide or protein sequence based, remain important as different approaches may provide different evolutionary hypotheses, as has been suggested before (Rivera et al., 1998, Jain et al., 1999, Glaeser and Kämpfer, 2015). The robust phylogeny obtained from this data will thus be invaluable for addressing questions pertaining to the evolutionary history of *Pantoea* and its related genera as this provides a framework for investigating how different biological traits, like pathogenicity and other potentially beneficial characteristics, have evolved in these different genera (Heath et al., 2008).

Acknowledgements

We would like to acknowledge the Centre for Bioinformatics and Computational Biology, University of Pretoria, for the use of the facility and server access. For genome sequencing, we want to acknowledge the Ion Torrent Sequencing Facility at the University of Pretoria and Markus Oggenfuss and Jürg E. Frey for sequencing at Agroscope (Wädenswil, Switzerland). We would also like to acknowledge the Genome Research Institute (GRI) as well as the Centre of Excellence in Tree Health Biotechnology (CTHB) at the University of Pretoria for additional funding. THMS and BD acknowledge the funding by the Swiss Federal Office of Agriculture ACHILLES project (BLW/FOAG Project ACHILLES) as part of the Agroscope Research Programme ProfiCrops and the Department of Life Sciences and Facility Management of ZHAW.

References

ABASCAL, F., ZARDOYA, R. & POSADA, D. 2005. ProtTest: selection of best-fit models of protein evolution. *Bioinformatics,* 21**,** 2104-2105.

ALVAREZ-PONCE, D., SABATER-MUÑOZ, B., TOFT, C., RUIZ-GONZÁLEZ, M. X. & FARES, M. A. 2016. Essentiality Is a Strong Determinant of Protein Rates of Evolution during Mutation Accumulation Experiments in *Escherichia coli*. *Genome Biology and Evolution,* 8**,** 2914-2927.

ANDA, M., OHTSUBO, Y., OKUBO, T., SUGAWARA, M., NAGATA, Y., TSUDA, M., MINAMISAWA, K. & MITSUI, H. 2015. Bacterial clade with the ribosomal RNA operon on a small plasmid rather than the chromosome. *Proceedings of the National Academy of Sciences,* 112**,** 14343-14347.

ANDAM, C. P. & GOGARTEN, J. P. 2011. Biased gene transfer in microbial evolution. *Nature Reviews Microbiology,* 9**,** 543-555.

ANGUS, A. A., AGAPAKIS, C. M., FONG, S., YERRAPRAGADA, S., ESTRADA-DE LOS SANTOS, P., YANG, P., SONG, N., KANO, S., CABALLERO-MELLADO, J., DE FARIA, S. M., DAKORA, F. D., WEINSTOCK, G. & HIRSCH, A. M. 2014. Plant-Associated Symbiotic *Burkholderia* Species Lack Hallmark Strategies Required in Mammalian Pathogenesis. *PLoS ONE,* 9**,** e83779.

AZIZ, R., BARTELS, D., BEST, A., DEJONGH, M., DISZ, T., EDWARDS, R., FORMSMA, K., GERDES, S., GLASS, E., KUBAL, M., MEYER, F., OLSEN, G., OLSON, R., OSTERMAN, A., OVERBEEK, R., MCNEIL, L., PAARMANN, D., PACZIAN, T., PARRELLO, B., PUSCH, G., REICH, C., STEVENS, R., VASSIEVA, O., VONSTEIN, V., WILKE, A. & ZAGNITKO, O. 2008. The RAST Server: Rapid Annotations using Subsystems Technology. *BMC Genomics,* 9**,** 75.

BENNETT, J. S., JOLLEY, K. A., EARLE, S. G., CORTON, C., BENTLEY, S. D., PARKHILL, J. & MAIDEN, M. C. J. 2012. A genomic approach to bacterial taxonomy: an examination and proposed reclassification of species within the genus *Neisseria*. *Microbiology,* 158**,** 1570-1580.

BERGSTEN, J. 2005. A review of long-branch attraction. *Cladistics,* 21**,** 163-193.

BLOM, J., KREIS, J., SPÄNIG, S., JUHRE, T., BERTELLI, C., ERNST, C. & GOESMANN, A. 2016. EDGAR 2.0: an enhanced software platform for comparative gene content analyses. *Nucleic Acids Research*.

BOTO, L. 2010. Horizontal gene transfer in evolution: facts and challenges. *Proceedings of the Royal Society B: Biological Sciences,* 277**,** 819-827.

BOUCHER, Y., DOUADY, C. J., SHARMA, A. K., KAMEKURA, M. & DOOLITTLE, F. W. 2004. Intragenomic heterogeneity and intergenomic recombination among haloarchaeal rRNA genes. *Journal of Bacteriology,* 186**,** 3980-3990.

BRADY, C., CLEENWERCK, I., VENTER, S., VANCANNEYT, M., SWINGS, J. & COUTINHO, T. 2008. Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). *Systematic and Applied Microbiology,* 31**,** 447-460.

BRADY, C., VENTER, S., CLEENWERCK, I., VANCANNEYT, M., SWINGS, J. & COUTINHO, T. 2007. A FALFP system for the improved identification of plant-pathogenic and plant-associated species of the genus Pantoea. *Syst Appl Microbiol,* 30.

BRADY, C. L., CLEENWERCK, I., VAN DER WESTHUIZEN, L., VENTER, S. N., COUTINHO, T. A. & DE VOS, P. 2012. *Pantoea rodasii* sp. nov., *Pantoea rwandensis* sp. nov. and *Pantoea wallisii* sp. nov., isolated from *Eucalyptus*. *International Journal of Systematic and Evolutionary Microbiology,* 62**,** 1457-1464.

BRADY, C. L., CLEENWERCK, I., VENTER, S. N., ENGELBEEN, K., DE VOS, P. & COUTINHO, T. A. 2010a. Emended description of the genus *Pantoea*, description of four species from human clinical samples, *Pantoea septica* sp. nov., *Pantoea eucrina* sp. nov., *Pantoea brenneri* sp. nov. and *Pantoea conspicua* sp. nov., and transfer of *Pectobacterium cypripedii* (Hori 1911) Brenner et al. 1973 emend. Hauben et al. 1998 to the genus as *Pantoea cypripedii* comb. nov. *International Journal of Systematic and Evolutionary Microbiology,* 60**,** 2430-2440.

BRADY, C. L., GOSZCZYNSKA, T., VENTER, S. N., CLEENWERCK, I., DE VOS, P., GITAITIS, R. D. & COUTINHO, T. A. 2011. *Pantoea allii* sp. nov., isolated from onion plants and seed. *International journal of systematic and evolutionary microbiology,* 61**,** 932-937.

BRADY, C. L., VENTER, S. N., CLEENWERCK, I., ENGELBEEN, K., VANCANNEYT, M., SWINGS, J. & COUTINHO, T. A. 2009. *Pantoea vagans* sp. nov., *Pantoea eucalypti* sp. nov., *Pantoea deleyi* sp. nov. and *Pantoea anthophila* sp. nov. *International journal of systematic and evolutionary microbiology,* 59**,** 2339-2345.

BRADY, C. L., VENTER, S. N., CLEENWERCK, I., VANDEMEULEBROECKE, K., DE VOS, P. & COUTINHO, T. A. 2010b. Transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoea terrea* to the genus *Tatumella* emend. as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terrea* comb. nov. and description of *Tatumella morbirosei* sp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 60**,** 484-494.

BREMER, K. R. 1994. Branch support and tree stability. *Cladistics,* 10**,** 295-304.

BROWN, S. D., UTTURKAR, S. M., KLINGEMAN, D. M., JOHNSON, C. M., MARTIN, S. L., LAND, M. L., LU, T.-Y. S., SCHADT, C. W., DOKTYCZ, M. J. & PELLETIER, D. A. 2012. Twenty-One Genome Sequences from *Pseudomonas* Species and 19 Genome Sequences from Diverse Bacteria Isolated from the Rhizosphere and Endosphere of *Populus deltoides*. *Journal of Bacteriology,* 194**,** 5991-5993.

CASTRESANA, J. 2000. Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Molecular Biology and Evolution,* 17**,** 540-552.

CHAN, J. Z. M., HALACHEV, M. R., LOMAN, N. J., CONSTANTINIDOU, C. & PALLEN, M. J. 2012. Defining bacterial species in the genomic era: insights from the genus *Acinetobacter*. *BMC microbiology,* 12**,** 302.

CLEENWERCK, I., VANDEMEULEBROECKE, K., JANSSENS, D. & SWINGS, J. 2002. Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 52**,** 1551-1558.

COENYE, T., GEVERS, D., VAN DE PEER, Y., VANDAMME, P. & SWINGS, J. 2005. Towards a prokaryotic genomic taxonomy. *Federation of European Microbiological Societies Microbiology Reviews,* 29**,** 147-167.

COHEN, O., GOPHNA, U. & PUPKO, T. 2011. The Complexity Hypothesis Revisited: Connectivity Rather Than Function Constitutes a Barrier to Horizontal Gene Transfer. *Molecular Biology and Evolution,* 28**,** 1481-1489.

CONLAN, S., THOMAS, P. J., DEMING, C., PARK, M., LAU, A. F., DEKKER, J. P., SNITKIN, E. S., CLARK, T. A., LUONG, K., SONG, Y., TSAI, Y.-C., BOITANO, M., DAYAL, J., BROOKS, S. Y., SCHMIDT, B., YOUNG, A. C., THOMAS, J. W., BOUFFARD, G. G., BLAKESLEY, R. W., MULLIKIN, J. C., KORLACH, J., HENDERSON, D. K., FRANK, K. M., PALMORE, T. N. & SEGRE, J. A. 2014. Single-molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing *Enterobacteriaceae*. *Science Translational Medicine,* 6**,** 254ra126-254ra126.

CONVILLE, P. S. & WITEBSKY, F. G. 2007. Analysis of multiple differing copies of the 16S rRNA gene in five clinical isolates and three type strains of *Nocardia* species and implications for species assignment. *Journal of Clinical Microbiology,* 45**,** 1146-1151.

CRUZ, A. T., CAZACU, A. C. & ALLEN, C. H. 2007. *Pantoea agglomerans* - A Plant Pathogen Causing Human Disease. *J. Clin. Microbiol.***,** JCM.00632-07.

DAUBIN, V., GOUY, M. & PERRIÈRE, G. 2002. A phylogenomic approach to bacterial phylogeny: evidence of a core of genes sharing a common history. *Genome Research,* 12**,** 1080-1090.

DE BAERE, T., VERHELST, R., LABIT, C., VERSCHRAEGEN, G., WAUTERS, G., CLAEYS, G. & VANEECHOUTTE, M. 2004. Bacteremic infection with *Pantoea ananatis*. *Journal of Clinical Microbiology,* 42**,** 4393-4395.

DE MAAYER, P., CHAN, W.-Y., BLOM, J., VENTER, S. N., DUFFY, B., SMITS, T. H. M. & COUTINHO, T. A. 2012. The large universal *Pantoea* plasmid LPP-1 plays a major role in biological and ecological diversification. *BMC Genomics,* 13**,** 625.

DE MAAYER, P., CHAN, W.-Y., RUBAGOTTI, E., VENTER, S. N., TOTH, I. K., BIRCH, P. R. J. & COUTINHO, T. A. 2014. Analysis of the *Pantoea ananatis* pan-genome reveals factors underlying its ability to colonize and interact with plant, insect and vertebrate hosts. *BMC Genomics,* 15**,** 1-28.

DESPER, R. & GASCUEL, O. 2002. Fast and Accurate Phylogeny Reconstruction Algorithms Based on the Minimum-Evolution Principle. *Journal of Computational Biology,* 9**,** 687-705.

DUTILH, B. E., HUYNEN, M. A., BRUNO, W. J. & SNEL, B. 2004. The Consistent Phylogenetic Signal in Genome Trees Revealed by Reducing the Impact of Noise. *Journal of Molecular Evolution,* 58**,** 527-539.

DUTILH, B. E., SNEL, B., ETTEMA, T. J. G. & HUYNEN, M. A. 2008. Signature Genes as a Phylogenomic Tool. *Molecular Biology and Evolution,* 25**,** 1659-1667.

EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research,* 32**,** 1792-1797.

FELSENSTEIN, J. 2005. SEQBOOT - bootstrap, jackknife or permutation resampling of molecular sequence, restriction site, gene frequency or character data.

FOUTS, D. E., MATTHIAS, M. A., ADHIKARLA, H., ADLER, B., AMORIM-SANTOS, L., BERG, D. E., BULACH, D., BUSCHIAZZO, A., CHANG, Y.-F., GALLOWAY, R. L., HAAKE, D. A., HAFT, D. H., HARTSKEERL, R., KO, A. I., LEVETT, P. N., MATSUNAGA, J., MECHALY, A. E., MONK, J. M., NASCIMENTO, A. L. T., NELSON, K. E., PALSSON, B., PEACOCK, S. J., PICARDEAU, M., RICALDI, J. N., THAIPANDUNGPANIT, J., WUNDER, E. A., JR., YANG, X. F., ZHANG, J.-J. & VINETZ, J. M. 2016. What Makes a Bacterial Species Pathogenic?:Comparative Genomic Analysis of the Genus *Leptospira*. *PLoS Negl Trop Dis,* 10**,** e0004403.

FOX, G. E., WISOTZKEY, J. D. & JURTSHUK, P. J. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *International Journal of Systematic Bacteriology,* 42**,** 166-170.

GALTIER, N. & DAUBIN, V. 2008. Dealing with incongruence in phylogenomic analyses. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 363**,** 4023-4029.

GALTIER, N. & GOUY, M. 1995. Inferring phylogenies from DNA sequences of unequal base compositions. *Proceedings of the National Academy of Sciences,* 92**,** 11317-11321.

GAVINI, F., HOLMES, B., IZARD, D., BEJI, A., BERNIGAUD, A. & JAKUBCZAK, E. 1989a. Numerical taxonomy of *Pseudomonas alcaligenes*, *P. pseudoalcaligenes, P. mendocina, P. stutzeri*, and related bacteria. *International Journal of Systematic and Evolutionary Microbiology,* 39**,** 135-144.

GAVINI, F., MERGAERT, J., BEJI, A., MIELCAREK, C., IZARD, D., KERSTERS, K. & DE LEY, J. 1989b. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. *Int J Syst Bacteriol,* 39.

GEVERS, D., COHAN, F. M., LAWRENCE, J. G., SPRATT, B. G., COENYE, T., FEIL, E. J., STACKEBRANDT, E., VAN DE PEER, Y., VANDAMME, P., THOMPSON, F. L. & SWINGS, J. 2005. Re-evaluating prokaryotic species. *Nature Reviews,* 3**,** 733-739.

GLAESER, S. P. & KÄMPFER, P. 2015. Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. *Systematic and Applied Microbiology,* 38**,** 237-245.

GOGARTEN, J. P., DOOLITTLE, W. F. & LAWRENCE, J. G. 2002. Prokaryotic Evolution in Light of Gene Transfer. *Molecular Biology and Evolution,* 19**,** 2226-2238.

GORDON, A. & HANNON, G. J. 2010. Fastx-toolkit. FASTQ/A short-reads pre-processing tools. *Unpublished http://hannonlab. cshl. edu/fastx_ toolkit*.

GUEULE, D., FOURNY, G., AGERON, E., LE FLÈCHE-MATÉOS, A., VANDENBOGAERT, M., GRIMONT, P. A. D. & CILAS, C. 2015. *Pantoea coffeiphila* sp. nov., cause of the 'potato taste' of Arabica

coffee from the African Great Lakes region. *International Journal of Systematic and Evolutionary Microbiology,* 65**,** 23-29.

GUINDON, S. P., DUFAYARD, J.-F. O., LEFORT, V., ANISIMOVA, M., HORDIJK, W. & GASCUEL, O. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology,* 59**,** 307-321.

HACKER, J. & CARNIEL, E. 2001. Ecological fitness, genomic islands and bacterial pathogenicity. *EMBO reports,* 2**,** 376-381.

HACKER, J. R. H., DOBRINDT, U. & KURTH, R. 2012. *Genome Plasticity and Infectious Diseases*, ASM Press.

HALL, T. 2011. BioEdit: an important software for molecular biology. *GERF Bull Biosci,* 2**,** 60-1.

HE, M., SEBAIHIA, M., LAWLEY, T. D., STABLER, R. A., DAWSON, L. F., MARTIN, M. J., HOLT, K. E., SETH-SMITH, H. M. B., QUAIL, M. A., RANCE, R., BROOKS, K., CHURCHER, C., HARRIS, D., BENTLEY, S. D., BURROWS, C., CLARK, L., CORTON, C., MURRAY, V., ROSE, G., THURSTON, S., VAN TONDER, A., WALKER, D., WREN, B. W., DOUGAN, G. & PARKHILL, J. 2010. Evolutionary dynamics of *Clostridium difficile* over short and long time scales. *Proceedings of the National Academy of Sciences,* 107**,** 7527-7532.

HEATH, T. A., HEDTKE, S. M. & HILLIS, D. M. 2008. Taxon sampling and the accuracy of phylogenetic analyses. *J Syst Evol,* 46**,** 239-257.

HILLIS, D. M. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Systematic Biology,* 47**,** 3-8.

HONG, K.-W., GAN, H. M., LOW, S.-M., LEE, P. K. Y., CHONG, Y.-M., YIN, W.-F. & CHAN, K.-G. 2012. Draft genome sequence of *Pantoea* sp. strain A4, a *Rafflesia*-associated bacterium that produces N-acylhomoserine lactones as quorum-sensing molecules. *Journal of Bacteriology,* 194**,** 6610.

JAIN, R., RIVERA, M. C. & LAKE, J. A. 1999. Horizontal gene transfer among genomes: The complexity hypothesis. *Proceedings of the National Academy of Sciences,* 96**,** 3801-3806.

JAIN, R., RIVERA, M. C., MOORE, J. E. & LAKE, J. A. 2002. Horizontal Gene Transfer in Microbial Genome Evolution. *Theoretical Population Biology,* 61**,** 489-495.

JEFFROY, O., BRINKMANN, H., DELSUC, F. D. R. & PHILIPPE, H. 2006. Phylogenomics: the beginning of incongruence? *TRENDS in Genetics,* 22**,** 225-231.

JOLLEY, K. A., BLISS, C. M., BENNETT, J. S., BRATCHER, H. B., BREHONY, C., COLLES, F. M., WIMALARATHNA, H., HARRISON, O. B., SHEPPARD, S. K., CODY, A. J. & MAIDEN, M. C. J. 2012. *Ribosomal Multi-Locus Sequence Typing: universal characterisation of bacteria from domain to strain.* PhD, University of Oxford.

JONES, D. T., TAYLOR, W. R. & THORNTON, J. M. 1992. The rapid generation of mutation data matrices from protein sequences. *Computer applications in the biosciences : CABIOS,* 8**,** 275-282.

KAMBER, T., SMITS, T. H. M., REZZONICO, F. & DUFFY, B. 2012. Genomics and current genetic understanding of *Erwinia amylovora* and the fire blight antagonist *Pantoea vagans*. *Trees,* 26**,** 227-238.

KIM, H. J., LEE, J. H., KANG, B. R., RONG, X., MCSPADDEN GARDENER, B. B., JI, H. J., PARK, C.-S. & KIM, Y. C. 2012. Draft genome sequence of *Pantoea ananatis* B1-9, a nonpathogenic plant growth-promoting bacterium. *Journal of Bacteriology,* 194**,** 729.

KIM, J. 1996. General Inconsistency Conditions for Maximum Parsimony: Effects of Branch Lengths and Increasing Numbers of Taxa. *Systematic Biology,* 45**,** 363-374.

KLENK, H. P. & GÖKER, M. 2010. En route to a genome-based classification of Archaea and Bacteria? *Systematic and Applied Microbiology,* 33**,** 175-182.

KONSTANTINIDIS, K. T. & TIEDJE, J. M. 2005. Towards a genome-based taxonomy for prokaryotes. *Journal of Bacteriology,* 187**,** 6258-6264.

KONSTANTINIDIS, K. T. & TIEDJE, J. M. 2006. Toward a more robust assessment of intraspecies diversity, using fewer genetic markers. *Applied and Environmental Microbiology,* 72**,** 7286-7293.

KONSTANTINIDIS, K. T. & TIEDJE, J. M. 2007. Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead. *Current Opinion in Microbiology,* 10**,** 504-509.

KOONIN, E. V. 2005. Orthologs, Paralogs, and Evolutionary Genomics. *Annual Review of Genetics,* 39**,** 309-338.

KOONIN, E. V. & WOLF, Y. I. 2006. Evolutionary systems biology: links between gene evolution and function. *Current Opinion in Biotechnology,* 17**,** 481-487.

KUCK, P. & LONGO, G. 2014. FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. *Frontiers in Zoology,* 11**,** 81.

LAN, R. & REEVES, P. R. 2000. Intraspecies variation in bacterial genomes: the need for a species genome concept. *Trends in Microbiology,* 8**,** 396-401.

LANG, J. M., DARLING, A. E. & EISEN, J. A. 2013. Phylogeny of Bacterial and Archaeal Genomes Using Conserved Genes: Supertrees and Supermatrices. *PLoS ONE,* 8**,** e62510.

LERAT, E., DAUBIN, V. & MORAN, N. A. 2003. From Gene Trees to Organismal Phylogeny in Prokaryotes:The Case of the γ-Proteobacteria. *PLoS Biol,* 1**,** e19.

LIM, J.-A., LEE, D. H., KIM, B.-Y. & HEU, S. 2014. Draft genome sequence of *Pantoea agglomerans* R190, a producer of antibiotics against phytopathogens and foodborne pathogens. *Journal of Biotechnology,* 188**,** 7-8.

LUKJANCENKO, O., WASSENAAR, T. & USSERY, D. 2012. Comparison of 61 sequenced *Escherichia coli* genomes. *Microbial Ecology,* 60**,** 708-720.

MA, H.-W. & ZENG, A.-P. 2004. Phylogenetic comparison of metabolic capacities of organisms at genome level. *Molecular Phylogenetics and Evolution,* 31**,** 204-213.

MA, Y., YIN, Y., RONG, C., CHEN, S., LIU, Y., WANG, S. & XU, F. 2016. *Pantoea pleuroti* sp. nov., Isolated from the Fruiting Bodies of *Pleurotus eryngii*. *Current microbiology,* 72**,** 207-212.

MAKAROVA, K., SLESAREV, A., WOLF, Y., SOROKIN, A., MIRKIN, B., KOONIN, E., PAVLOV, A., PAVLOVA, N., KARAMYCHEV, V., POLOUCHINE, N., SHAKHOVA, V., GRIGORIEV, I., LOU, Y., ROHKSAR, D., LUCAS, S., HUANG, K., GOODSTEIN, D. M., HAWKINS, T., PLENGVIDHYA, V., WELKER, D., HUGHES, J., GOH, Y., BENSON, A., BALDWIN, K., LEE, J. H., DÍAZ-MUÑIZ, I., DOSTI, B., SMEIANOV, V., WECHTER, W., BARABOTE, R., LORCA, G., ALTERMANN, E., BARRANGOU, R., GANESAN, B., XIE, Y., RAWSTHORNE, H., TAMIR, D., PARKER, C., BREIDT, F., BROADBENT, J., HUTKINS, R., O'SULLIVAN, D., STEELE, J., UNLU, G., SAIER, M., KLAENHAMMER, T., RICHARDSON, P., KOZYAVKIN, S., WEIMER, B. & MILLS, D. 2006. Comparative genomics of the lactic acid bacteria. *Proceedings of the National Academy of Sciences,* 103**,** 15611-15616.

MARGULIES, M., EGHOLM, M., ALTMAN, W. E., ATTIYA, S., BADER, J. S., BEMBEN, L. A., BERKA, J., BRAVERMAN, M. S., CHEN, Y.-J. & CHEN, Z. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature,* 437**,** 376-380.

MERGAERT, J., VERDONCK, L. & KERSTERS, K. 1993. Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int J Syst Bacteriol,* 43.

MITCHELL, A., MITTER, C. & REGIER, J. C. 2000. More Taxa or More Characters Revisited: Combining Data from Nuclear Protein-Encoding Genes for Phylogenetic Analyses of Noctuoidea (Insecta: Lepidoptera). *Systematic Biology,* 49**,** 202-224.

NABHAN, A. R. & SARKAR, I. N. 2012. The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. *Briefings in Bioinformatics,* 13**,** 122-134.

NAUM, M., BROWN, E. W. & MASON-GAMER, R. J. 2008. Is 16S rDNA a reliable phylogenetic marker to characterize relationships below the family level in the enterobacteriaceae? *Journal of molecular evolution,* 66**,** 630-642.

NEI, M. & KUMAR, S. 2000. *Molecular evolution and phylogenetics*, Oxford university press.
PALMER, M., DE MAAYER, P., POULSEN, M., STEENKAMP, E. T., VAN ZYL, E., COUTINHO, T. A. & VENTER, S. N. 2016. Draft genome sequences of *Pantoea agglomerans* and *Pantoea vagans* isolates associated with termites. *Standards in Genomic Sciences,* 11**,** 1-11.
PHILIPPE, H., BRINKMANN, H., LAVROV, D. V., LITTLEWOOD, D. T. J., MANUEL, M., WÖRHEIDE, G. & BAURAIN, D. 2011. Resolving difficult phylogenetic questions: why more sequences are not enough. *PLoS Biol,* 9**,** e1000602.
PHILIPPE, H. & FORTERRE, P. 1999. The rooting of the universal tree of life is not reliable. *Journal of Molecular Evolution,* 49**,** 509-523.
POND, S. L. K. & MUSE, S. V. 2005. HyPhy: Hypothesis Testing Using Phylogenies. *Statistical Methods in Molecular Evolution.* New York, NY: Springer New York.
POPP, A., CLEENWERCK, I., IVERSEN, C., DE VOS, P. & STEPHAN, R. 2010. *Pantoea gaviniae* sp. nov. and *Pantoea calida* sp. nov., isolated from infant formula and an infant formula production environment. *International journal of systematic and evolutionary microbiology,* 60**,** 2786-2792.
PRAKASH, O., NIMONKAR, Y., VAISHAMPAYAN, A., MISHRA, M., KUMBHARE, S., JOSEF, N. & SHOUCHE, Y. S. 2015. *Pantoea intestinalis* sp. nov., isolated from the human gut. *International journal of systematic and evolutionary microbiology,* 65**,** 3352-3358.
PRASANNA, A. N. & MEHRA, S. 2013. Comparative Phylogenomics of Pathogenic and Non-Pathogenic *Mycobacterium*. *PLoS ONE,* 8**,** e71248.
PRICE, M. N., DEHAL, P. S. & ARKIN, A. P. 2010. FastTree 2 - Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE,* 5**,** e9490.
REZZONICO, F., SMITS, T. H., MONTESINOS, E., FREY, J. E. & DUFFY, B. 2009. Genotypic comparison of *Pantoea agglomerans* plant and clinical strains. *BMC Microbiology,* 9**,** 204.
REZZONICO, F., SMITS, T. H. M., BORN, Y., BLOM, J., FREY, J. E., GOESMANN, A., CLEENWERCK, I., DE VOS, P., BONATERRA, A., DUFFY, B. & MONTESINOS, E. 2016. *Erwinia gerundensis* sp. nov., a cosmopolitan epiphyte originally isolated from pome fruit trees. *International Journal of Systematic and Evolutionary Microbiology,* 66**,** 1583-1592.
RICHTER, M. & ROSSELLÓ-MÓRA, R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences,* 106**,** 19126-19131.
RIVERA, M. C., JAIN, R., MOORE, J. E. & LAKE, J. A. 1998. Genomic evidence for two functionally distinct gene classes. *Proceedings of the National Academy of Sciences,* 95**,** 6239-6244.
RONG, C., MA, Y., WANG, S., LIU, Y., CHEN, S., HUANG, B., WANG, J. & XU, F. 2016. *Pantoea hericii* sp. nov., Isolated from the Fruiting Bodies of *Hericium erinaceus*. *Current microbiology,* 72**,** 738-743.
SALICHOS, L. & ROKAS, A. 2013. Inferring ancient divergences requires genes with strong phylogenetic signals. *Nature,* 497**,** 327-333.
SARKAR, S. F. & GUTTMAN, D. S. 2004. Evolution of the Core Genome of *Pseudomonas syringae*, a Highly Clonal, Endemic Plant Pathogen. *Applied and Environmental Microbiology,* 70**,** 1999-2012.
SEGATA, N. & HUTTENHOWER, C. 2011. Toward an Efficient Method of Identifying Core Genes for Evolutionary and Functional Microbial Phylogenies. *PLoS ONE,* 6**,** e24704.
SMITS, T. H. M., REZZONICO, F., KAMBER, T., BLOM, J., GOESMANN, A., ISHIMARU, C. A., FREY, J. E., STOCKWELL, V. O. & DUFFY, B. 2011. Metabolic Versatility and Antibacterial Metabolite Biosynthesis Are Distinguishing Genomic Features of the Fire Blight Antagonist *Pantoea vagans* C9-1. *PLOS ONE,* 6**,** e22247.
SMITS, T. H. M., REZZONICO, F., KAMBER, T., GOESMANN, A., ISHIMARU, C. A., STOCKWELL, V. O., FREY, J. E. & DUFFY, B. 2010. Genome sequence of the biocontrol agent *Pantoea vagans* strain C9-1. *Journal of Bacteriology,* 192**,** 6486-6487.
SMITS, T. H. M., REZZONICO, F., LÓPEZ, M. M., BLOM, J., GOESMANN, A., FREY, J. E. & DUFFY, B. 2013. Phylogenetic position and virulence apparatus of the pear flower necrosis pathogen

Erwinia piriflorinigrans CFBP 5888T as assessed by comparative genomics. *Systematic and Applied Microbiology,* 36**,** 449-456.

STALEY, J. T. 2006. The bacterial species dilemma and the genomic-phylogenetic species concept. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 361**,** 1899-1909.

STAMATAKIS, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics,* 30**,** 1312-1313.

SWOFFORD, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version4,* Sunderland, Massachesetts, Sinauer Associates.

TAJIMA, F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics,* 135**,** 599-607.

TAMBONG, J. T., XU, R., KANEZA, C.-A. & NSHOGOZABAHIZI, J.-C. 2014. An In-depth Analysis of a Multilocus Phylogeny Identifies leuS As a Reliable Phylogenetic Marker for the Genus *Pantoea*. *Evolutionary Bioinformatics Online,* 10**,** 115-125.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution,* 30**,** 2725-2729.

TANAKA, Y. K., HORIE, N., MOCHIDA, K., YOSHIDA, Y., OKUGAWA, E. & NANJO, F. 2015. *Pantoea theicola* sp. nov., isolated from black tea. *International journal of systematic and evolutionary microbiology,* 65**,** 3313-3319.

TAVARÉ, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on mathematics in the life sciences,* 17**,** 57-86.

TIAN, H. & JING, C. 2014. Genome Sequence of the Aerobic Arsenate-Reducing Bacterium *Pantoea* sp. Strain IMH. *Genome Announcements,* 2.

WALTERSON, A. M. & STAVRINIDES, J. 2015. *Pantoea*: insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiology Reviews,* 39**,** 968-984.

WAN, X., HOU, S., PHAN, N., MALONE MOSS, J. S., DONACHIE, S. P. & ALAM, M. 2015. Draft Genome Sequence of *Pantoea anthophila* Strain 11-2 from Hypersaline Lake Laysan, Hawaii. *Genome Announcements,* 3.

WANG, X., YANG, F. & VON BODMAN, S. B. 2011. The genetic and structural basis of two distinct terminal side branch residues in stewartan and amylovoran exopolysaccharides and their potential role in host adaptation. *Molecular Microbiology,* 83**,** 195-207.

WOESE, C. R. 2000. Interpreting the universal phylogenetic tree. *Proceedings of the National Academy of Sciences,* 97**,** 8392-8396.

WOLF, Y. I., ROGOZIN, I. B., GRISHIN, N. V., TATUSOV, R. L. & KOONIN, E. V. 2001. Genome trees constructed using five different approaches suggest new major bacterial clades. *BMC Evolutionary Biology,* 1**,** 8.

WU, Q., DU, J., ZHUANG, G. & JING, C. 2013. *Bacillus* sp. SXB and *Pantoea* sp. IMH, aerobic As (V) ‐ reducing bacteria isolated from arsenic ‐ contaminated soil. *Journal of applied microbiology,* 114**,** 713-721.

XIA, X. & XIE, Z. 2001. DAMBE: Software Package for Data Analysis in Molecular Biology and Evolution. *Journal of Heredity,* 92**,** 371-373.

XIA, X., XIE, Z., SALEMI, M., CHEN, L. & WANG, Y. 2003. An index of substitution saturation and its application. *Molecular phylogenetics and evolution,* 26**,** 1-7.

ZHANG, Y. & QIU, S. 2015. Examining phylogenetic relationships of *Erwinia* and *Pantoea* species using whole genome sequence data. *Antonie van Leeuwenhoek,* 108**,** 1037-1046.

ZWICKL, D. J. & HILLIS, D. M. 2002. Increased Taxon Sampling Greatly Reduces Phylogenetic Error. *Systematic Biology,* 51**,** 588-598.