Research Letters

Study of mesophilic *Aeromonas salmonicida* A527 strain sheds light on the species lifestyles and taxonomic dilemma

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ABSTRACT

The Gram-negative bacterium *Aeromonas salmonicida* contains five subspecies: *salmonicida*, *smithia*, *achromogenes*, *masoucida* and *pectinolytica*. *Pectinolytica* is a mesophilic subspecies with the ability to thrive at a wide range of temperatures, including 37°C, while the four other subspecies are psychrophilic, thus restricted to lower temperatures. The psychrophilic subspecies are known to infect a wide range of fishes. However, there is no evidence of pathogenicity for the mesophilic subspecies *pectinolytica*. Study of the differences between the mesophilic and psychrophilic subspecies is hampered by the lack of completely sequenced and closed genomes from the mesophilic subspecies. A previous study reported that insertion sequences, which can induce genomic rearrangements at temperatures around 25°C, could be one of the determinants explaining the differences in lifestyle (mesophilic or psychrophilic) between the subspecies. In this study, the genome of mesophilic strain A527 of *A. salmonicida* was sequenced, closed and analyzed to investigate the mesophilic/psychrophilic discrepancy. This reference genome supports the hypothesis that insertion sequences are major determinants of the lifestyle differences between the *A. salmonicida* subspecies. Moreover, the phylogenetic analysis done to position strain A527 within the taxonomy raises an issue regarding the intraspecies structure of *A. salmonicida*.

INTRODUCTION

The first mention of the Gram-negative bacterium *Aeromonas salmonicida* was in 1894 (Emmerich and Weibel 1894). It was reported to be the subspecies *salmonicida* (named *Bacillus der Forellenseuche* at that time), causing the fish disease furunculosis. As detailed elsewhere (Austin and Austin 2016), the taxonomic positioning and the name of the bacterium became confusing over time, due to some articles that were written using different names to describe the bacterium, and in other research, the creation of novel bacterial families, including *Aeromonadaceae* (Colwell, Macdonell and De 1986).

Currently, there are five officially recognized *A. salmonicida* subspecies: *salmonicida*, *smithia*, *achromogenes*, *masoucida,* and *pectinolytica* (Martin-Carnahan and Joseph 2005). The subspecies *salmonicida* is considered to be the usual etiologic agent of furunculosis in salmonids, while the four other subspecies are designated as "atypical" according to their phenotypic and biochemical differences and their ability to infect a wide range of fishes (Martin-Carnahan and Joseph 2005; Dallaire-Dufresne *et al.* 2014; Austin and Austin 2016). Strains of the subspecies *salmonicida*, *smithia*, *achromogenes* and *masoucida* are psychrophilic and thus, their growth is restricted to temperatures around 25°C (Austin and Austin 2016).

In 2000, the *pectinolytica* subspecies was isolated from a polluted river (Matanza River, Argentina) but it does not have any known (fish) host (Pavan *et al.* 2000) and there is no record of its pathogenicity. This subspecies, by having the ability to grow well at 37°C, is considered mesophilic and challenged the current knowledge on *A. salmonicida*, thereby suggesting a greater diversity.

More recently, a study reported the characterization of three Indian mesophilic *A. salmonicida* strains (Y47, Y567 and Y577) from undetermined subspecies and suggested that insertion sequences could be major determinants in the temperature-related lifestyle dichotomy

between species of *A. salmonicida* (Vincent *et al.* 2016b). This hypothesis is mainly due to the fact that genomes from mesophilic strains seem to have fewer insertion sequences and that their repertoire is different from the one of psychrophilic strains. Several studies reported experimental evidence that insertion sequences in *A. salmonicida* can be the cause of major genomic disturbance events when the strains are grown at temperatures above their optimal growth temperature. For example, growing *A. salmonicida* subsp. *salmonicida* at temperatures around 25°C resulted in the disruption of genes (Gustafson, Chu and Trust 1994), the loss of the type three secretion system (TTSS) locus (Daher *et al.* 2011), the loss of the small pAsal1 plasmid (Tanaka *et al.* 2012), and reshaping of the pAsa4 plasmids (Tanaka *et al.* 2016). However, complete and closed genomic sequences are only available for reference strain A449, of the *salmonicida* subspecies, (Reith *et al.* 2008) and for three likely psychrophilic strains [S44 (BioSample: SAMN07276874), S68 (BioSample: SAMN07276873) and S121 (BioSample: SAMN07276469)]. The taxonomy for these three strains is unclear, which limits genomic interpretation and analysis.

In this study, the complete genome of the mesophilic strain *A. salmonicida* A527 was sequenced, closed and analyzed to shed light on the unusual lifestyle dichotomy of the species. The genomic information gained herein also raised a taxonomic issue about the subspecies delineation of *A. salmonicida*.

MATERIAL AND METHODS

A. salmonicida strain A527 was isolated in a market in Mumbai (India) from a dead giant river prawn (*Macrobrachium rosenbergii*) (Nagar, Shashidhar and Bandekar 2011). As indicated previously (Nagar, Shashidhar and Bandekar 2011), 25 g of the prawn was added to 225 mL of tryptic soya broth, homogenized, and incubated at 30°C for 24 h. The resulting culture was then plated on starch ampicillin agar and incubated again at 30°C for 24 h. A single colony that showed typical *Aeromonas* characteristics was picked at random and re-streaked as above. A first taxonomic assignment was made by sequencing the 16S rRNA gene and then by searching homologous sequences in GenBank database. Finally, a pulsed field gel electrophoresis was performed to obtain a DNA fingerprint of the strain and to compare it with reference strains of various *Aeromonas* species (Nagar, Shashidhar and Bandekar 2011).

Bacterial samples were recovered from frozen stocks at –80°C, plated on TSA, and incubated at 18°C for 48 h before being used in subsequent analyses. The growth kinetics were realized at 18°C and 37°C in a Tecan Infinite F200 PRO microplate reader (Tecan, USA) as already described elsewhere (Vincent *et al.* 2016b). Phenotypic tests were performed using API 20E strips (bioMérieux) as described by the manufacturer. The strips were incubated for 72 h at 18°C and analyzed. The tests for motility, production of pigments, catalase, oxidase, utilization of gluconate, haemolytic capacity, degradation of casein and tributyrin, growth on MacConkey agar and finally Gram/KOH were also performed as described elsewhere (Gerhardt 1994; Cowan *et al.* 2003).

The DNA was extracted from strain A527 with phenol and chloroform, following a protocol called *Extracting DNA Using Phenol-Chloroform*, provided by Pacific Biosciences (http://www.pacb.com). The SMRT long-reads technology from PacBio was chosen to generate a high quality assembly able to bypass the large, repeated elements, such as insertion sequences

and ribosomal operons (Vincent *et al.* 2014a). PacBio reads were processed and *de novo* assembled with the RS_HGAP_Assembly.3 pipeline as implemented in SMRT Analysis 2.3.0 [https://github.com/PacificBiosciences/SMRT-Analysis/wiki/SMRT-Analysis-Software-Installation-v2.3.0]. The tool Circlator version 1.5.1 (Hunt *et al.* 2015) was used to circularize the chromosome sequence. The resulting sequence was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) of the NCBI and was deposited in GenBank under the accession number CP022550. The insertion sequences contained in the *A. salmonicida* A527 genome were annotated using ISsaga (Varani *et al.* 2011) and manually curated.

The pan-genome of all available *A. salmonicida* genomes (a total of 32, including the genome of strain A527 and at least one representative of each of the five official subspecies), in addition to representatives of all other *Aeromonas* species (a total of 28), was evaluated using GET_HOMOLOGUES version 20170609 (Contreras-Moreira and Vinuesa 2013) (see Supp. Fig. S1 and Table S1). The sequences of the soft core genes (genes that are present in more than 95% of taxa) were aligned by codons with TranslatorX version 1.1 (Abascal, Zardoya and Telford 2010), and filtered using BMGE version 1.12 (Criscuolo and Gribaldo 2010). Finally, the resulting 2,044 sequences (after removing the paralogous genes) were concatenated into a partitioned supermatrix with 882,290 positions. The phylogenetic analysis itself was performed with IQ-TREE version 1.6.beta2, where the best-fit model was found for each partition by performing 10,000 ultrafast bootstraps (UFBoot) (Nguyen *et al.* 2015). The resulting tree was visualized and midpoint-rooted by FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). Average Nucleotide Identity (ANI) values were obtained with pyani (https://github.com/widdowquinn/pyani).

The tools TXSScan (Abby *et al.* 2016) and PHASTER (Arndt *et al.* 2016) were used to predict secretion systems and prophage sequences, respectively. The antibiotic resistance genes

were predicted by the Resistance Gene Identifier (RGI) available online through the Comprehensive Antibiotic Resistance Database (CARD) (Jia *et al.* 2016). Finally, the presence of CRISPR clusters was assessed using CRISPRFinder online (Grissa, Vergnaud and Pourcel 2007).

RESULTS AND DISCUSSION

General features

A. salmonicida strain A527 was isolated from a dead giant river prawn (*Macrobrachium rosenbergii*) that showed no sign of disease during a sampling campaign for a study that evaluated the presence of *Aeromonas* bacteria at various food retailers in Mumbai (India) (Nagar, Shashidhar and Bandekar 2011). It is unclear if *M. rosenbergii* is A527's host, or if it was contaminated with the *A. salmonicida* strain. Therefore, the host is considered as unknown. Given the reported mesophilic/psychrophilic lifestyle dichotomy present in the *A. salmonicida* species (Vincent *et al.* 2016b), A527's capacity to grow at 18°C and 37°C was tested. Predictably, as the strain was initially recovered at 30°C (Nagar, Shashidhar and Bandekar 2011), A527 grew efficiently at both temperatures (Fig. 1).

A. salmonicida strain A527 shares some signature metabolic activities with other mesophilic *A. salmonicida* strains (see Supp. Fig. S2), such as the production of acid from sorbitol and arabinose, and the presence of active L-lysine decarboxylase and β-galactosidase. Both A527 and strain 34mel^T of the subspecies *pectinolytica* were cytochrome *c* oxidase positive, as are the psychrophilic *A. salmonicida* strains [NBRC 13784^T (subspecies *masoucida*) and 01-B526 (subspecies *salmonicida*)], meaning that they can reduce molecules of oxygen to water in the aerobic respiratory chain (Iwata 1998). Other mesophilic Indian strains Y577, Y567 and Y47 are cytochrome *c* oxidase negative. Notwithstanding, a clustering based on phenotypes permitted the generation of two distinct groups (see Supp. Fig. S2): the first containing the psychrophilic

subspecies *masoucida* and *salmonicida*, and a second group with the mesophilic Indian *A. salmonicida* strains (A527, Y47, Y567 and Y577) and the mesophilic subspecies *pectinolytica*. However, it is actually impossible to assign a known subspecies to the strain A527 based on the 35 tests performed in the present study.

Since no closed genome from a mesophilic strain of *A. salmonicida* was available, the A527 genome was sequenced by PacBio, which led to the assembly of a single contig. This 4,806,250 bp-long genome has a guanine-cytosine (GC) content of 58.66%. These values are close to the ones reported for the four other *A. salmonicida* closed genomes (see Supplementary Table S2). However, a comparison between these genomes showed major differences within their architecture (Fig. 2). An investigation of the A527 genome using TXSScan (Abby *et al.* 2016) predicted genes coding for proteins involved in putative complete Type I and II secretion systems, and Type IV pilus. The same tool also predicted the mandatory genes to produce flagella.

Recent studies reported antibiotic resistance as a major issue for treating *A. salmonicida* subsp. *salmonicida* infections (Vincent *et al.* 2014b, 2016a; Piotrowska and Popowska 2015; Trudel *et al.* 2016). However, little is known about the antibiotic resistance of atypical *A. salmonicida* strains (L'Abée-Lund and Sørum 2001; Casas *et al.* 2005). The genome of *A. salmonicida* strain A527 possesses several genes that code for efflux pumps putatively involved in antibiotic resistance (see Supp. Fig. S3). PHASTER (Arndt *et al.* 2016) predicted a complete prophage in the genome of A527 (including *attL* and *attR* sequences). Three distinct prophages were already reported in genomes of strains of *A. salmonicida* subsp. *salmonicida*. One of them, named Prophage 3 (Emond-Rheault *et al.* 2015), exhibits sequelog sequences (Varshavsky 2004) with the prophage found in A527. No CRISPR was found in the genome of A527.

Phylogenetic analysis

The phylogenetic position of this new strain was assessed by molecular phylogeny. Strain A527 clustered along with other mesophilic strains (*pectinolytica* 34mel^T, Y577, Y567 and Y47) (Fig. 3). The positions of strains of the subspecies *salmonicida*, *smithia*, *achromogenes* and *masoucida* respect the previously proposed topology (Vincent *et al.* 2016b; Vincent and Charette 2017). The two newly published *A. salmonicida* subsp. *salmonicida* Chinese strains BG and YK (Long *et al.* 2016) were basal to those from Canada and Europe. Strains S44, S68 and S121, for which the genomes are closed, form a clade with the subspecies *masoucida*. However, there is currently no additional information on these latter strains, preventing conclusions about their phylogenetic position.

We correlated the phylogenetic positions with the average nucleotide identity (ANI), a measure that helps to define species boundaries (Richter and Rosselló-Móra 2009). It was previously estimated that an ANI cutoff value of 96% is appropriate for *Aeromonas* species delineations (Colston *et al.* 2014). The present analysis showed, as expected, that all *A. salmonicida* strains belong to the same species and that the psychrophilic and mesophilic strains form two distinct groups, as suggested by the clustering based on the biochemical tests (see Supp. Fig. S2). In addition, all psychrophilic strains share high ANI values, while mesophilic strains are much more distant, even between each other (Fig. 3). For example, strain A527 shares ANI values of 0.974 and 0.975 with strain 34mel^T of *pectinolytica* and Y577, respectively, and 0.970 with A449, of the subspecies *salmonicida*.

The fact that the ANIs between the genomes of A527 and its closest relatives are almost as distant as with a strain of a psychrophilic subspecies such as *salmonicida* suggest that strain A527 should possibly be considered to be a member of a new subspecies. However, this

reasoning could also be applied to Y577, Y567 and Y47, which are also distant. In correlation with the ANI values, all the mesophilic strains harbour long branch lengths compared to the psychrophilic strains (see Supp. Fig. S4). The fact that the psychrophilic strains have short branch lengths is of interest, because the genome architecture of reference strain A449 of subspecies *salmonicida* was shown to be divergent from those of strains S44, S68 and S121 (Fig. 2).

Taken altogether, the above data presents a taxonomic dilemma, specifically about the intraspecies structure of *A. salmonicida* (Austin 2011; Austin and Austin 2016). Since the psychrophilic subspecies *salmonicida*, *smithia*, *achromogenes* and *masoucida* are considered taxonomically different despite their high ANI values (~99%), each mesophilic strain should perhaps also be considered to be a different subspecies, as they are even more divergent. Although this could be due to a sampling bias, only one member of the many putative new subspecies has been isolated, which seems unusual.

As shown in Figure 3, there is a clear sequencing bias towards *A. salmonicida* subsp. *salmonicida*, mainly given its recurrent presence in fish farms (Dallaire-Dufresne *et al.* 2014). More genomes from strains belonging to other subspecies should be sequenced to learn about their genomic signatures. We now know that some strains of the species *salmonicida* are mesophilic. This opens the door to sample environments that may previously not have been considered likely locations for *A. salmonicida*.

The level of diversity and heterogeneity in the biochemical characteristics of the *A. salmonicida* strains is not an exact reflection of the genomic diversity and heterogeneity. As indicated above, only a few features can distinctively separate psychrophilic and mesophilic *A. salmonicida* isolates. However, analysis of other mesophilic *A. salmonicida* strains is required to robustly assign subspecies and biochemical signatures to them, as was done for the psychrophilic *A. salmonicida* subspecies (Austin, McIntosh and Austin 1989). For all of these

reasons, strain A527 has not yet been associated with a subspecies. The classification scheme for *A. salmonicida* must be clarified before considering defining new subspecies.

Investigation of the insertion sequences

Obtaining the complete closed genome of strain A527 was an opportunity to substantiate the reported trend that genomes from mesophilic *A. salmonicida* strains harbour fewer insertion sequences than those from psychrophilic subspecies, and that their insertion sequence repertoire is divergent (Vincent *et al.* 2016b). The genome of A527 was predicted to harbour 74 complete and 21 fragments of insertion sequences distributed in 19 types and 10 families (Table 1). These high numbers are close to those of well-annotated strain A449 of the subspecies *salmonicida*, which has 88 complete and 14 partial insertion sequences (Reith *et al.* 2008). According to the ISfinder database (Siguier *et al.* 2006), several predicted insertion sequences in A527 were previously found in *A. salmonicida* (Table 1). However, there is no mention regarding the strains or even the subspecies in the database. Although A527's genome includes a high number of insertion sequences, as in the genome of the A449 strain, its repertoire is different. Of the 19 types in A527, only IS*Ahy2* (annotated as IS*AS4* in A449) and IS*As19* (not annotated) are found in the genome of A449. It is interesting to note that IS*As18*, IS*As23*, IS*As24*, IS*As30* and IS*As31* were listed to be specific to mesophilic *A. salmonicida* (Vincent *et al.* 2016b).

Although the insertion sequences are not directly annotated in the genomes of S44, S68 and S121 as they are for *A. salmonicida* subsp. *salmonicida* A449, we investigated their presence and those shared with A527's genome. Again, only a few shared insertion sequences were found. In strain S44, insertion sequences IS*5*, IS*Kpn3* and IS*Ec35* were found to be present on large plasmid pS44-1 (NZ_CP022176.1). Interestingly, three IS*Kpn3*s are predicted to be present in A527's genome. This insertion sequence was originally found in *Klebsiella pneumoniae* plasmid

pRDDHA (Verdet *et al.* 2006) and more recently in the large plasmid pAsa4c of *A. salmonicida* subsp. *salmonicida* strain JF2267 (Tanaka *et al.* 2016). Notably, several freestanding transposase genes, not clearly associated with known insertion sequences, were found in A527's genome. This highlights that putative new insertion sequences could eventually be found in the A527 genome and that the ISfinder database should continue to be updated (Siguier *et al.* 2006).

Insertion sequences can drive novelty in terms of adaptation and genome plasticity (Vandecraen *et al.* 2017) and were already known to cause major genomic alterations in *A. salmonicida*, such as plasmid reshaping (Tanaka *et al.* 2016, 2017) and the disruption of *vapA* (Gustafson, Chu and Trust 1994), a gene coding for a protein involved in the A-layer virulence factor (Chu *et al.* 1991). *A. salmonicida* genomes display high rearrangement capabilities while having a slow mutation rate for coding sequences (Fig. 2 and Supp. Fig. S4). Insertion sequences could be one of the determinants causing this asymmetric evolution by driving recombination. Also, it is known that insertion sequences can modify regulation/transcription of genes (Vandecraen *et al.* 2017) and further studies should assess if these elements are involved in similar alterations in *A. salmonicida*.

Conclusion

This study describes the first complete sequenced and closed genome of a mesophilic *A. salmonicida* strain, as a reference to investigate the dichotomy between psychrophilic and mesophilic *A. salmonicida* subspecies. As already suggested by another study (Vincent *et al.* 2016b), the content in insertion sequences is a major difference between the genomes of mesophilic and psychrophilic *A. salmonicida* strains, and further studies are needed to assess if these elements are involved in creating the lifestyle differences between psychrophilic and mesophilic *A. salmonicida* subspecies. To get a more complete picture of the evolution of

A. salmonicida and to help clarify the taxonomy of this species, reference closed genomes for subspecies *achromogenes*, *smithia*, *masoucida* and *pectinolytica* are required.

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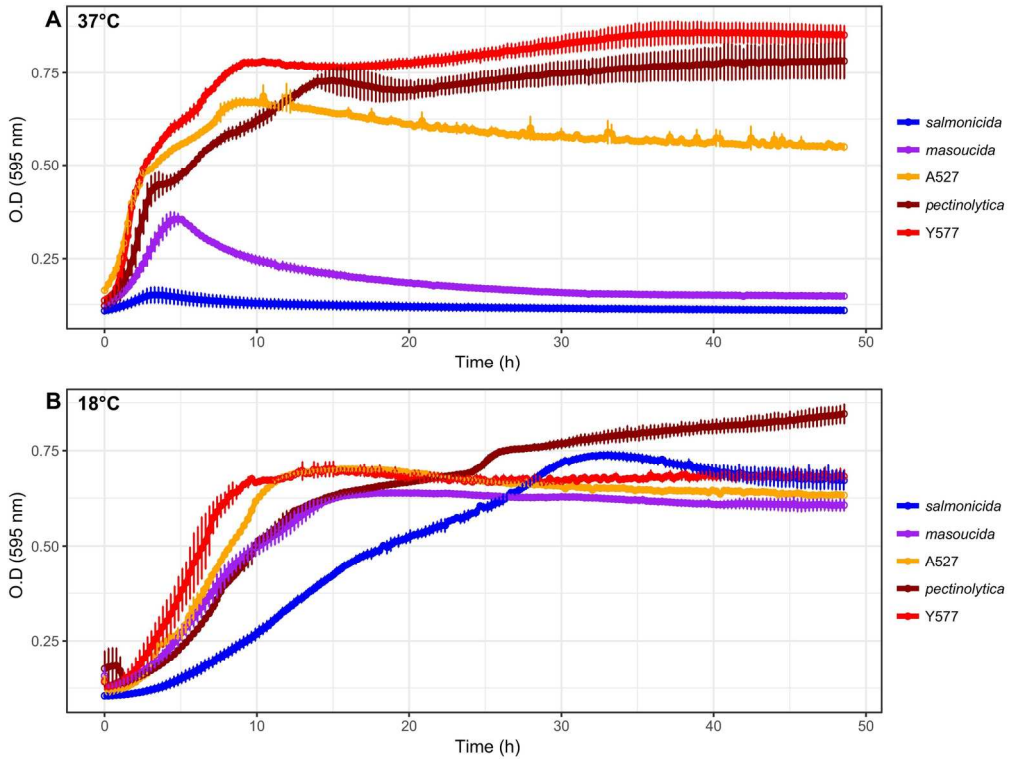
Figure 1. Growth profiles of selected *A. salmonicida* strains. Growth profiles of A527 and selected mesophilic (*pectinolytica* 34mel^T and Y577) and psychrophilic (*salmonicida* 01-B526) *A. salmonicida* strains at 37°C (A) and 18°C (B). The strain NBRC 13784^T of the *masoucida* subspecies was also added given its intermediate ability to grow at 37°C (Vincent *et al.* 2016b). All the curves were done in triplicate and the standard error bars are shown.

Figure 2. Comparison between the complete closed genomes of *A. salmonicida* strains. Only the genomes of strains A527 (CP022550), S121 (NZ_CP022175.1), S44 (NZ_CP022181.1), S68 (NZ_CP022186.1) and A449 (NC_009348.1) are compared since they are the only closed genomes available for *A. salmonicida*. The direct and inverted matches are in orange and blue, respectively. The strain A449 is the only strain in this figure with an official subspecies assignment (*salmonicida*).

Figure 3. Cladogram showing the phylogenetic relations between all available *A. salmonicida* strains with a sequenced genome (draft or closed). Only bootstrap values inferior to 100 are shown at the corresponding nodes. The heatmap represents the ANI values. The complete tree with all 60 taxa is shown in supplementary material (Figure S4). The strains of the *salmonicida* subspecies cluster according to the geographical regions where they have been isolated: China, Europe (Eur.), United States (U.S.) and the Canadian provinces of New-Brunswick (N.B.) and Quebec (Que.).

Fig. 1



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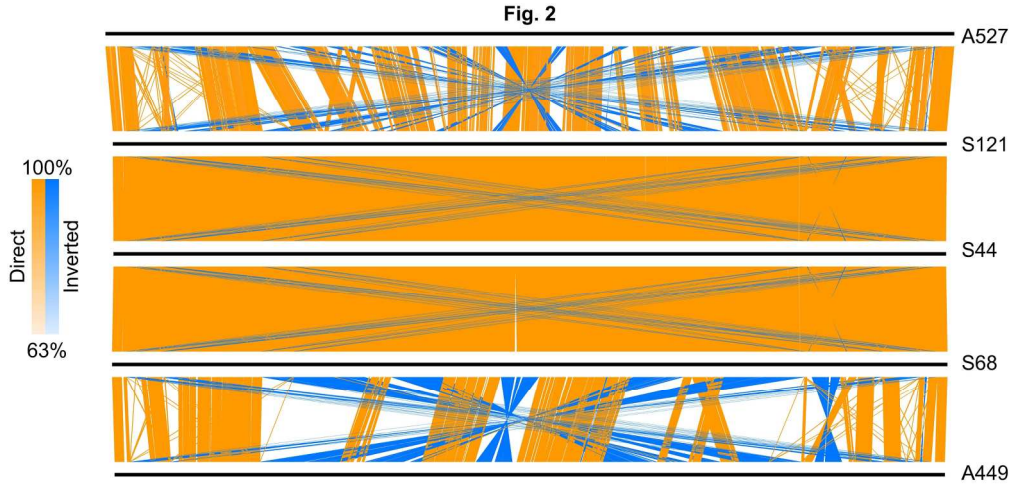

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Fig. 3

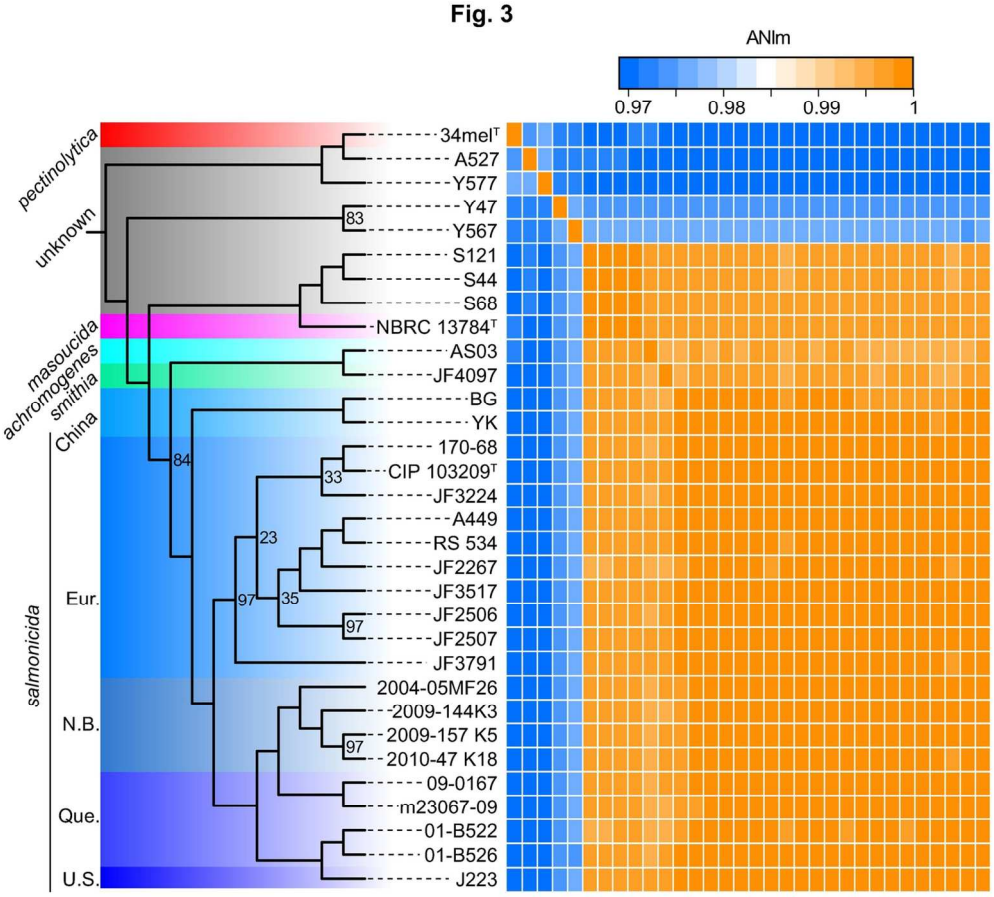

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Table 1. Complete and partial ISs found in the genome of *A. salmonicida* A527

IS	Family	Complete	Partial	Host[a]	Presence in other closed *A. salmonicida* genomes			
					A449	S44	S68	S121
IS*1396*	IS*L3*	0	1	*Serratia marcescens*	No	No	No	No
IS*Aeca4*	IS*3*	7	2	*Aeromonas caviae*	No	No	No	No
IS*Aeme5*	IS*66*	0	1	*Aeromonas media*	No	No	No	Yes
IS*Aeme17*	IS*21*	1	0	*Aeromonas media*	No	No	No	No
IS*Aeme21*	IS*481*	0	2	*Aeromonas media*	No	No	No	No
IS*Ahy2*	IS*630*	8	2	*Aeromonas hydrophila*	Yes[b]	Yes	Yes	Yes
IS*As13*	IS*5*	3	0	*Aeromonas salmonicida*	No	No	No	No
IS*As15*	IS*5*	1	1	*Aeromonas salmonicida*	No	No	No	No
IS*As18*	IS*4*	5	1	*Aeromonas salmonicida*	No	No	No	No
IS*As19*	IS*481*	2	2	*Aeromonas salmonicida*	Yes[c]	No	No	No
IS*As23*	IS*1595*	3	2	*Aeromonas salmonicida*	No	No	No	No
IS*As24*	IS*110*	4	4	*Aeromonas salmonicida*	No	No	No	No
IS*As30*	IS*4*	4	1	*Aeromonas salmonicida*	No	No	No	No
IS*As31*	IS*3*	10	0	*Aeromonas salmonicida*	No	No	No	No
IS*Ave3*	IS*3*	1	0	*Aeromonas veronii*	No	No	No	No
IS*Ec35*	IS*5*	0	1	*Escherichia coli*	No	Yes	No	No
IS*Kpn3*	IS*1595*	2	1	*Klebsiella pneumoniae*	No	Yes	No	No
IS*UnCu16*	IS*66*	20	0	*uncultured bacterium*	No	No	No	No
IS*5*	IS*5*	3	0	*Escherichia coli*	No	Yes	No	Yes
Total		**74**	**21**					

a: Host in which the insertion sequence is listed in ISfinder.
b: IS*Ahy2* showed a high identity with IS*AS4* of the *A. salmonicida* subsp. *salmonicida* A449 genome.
c: This insertion sequence is currently not annotated in the *A. salmonicida* subsp. *salmonicida* A449 genome.

Supplementary material

Study of mesophilic *Aeromonas salmonicida* A527 strain sheds light on the species lifestyles and taxonomic dilemma

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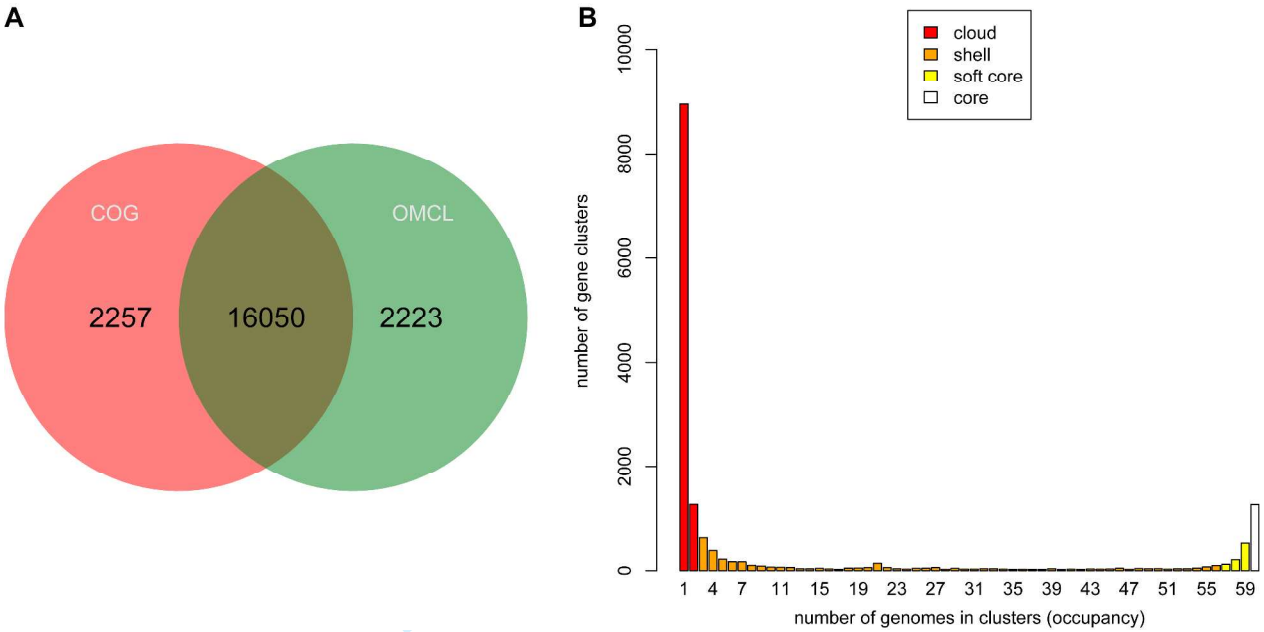

Figure S1. Pan-genome determination of all available *A. salmonicida* genomes. (A) Gene clusters found by COG [1] and OMCL [2] algorithms (through GET_HOMOLOGUES [3]). Only the clusters found by both were used in the present study. (B) Pan-genome distribution between four categories: cloud, shell, soft core and core, as defined by GET_HOMOLOGUES.

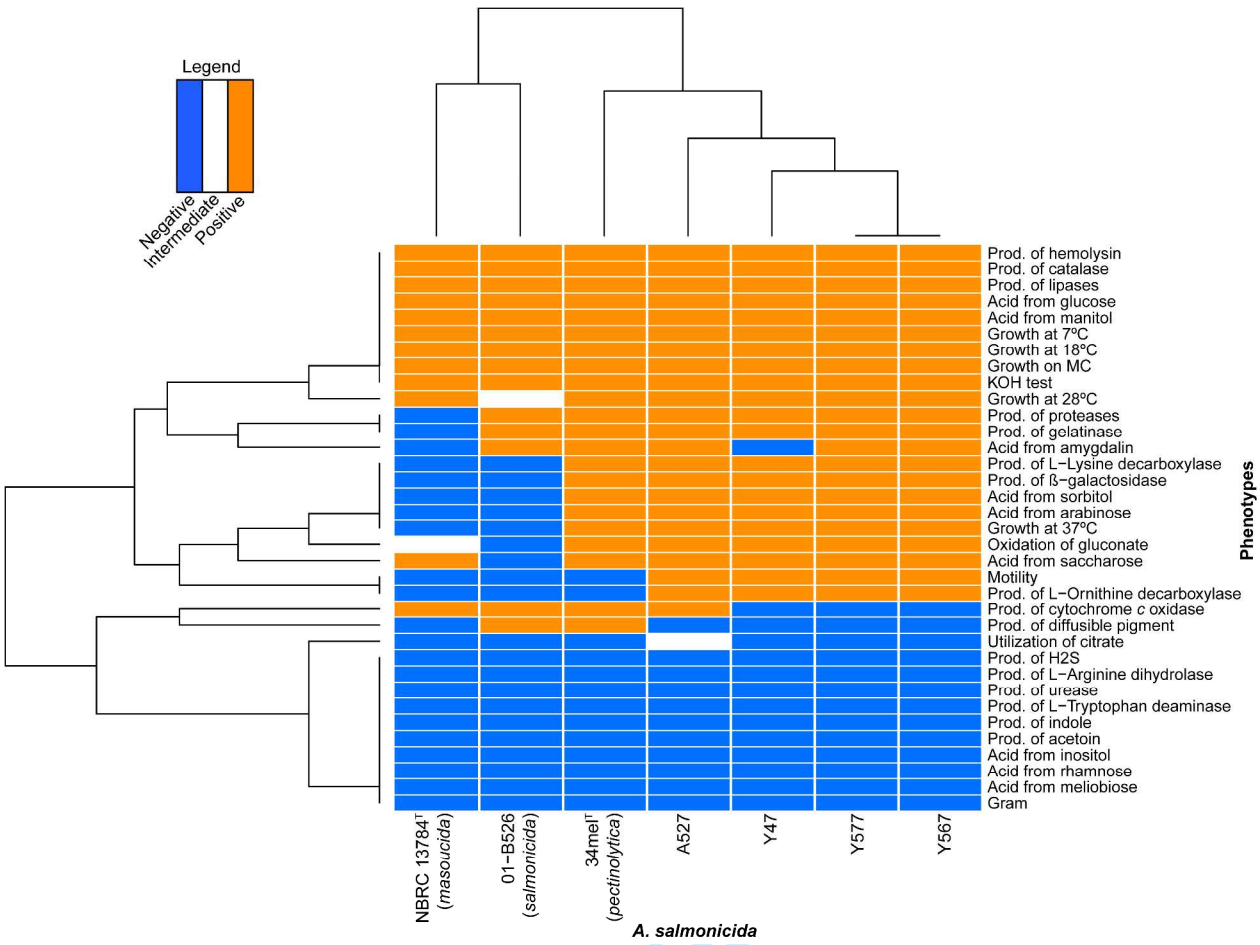

Figure S2. Clustering based on observed phenotypes of some *A. salmonicida* strains, including A527.

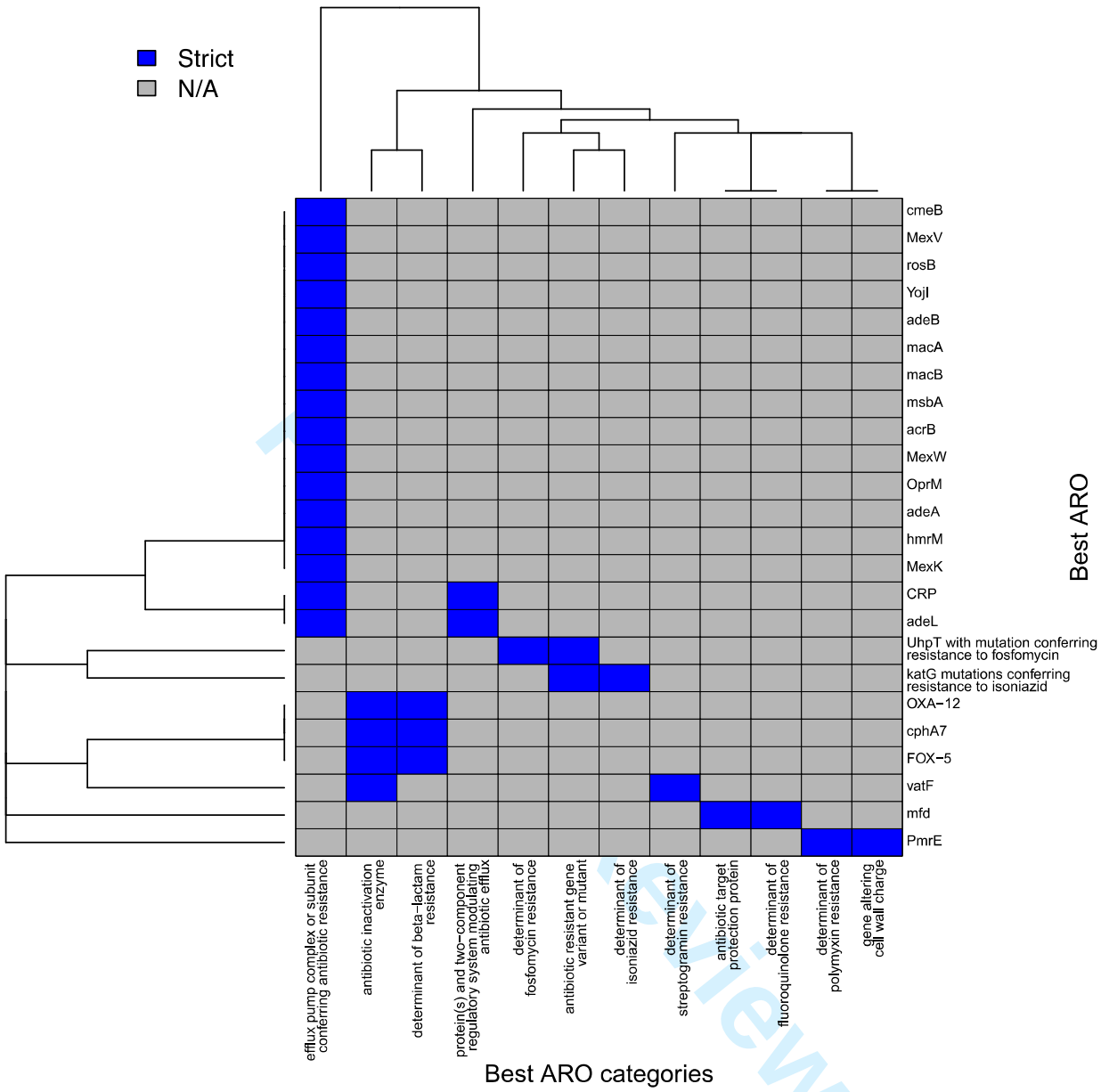

Figure S3. Antibiotic resistance genes in A527 genome. Heatmap showing the putative presence (blue squares) of antibiotic resistance genes (ordinate) and their Antibiotic Resistance Ontology (ARO) categories (abscissa) for *A. salmonicida* A527 as analyzed by CARD [4].

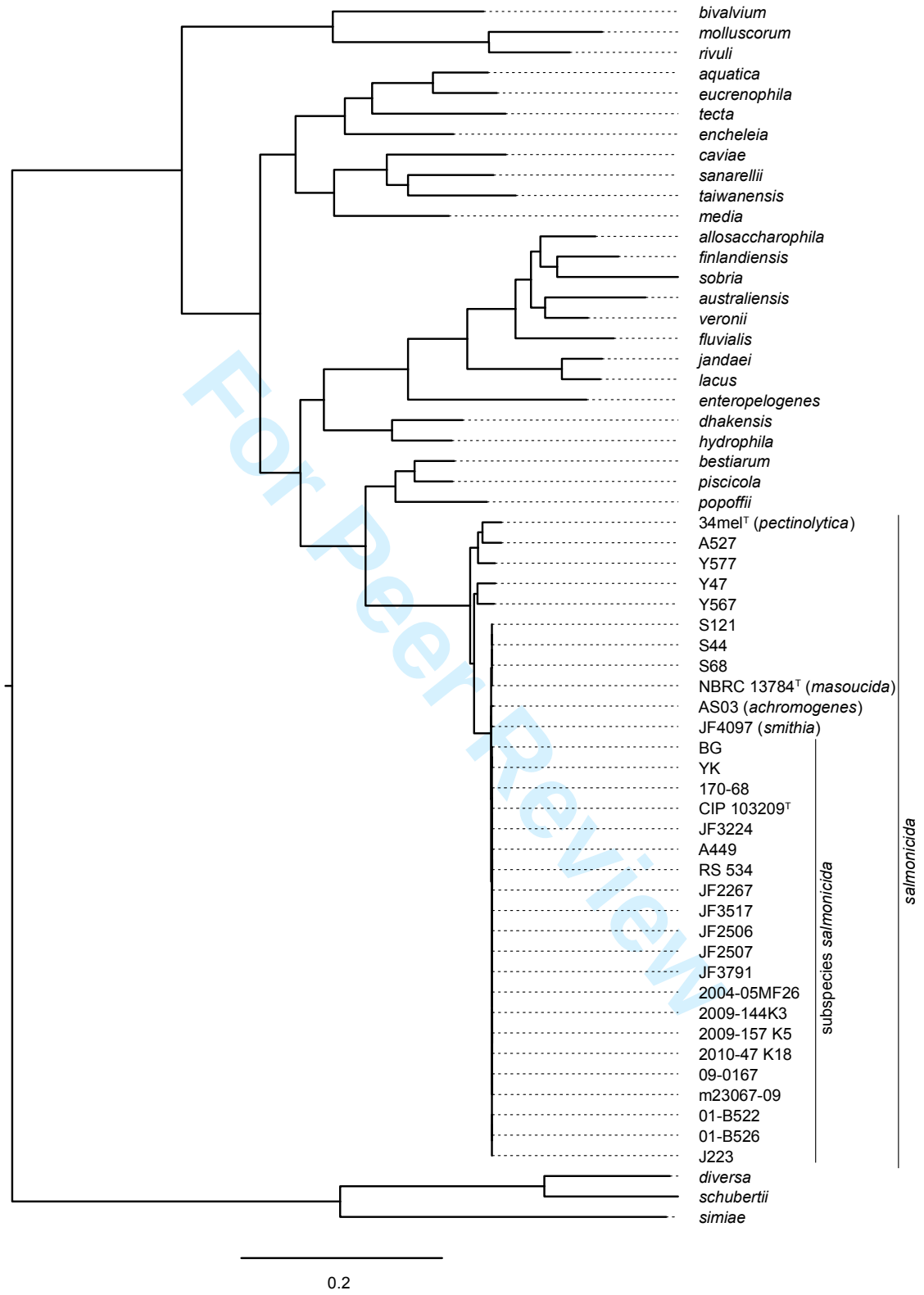

Figure S4. Phylogenetic tree containing all the 60 taxa analyzed. All the bootstrap values for the species other than *salmonicida* were at 100, while those for this subspecies are shown only in the Figure 3 of the main manuscript for clarity purpose.

Table S1. Genomes used for the phylogenetic analysis

Species	Strain	GenBank	Reference
allosaccharophila	CECT 4199^T	NZ_CDBR00000000	[5]
aquatica	AE235	NZ_JRGL00000000	[6]
australiensis	CECT 8023^T	NZ_CDDH00000000	[5]
bestiarum	CECT 4227^T	NZ_CDDA00000000	[5]
bivalvium	CECT 7113^T	NZ_CDBT00000000	[5]
caviae	YL12	NZ_JOVP00000000	N/A[a]
dhakensis	AAK1	NZ_BAFL00000000	[7]
diversa	CDC 2478-85^T	NZ_APVG00000000	[8]
encheleia	CECT 4342^T	NZ_CDDI00000000	[5]
enteropelogenes	CECT 4255^T	NZ_CDDE00000000	[5]
eucrenophila	CECT 4224^T	NZ_CDDF00000000	[5]
finlandiensis	4287D	NZ_JRGK00000000	[9]
fluvialis	LMG 24681^T	NZ_CDBO00000000	[5]
hydrophila	ATCC 7966^T	NC_008570.1	[10]
jandaei	CECT 4228^T	NZ_CDBV00000000	[5]
lacus	AE122	NZ_JRGM00000000	[9]
media	WS	NZ_CP007567.1, NZ_CP007568.1	[11]
molluscorum	848^T	NZ_AQGQ00000000	[12]
piscicola	LMG 24783^T	NZ_CDBL00000000	[5]
popoffii	CIP 105493^T	NZ_CDBI00000000	[5]
rivuli	DSM 22539^T	NZ_CDBJ01000000	[5]
sanarellii	LMG 24682^T	NZ_CDBN00000000	[5]
schubertii	WL1483	NZ_CP013067.1	[13]
simiae	CIP 107798	NZ_CDBY00000000	[5]
sobria	CECT 4245^T	NZ_CDBW01000000	[5]
taiwanensis	LMG 24683^T	NZ_BAWK00000000	[14]
tecta	CECT 7082^T	NZ_CDCA00000000	[5]
veronii	B565	NC_015424.1	[15]
salmonicida	A527	CP022550	This study
salmonicida* subsp. *salmonicida	01-B522	MIIM00000000	[16]
salmonicida* subsp. *salmonicida	01-B526	AGVO00000000	[17]
salmonicida* subsp. *salmonicida	09-0167	LMTK00000000	[16]
salmonicida* subsp. *salmonicida	170-68	MIIN01000000	[16]
salmonicida* subsp. *salmonicida	2004-05MF26	JRYW00000000	[18]
salmonicida* subsp. *salmonicida	2009-144K3	JRYV00000000	[18]
salmonicida* subsp. *salmonicida	2009-157 K5	MIIQ00000000	[16]

salmonicida* subsp. *salmonicida	2010-47 K18	MIIR00000000	[16]
salmonicida* subsp. *pectinolytica	34mel^T	ARYZ00000000	[19]
salmonicida* subsp. *salmonicida	A449	CP000644.1, CP000645.1, CP000646.1, AY301063.1, AY301064.1, AY301065.1	[20,21]
salmonicida* subsp. *achromogenes	AS03	AMQG00000000	[22]
salmonicida* subsp. *salmonicida	BG	LUHO00000000	[23]
salmonicida* subsp. *salmonicida	CIP 103209^T	CDDW00000000	[5]
salmonicida* subsp. *salmonicida	J223	LSGV00000000	N/A[a]
salmonicida* subsp. *salmonicida	JF2267	MIIO01000000	[16]
salmonicida* subsp. *salmonicida	JF2506	MIIS01000000	[16]
salmonicida* subsp. *salmonicida	JF2507	MIIT00000000	[16]
salmonicida* subsp. *salmonicida	JF3224	JXTA00000000	[24]
salmonicida* subsp. *salmonicida	JF3517	MIIU01000000	[16]
salmonicida* subsp. *salmonicida	JF3791	JYFG01000000, KU499859.1	[25]
salmonicida* subsp. *smithia	JF4097	JZTI00000000	[26]
salmonicida* subsp. *salmonicida	m23067-09	MIIP00000000	[16]
salmonicida* subsp. *masoucida	NBRC 13784^T	BAWQ01000000	N/A
salmonicida* subsp. *salmonicida	RS 534	JYFF00000000	[26]
salmonicida	S121	NZ_CP022175.1, NZ_CP022170.1, NZ_CP022171.1, NZ_CP022172.1, NZ_CP022173.1, NZ_CP022174.1	N/A
salmonicida	S44	NZ_CP022181.1, NZ_CP022176.1, NZ_CP022177.1, NZ_CP022178.1, NZ_CP022179.1, NZ_CP022180.1	N/A
salmonicida	S68	NZ_CP022186.1, NZ_CP022182.1, NZ_CP022183.1, NZ_CP022184.1, NZ_CP022185.1	N/A
salmonicida	Y47	JZTF00000000	[26]
salmonicida	Y567	JZTG00000000	[26]
salmonicida	Y577	JZTH00000000	[26]
salmonicida* subsp. *salmonicida	YK	LUHP00000000	[23]

a: N/A means not-applicable

Table S2. General features of the *A. salmonicida* available closed chromosomes

Strain	Length (Mb)	GC %	Proteins	**rRNA**	tRNA
A527	4.8	58.6	4,308	31	125
S121	4.7	58.6	3,955	31	120
S44	4.7	58.6	3,963	31	120
S68	4.7	58.6	3,978	31	120
A449[a]	4.7	58.5	4,007	28	108

a: The strain A449 is the only strain in this table with an official subspecies assignment (*salmonicida*)

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