The *Aeromonas salmonicida* plasmidome: a model of modular evolution and genetic diversity

Antony T. Vincent[1], Nava Hosseini[2,3,4] and Steve J. Charette[2,3,4*]

1. Département des sciences animales, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, 2425, rue de l'Agriculture, Quebec City, QC, Canada G1V 0A6
2. Institut de biologie intégrative et des systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, 1030 avenue de la Médecine, Quebec City, QC, Canada, G1V 0A6
3. Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec (Hôpital Laval), 2725 Chemin Sainte-Foy, Quebec City, QC, Canada, G1V 4G5
4. Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, 1045 avenue de la Médecine, Quebec City, QC, Canada G1V 0A6

* Correspondence to: Steve J. Charette, Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Quebec City, QC, Canada, G1V 0A6, telephone: 418-656-2131, ext. 406914, email: steve.charette@bcm.ulaval.ca

Running Head: *Aeromonas salmonicida* plasmidome

Keywords: *Aeromonas salmonicida*; plasmid; antibiotic resistance; virulence, plasmidome

ABSTRACT

High-throughput genomic sequencing has helped to reveal the plasmidome of *Aeromonas salmonicida*. This literature review provides an overview of the *A. salmonicida*'s rich plasmidome by presenting all the plasmids identified so far, addressing their biological importance and the functional links between them. The plasmids of *A. salmonicida*, especially those bearing antibiotic resistance genes, can provide clues about interactions of this species with other pathogens (animals and humans) as it is the case for pRAS3-3432 with *Chlamydia suis* or pSN254b with *Salmonella enterica*. In addition to antibiotic resistance, plasmids play an important role in the virulence of *A. salmonicida*, and more particularly for the subspecies *salmonicida* with the plasmid pAsa5 carrying the genes of the type three secretion system, a virulence factor essential for the bacterium. The *A. salmonicida* plasmidome also has many cryptic plasmids with no known biological function, but which can be used for the acquisition of new genetic elements. Striking examples are pAsa7 and pAsaXII that provide respectively resistance to chloramphenicol and formaldehyde and that are derivatives of cryptic pAsa2.

MAIN TEXT

Where are we in the study of plasmids?

With the advance of Next-Generation Sequencing (NGS), we are now able to unravel bacterial genomes at an unprecedented pace.[1–3] Commonly, bacterial genomes harbor a single circular chromosome and may contain plasmids, which are defined as extra-chromosomal and autonomously replicating genetic elements.[4] The chromosome contains the major essential genes while the plasmids are usually non-essential and considered to be part of the accessory genome. Even though the vast majority of plasmids are nonessential, many of them convey a clear advantage to the bacteria that bear them, compared to cells without plasmids. The biological functions of plasmids are extremely diverse and may help to enhance cells by conferring metabolic capabilities, pathogeny, resistance against antimicrobial agents, and other host/environmental adaptation factors.[5]

In 2019, Brooks et al. published an impressive curated plasmid sequence database including 10,892 complete plasmid sequences extracted from bacterial genomes sequenced with NGS.[6] Some of the bacteria have highly diverse plasmidomes containing various members. For

example, analysis using NGS and the tool PLACNET[7] of the well-known bacterial model *Escherichia coli* revealed around 255 plasmids in a total of 61 genomes.[8] However, plasmidome diversity is not exclusive to model bacteria and non-model bacterial species can also harbor an impressive plasmid repertoire.

The non-model bacterium *Aeromonas salmonicida*

One of the current challenges is the study of plasmidome diversity for non-model bacteria like *A. salmonicida*. Bacterial strains from this species have a surprisingly diverse plasmidome. This species, which belongs to the *Gammaproteobacteria* class, contains five official subspecies: *pectinolytica*,[9] *masoucida*,[10,11] *achromogenes*,[12] *smithia*,[13] and *salmonicida*,[14] and potentially many others.[15–19]

A. salmonicida is of clinical importance since many members belonging to various subspecies are known to infect fish,[20] thus causing important economic losses worldwide.[21] The most studied subspecies of this bacterium is *salmonicida*, which is sometimes qualified as "typical," and which is well known to cause furunculosis, a worldwide fish disease that mainly affects salmonids. The

other subspecies are qualified as “atypical” and infect a wide range of hosts.[21] Contrary to psychrophilic subspecies (*masoucida*, *achromogenes*, *smithia* and *salmonicida*), which grow at temperatures around 18°C, *A. salmonicida* subsp. *pectinolytica*, which is the official mesophilic subspecies for this species, can grow up to 37°C and even higher.[9,15,19] There have also been several reports describing mesophilic isolates attributed to *A. salmonicida* over the past decades.[15,16,18,19]

Compared to the *A. salmonicida* psychrophilic subspecies which have long been characterized, the study of the mesophilic strains was more recent and no putative host was known until 2008, where there have been reports of the isolation of putative mesophilic *A. salmonicida* strains from humans.[22–26] The phylogenomic analysis of the JF2480 strain that grow both at 18°C and 40°C temperatures, isolated from a dead infected *Recurvirostra avosetta* migratory bird, revealed an even wider host range for *A. salmonicida*.[19] Based on the proven virulence of mesophilic *A. salmonicida* strains in a mammal model, it is a potential human emerging pathogen and it has been recommended to include this bacterium in diagnostic microbiology tests.[18] However, recovering *A. salmonicida* from human samples seems to remain a rare event.

In addition to the *A. salmonicida* subsp. *salmonicida* reference isolate A449, which was completely sequenced and assembled in 2008,[27] 64 other *A. salmonicida* isolates have their genome sequenced and deposited in the public database GenBank (April 2020), with approximately half of them belonging to the *salmonicida* subspecies. This increase in sequenced genomes has permitted accurate study of *A. salmonicida's* genomic content, including the mobilome, which contains the plasmid repertoire.

In this paper, considering the essential role of plasmids in virulence, antibiotic resistance and adaptability potential for *A. salmonicida*, we will review all aspects of the plasmidome of this species.

The first plasmid descriptions in *A. salmonicida*

Furunculosis was first described in a German journal in 1890,[28] but it was 81 years before a paper discussed plasmids in *A. salmonicida*, described as R factors.[29] The word "plasmid" was first used in a scientific publication by Lederberg in 1952.[30] Three decades of studies followed Aoki's

1971 publication, mainly using classical molecular biology techniques to characterize the plasmidome of *A. salmonicida* (Table 1). Some of the studies were about the plasmids causing antibiotic resistance,[29,31–40] while just a few verified the importance of plasmids in the virulence of *A. salmonicida*, without any clear success.[41,42]

The typing of bacterial strains is fundamental in microbiology since this action makes it possible to make links between genetic and phenotypic diversity and other characteristics, for example epidemiological. Given the importance of this field of study, many microbiological and molecular methods have been developed.[43,44] Relatively early in the history of *A. salmonicida* research, studies evaluated the potential of plasmids as epidemiological markers for this bacterium. Many of these studies stated that the plasmidome of typical *A. salmonicida* was too conserved and without enough variations to be used as a marker in an epidemiological context.[45–47] Nielsen *et al*. did a large-scale study and also reported a uniform plasmidome among the isolates, but some variations were identified due to their large strains sampling (124 strains were tested).[48] Consistent with this observation, it was reasonable to believe that the use of *A. salmonicida* plasmids in an epidemiological context was still possible. On the other hand,

118 atypical *A. salmonicida* bacteria were found to have a variable plasmid repertoire.[45,46,49] Even if it
119 was possible to have an approximation of the plasmid repertoire no complete plasmid sequence
120 was reported.
121
122 The year 1977 was a turning point in modern science when the complete genome of the
123 bacteriophage ΦX174 was sequenced by Frederick Sanger.[50] Two years later, the well-known
124 *E. coli* cloning vector plasmid pBR322 was sequenced.[51] The first plasmids of *A. salmonicida* to
125 be fully sequenced were pRAS3.1 and pRAS3.2; two variants conferring tetracycline resistance.[32]
126 Since this milestone, numerous *A. salmonicida* plasmids have been sequenced, and the
127 plasmidome of this species is presented in Table 2 and Table S1.
128
129 **General features of the sequenced plasmids**
130 Biology has entered a sequencing era with the advent of high-throughput sequencing.[1,2] Thanks
131 to this technological advance, 61 *A. salmonicida* plasmids have been completely sequenced
132 (Table 2 and Table S1), almost the same number as found by Nielsen in 1993 by using low-

133 resolution approach.[48] The known plasmidome of *A. salmonicida* contains plasmids of various
134 lengths, ranging from 5.2 to >180 kb (Table 2).
135
136 All plasmids of *A. salmonicida* known so far can be roughly categorized within three phenotypic
137 groups, which either (1) confer drug resistance, (2) confer virulence, or (3) are cryptic, meaning
138 that no known function was attributed to those plasmids. In the next sections, we will review
139 plasmids from these three categories, and will review specific plasmid features, such as genome
140 stability, classification of the IncA/C plasmids, and the geographical distribution of the small
141 plasmids.
142
143 **Drug resistance**
144 The R-plasmids, which confer antimicrobial resistance, were the first of the *A. salmonicida*
145 plasmids to be studied.[29,31] The reason is simple: they display an important and easy-to-see
146 phenotype. The rise of antimicrobial-resistant bacterial strains, named superbugs, is alarming
147 worldwide.[52] The intensive use of antibiotics in aquaculture has increased the number of fish
148 pathogens that are resistant or even multi-resistant to the administered drugs.[53,54] It has been well

149 explained that *Aeromonas*, which is a waterborne bacterium, might play an important role in the
150 spreading of resistance genes since they can acquire resistance plasmids from allochthonous
151 bacteria and in turn, behave as reservoirs of resistance genes for other susceptible bacteria.[55]
152
153 In *A. salmonicida* alone, six non-sequenced and 13 completely sequenced plasmids are known to
154 confer resistance to one or many antibiotics. In the following section we will discuss these
155 plasmids.
156
157 The non-sequenced R-plasmids
158 The first R-plasmid was reported in 1971 in *A. salmonicida* strain Ar-32, which was resistant to
159 various drugs: sulfathiazole, chloramphenicol and streptomycin.[29] In 1986 Aoki et al. followed
160 up with a more extensive characterization of this plasmid, named pAr-32.[34] This plasmid was
161 estimated to have a length around 47 kb, to be in the incompatibility group U (IncU), and to bear
162 repeated sequences.[34] They also reported this plasmid to be similar to RA3, a plasmid found in
163 the human pathogen *Aeromonas hydrophila*, and which is the reference plasmid of the IncU
164 group.[56] This led to the postulate that R-plasmids can be transferred between *A. salmonicida* and

165 *A. hydrophila*. Later, pAr-32 was found to have a class 1 integron and to share some common

166 DNA with pRAS1 and the pASOTs (see below). Finally, further characterization revealed that

167 pAr-32 has an In6-like integron similar to that of pSa, a plasmid found in *Shigella*.[57]

168

169 Another non-sequenced R-plasmid is the conjugable pRAS1, approximately 45 kb, also related to

170 the IncU group.[58] This plasmid has a complete class 1 integron (In4-like) and a fragmented

171 transposon Tn*1721*. The combination of both elements confers drug resistance genes: *dfrA16*,

172 *qacEΔ1*, *sul1* and finally *tetA* and *tetR*.[59] Interestingly, pRAS1 was found in atypical and typical

173 *A. salmonicida*.[59] Another not-yet sequenced R-plasmid is pRAS2 (~48 kb). This plasmid was

174 found in *A. salmonicida* subsp. *salmonicida* strain 1682/92 and bears transposon Tn*5393c*, which

175 confers resistance to streptomycin, sulfonamide and tetracycline.[40]

176

177 Three other plasmids, related to the incompatibility group IncU and causing resistance to

178 oxytetracycline, were reported: pASOT (~47kb), pASOT2 (~47kb) and pASOT3 (~39 kb).[39]

179 Plasmidic profiles showed that pASOT and pASOT2 have a significant homology with pRAS1,

while pASOT3 is more distant. These plasmids bear a class 1 integron, which may differ in the cassettes region.[60]

The sequenced R-plasmids

The smallest known sequenced antibiotic-resistance-bearing plasmid is the ColE1-like pAsa7 (Table 2), which has only been found in the *A. salmonicida* subsp. *salmonicida* Swiss isolate JF3791. This plasmid confers chloramphenicol (CHL) resistance due to the presence of a *cat* gene, encoding a chloramphenicol acetyltransferase.[61] This small, 5276 bp, plasmid has a similar backbone to pAsa2, a cryptic plasmid usually found in *A. salmonicida* subsp. *salmonicida* (see below the section concerning the cryptic plasmids).[62] However, as stated in the article describing this plasmid, it is unclear if one is derived from the other.[63] This is mainly because it is impossible to explain the evolutionary scenario between these plasmids. As other ColE1-type replicon plasmids, pAsa7 is in a high copy numbers, estimated between 27 to 34 copies per cell.

Since this plasmid bearing a *cat* gene is in high copy numbers, it was reasonable to think that the resistance to CHL would have been high as well. Surprisingly, an analysis of a representative

196 panel of strains resistant to CHL revealed that the minimum inhibitory concentration (MIC) of
197 CHL of the large single-copy plasmid pAsa4, which also bears a *cat* gene, was much higher than
198 pAsa7.[61] Analysis of the expression of both genes demonstrated that the one on pAsa4 was
199 transcribed 50 times higher than the one on pAsa7, which compensated for the low copy number
200 of pAsa4.
201
202 The high expression level of the *cat* gene of pAsa4 was first observed in 1989.[46] They found that
203 CAT-pAsa4 was a predominant protein in whole-cell lysates of *E. coli* that bore a part of the
204 pAsa4 plasmid containing *cat*-pAsa4 gene and that the recipient cells were also highly resistant to
205 CHL.[46]
206
207 The complete sequence of pAsa4 was published 19 years later.[27] This large plasmid of ~166 kb
208 harbors a transposon Tn*21* with an In2 integron, encoding resistance to
209 streptomycin/spectinomycin (*aadA*), sulfonamide (*sul1*) and chloramphenicol (*cat*). In addition to
210 this resistance encoded by Tn*21*, pAsa4 also has genes related to tetracycline resistance: *tetA*(E)
211 and *tetR*(E).

212

213 Although pAsa4 is not a common plasmid, two variants named pAsa4b and pAsa4c were found,

214 sequenced and characterized.[64] The three pAsa4 variants exhibited a high identity at the

215 nucleotide level (94 to 99%), however they had quite varied architecture. The pAsa4s plasmid

216 contains a high proportion of insertion sequences (ISs), which promote large-scale

217 rearrangements. The genome instability confered by the ISs was also previously reported for

218 other plasmids in *A. salmonicida* subsp. *salmonicida* (see the section regarding virulence, more

219 precisely on pAsa5). The structural variations affect the repertoire of antibiotic resistance genes,

220 with the original pAsa4 bearing most of the antibiotic resistance genes (Table 2). The variant

221 pAsa4c lacks only the gene causing resistance to tetracycline, *tetA*(E), while pAsa4b does not

222 bear the genes *aadA* and *cat*, which, respectively, confer resistance to

223 streptomycin/spectinomycin and chloramphenicol.

224

225 The incompatibility group of pAsa4 is still unknown. Studies reported pAsa4 as an IncA/C-

226 related plasmid,[65] however, some authors classify pAsa4 as a real IncA/C.[66] In 2008, an IncA/C

227 plasmid was reported in Canadian *A. salmonicida* subsp. *salmonicida* isolates.[39] This conjugative

228 plasmid was shown to have multiple drug resistance cassettes organized similarly to those in
229 pSN254,[67] which is an IncA/C plasmid found in *Salmonella enterica*.[65] The complete sequence of
230 this plasmid, named pSN254b, was further characterized in 2014.[68] This study revealed the
231 presence of a Tn*21* transposon, similar to those found in the plasmids pAsa4, pAsa4b and
232 pAsa4c.[27,64] It is interesting to mention that this transposon, in addition to possessing genes for
233 resistance to antibiotics, also has genes for resistance to mercury. Although resistance to heavy
234 metals is less frequently found than resistance to antibiotics in strains of *A. salmonicida* subsp.
235 *salmonicida*, the presence of mercury resistance genes suggests that this transposon may allow
236 adaptation to environments polluted by various components. A large-scale study of 100 *A.*
237 *salmonicida* subsp. *salmonicida* strains, mainly from Quebec (Canada), showed by PCR-
238 genotyping that this plasmid is the most prevalent within Canadian isolates.[69]
239
240 The phylogenetic position of pSN254 among other IncA/C was inferred in 2009.[70,71] The pAsa4
241 plasmid found in reference strain A449 was added to Fricke et al.'s analysis,[71] allowing them to
242 locate its basal position. However, the pSN254b plasmid and variants of pAsa4 (see above) were
243 sequenced after the publication of this article. Consequently, to shed light on the positions of

these plasmids within the IncA/C group, we found the pan-genome of 14 IncA/C plasmids in addition to pAsa4 and its variants. Interestingly, as shown in Figure 1, a hierarchical clustering confirmed our observation that IncA/C plasmids are grouped depending on if they are lacking or possessing a region containing bla_{CMY-2}, *blc*, *sugE* and *dsbC*.[72] Moreover, as previously observed,[71] the pAsa4s clearly clustered at a basal position, meaning that their relation to the IncA/C group is questionable.

The paper reporting pSN254b also characterized two other plasmids: pAB5S9b and pRAS3.3.[68] pAB5S9b is a variant of pAB5S9, reported in *Aeromonas bestiarum*.[55] The plasmid in *A. bestiarum* was shown to bear multiple antibiotic-resistance genes, many of which were in two regions similar with a segment of the conjugative integrative SXT element of *Vibrio cholerae*.[73] However, this segment is truncated in pAB5S9 by two genes: *tetR* and *tet*(Y). Interestingly, pAB5S9b is very closely related to pAB5S9, but the SXT segment is not truncated as it is for pAB5S9.[68]

pRAS3.3 is another plasmid variant,[68] which is close to IncQ plasmids pRAS3.1 and pRAS3.2.[32] Interestingly, restriction enzymatic digestion and hybridization indicated that pRAS3.2 and pJA8102-2 published in 1986 were identical.[32,34] The three pRAS3 plasmids harbor the genes *tetA* and *tetR*, which confer resistance to tetracycline. The backbones of these plasmids only differ by short repetitions in two regions: (1) in the promoter region of the *mobB-mobA*/*repB* genes and (2) near *oriV*. The number of repetitions can vary the plasmid copy number.[74] A study made on *E. coli* revealed that the pRAS3s with a lower copy number placed a lower metabolic burden on cells, and can consequently increase the population's fitness.[75] The study revealing the existence of pRAS3.3 also revealed pRAS3.4, which is an additional variant having a different number of repetitions.[68]

The fifth pRAS3 variant, named pRAS3-3432, was obtained from the SHY16-3432 strain.[76] It is distinct from the previous ones due to the presence of a unique insertion element that has been never found in any *A. salmonicida* strains before, though it is known from a totally different bacterium: the swine pathogen *Chlamydia suis*.[77] This insertion element (IS*cs605*) is a part of bigger *tetC* island, which is responsible for tetracycline resistance. Except for a few subtle

275 differences, the *tetC* island and pRAS3-3432 variant share high nucleotide identity. So, the
276 existence of IS*cs605* in this pRAS3 variant suggests horizontal gene transfer between
277 *A. salmonicida* subsp. *salmonicida* and *C. suis*. Various scenarios are possible, including 1) both
278 bacteria were confronted during fish-based food digestion, or 2) water containing *A. salmonicida*
279 subsp. *salmonicida* passed through a pig's gastrointestinal tract.
280
281 Another large R-plasmid found in *A. salmonicida* subsp. *salmonicida* is pAsa8.[69] This plasmid
282 has been listed in only two Canadian isolates (M16474-11 and M15448-11) and bears a large
283 panel of antibiotic resistance genes (Table 2). The architecture of pAsa8 is composed of multiple
284 mobile genetic elements (MGE) nested inside each other.[69] The antibiotic resistance genes of this
285 plasmid are located on both a Tn*1721* transposon and in a complex class 1 integron (In104-like
286 showing similarity with In4), which exhibits an important likelihood with the one of the
287 *Salmonella* genomic island 1 (SGI) (well reviewed in [78]) found in *Salmonella enterica* and
288 *Proteus mirabilis*.[79,80] This plasmid is another confirmation that *A. salmonicida* subsp.
289 *salmonicida* could be an important reservoir for MGEs and antibiotic resistance genes.
290 Interestingly, this situation is near to the one found in pRAS1, where a Tn*1721* and an In4-like

provide antibiotic resistance genes.[59] However, since pRAS1 is not sequenced, it is hazardous to postulate any evolutionary links between these two plasmids.

The transfer of MGEs carrying significant resistance genes by plasmid recombination can lead to the production of new plasmid variants. Long-read sequencing (PacBio SMRT technology) has helped to fully assemble pAsa5-3432 from SHY16-3432 strain, which was a new variant of the classical pAsa5 plasmid.[76] This plasmid is essential in the virulence of *A. salmonicida* subsp. *salmonicida,* and is described later in this paper. The main noticeable difference among pAsa5-3432 and the original plasmid is the existence of an additional multiple antibiotic resistance gene regions in the variant. Interestingly, the region containing Tn*1721*, In104-like and *IS5* in pAsa8 was also found in pAsa5-3432.[69] The similarity and high-level of homology among the two regions, with only subtle differences, have led to the conclusion that maybe the origin of this unusual region in the pAsa5 variant is from pAsa8.[76]

Tn*1721* contains significant antibiotic resistance genes and is able to integrate with other mobile genetic elements. The acquisition of plasmids containing Tn*1721* seems to help *A. salmonicida*

subsp. *salmonicida* strains to be equipped for environmental pressures. Plasmid variants include this transposon in their genomes, either completely or partially. pAsa8 is the example for complete acquisition of Tn*1721* transposon, while pAsa10 bears a partial part of the transposon.[81,82]

The discovery of pAsa10 goes back to the observation of incompatibility between the tetracycline resistance profile and PCR assay results of the SHY15-2743 strain.[82] The result of sequencing the genome of this strain was the discovery of a 10-kb plasmid known as pAsa10. The tetracycline resistance provided by pAsa10 comes from the *tet* region found in a partial Tn*1721*. The reason that the partial acquisition of Tn*1721* took place in this plasmid may lie in the existence of a peculiar reverse orientation of inverted repeats (IRs) in this plasmid sequence.

The plasmids implicated in virulence

The concept underlying virulence is much more complex than for antibiotic resistance. In general, genes causing resistance to drugs are well known and defined. For example, the gene *cat* encoding a chloramphenicol acetyltransferase, as found on pAsa7, confers resistance to

chloramphenicol.[61] Roughly, one gene equals resistance to an antibiotic family. What about virulence factors? The database VFDB is specialized for virulence,[83] and gives the following definition: "Virulence factors refer to the properties (i.e., gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease." (http://www.mgc.ac.cn/VFs/main.htm).

Every gene that encodes a product which enhances the pathogenic potential of a bacterium might fall under the virulence factor category. Virulence factors can be classified into five subcategories[84]: (1) membrane proteins, (2) capsule, (3) secretory proteins, (4) cell wall and outer membrane components and finally (5) others, which include biofilm, iron acquisition factors, and the PhoP/PhoQ two-component system. Whereas in antibiotic resistance, normally one gene confers resistance, some virulence factors such as secretion systems require many proteins to assemble into a complex biological machinery.[85]

Here, we will explore the plasmids known to be implied in the pathogenicity of *A. salmonicida*. We redirect the readers to other reviews for a more complete vision of the main pathogenic factors found in the genus *Aeromonas*.[86,87]

As is well reviewed elsewhere, one of the major virulence factors in *A. salmonicida* subsp. *salmonicida* is the type-three secretion system (TTSS).[88] As with other secretion systems, the TTSS exports effectors from the bacterial cytoplasm to the extracellular area or even into a target cell using complex protein machinery.[85] The TTSS of *A. salmonicida* subsp. *salmonicida* was found to be located on a large plasmid (~140 kb), named pASvirA.[89] This plasmid also encodes three known effectors and their chaperones (AopH/SycH, Ati2/Ati1 and AopO/SycO) and a putative one, AopX, for which the gene is truncated due to 20-bp duplication.[88,90] Other effectors, AexT and AopP, are coded on the chromosome and the small plasmid pAsal1, respectively.[91,92] There is also another putative chromosome-located gene encoding TTSS effector, AopS, which is truncated in *A. salmonicida* subsp. *salmonicida,* but intact in *A. salmonicida* subsp. *achromogenes*.[88]

353 Plasmid pASvirA, also named pAsa5, is essential for the virulence of *A. salmonicida* subsp.

354 *salmonicida*. More specifically, it was shown that the inactivation of one structural gene in the

355 TTSS locus on this plasmid is enough to totally abrogate the virulence of the bacterium. This was

356 nicely illustrated by the work of Dacanay and colleagues in 2007 using an Atlantic salmon

357 model.[93] In this study, it was shown that inactivating effectors *aopO*, *aopH* or *aexT* was not

358 sufficient to completely inhibit bacterial virulence compared to inactivation of the *ascC* gene

359 resulting in an avirulent mutant not able to provoke clinical symptoms by both intraperitoneal

360 injection and immersion.

361

362 It is also important to note that pAsa5 was reported to be thermolabile: the plasmid is lost when

363 the bacterium bearing it is grown at temperature of 25°C or above, resulting in a loss of the

364 virulence.[89] Further investigations based on PCR genotyping of 20 *A. salmonicida* subsp.

365 *salmonicida* strains showed that only the TTSS locus was systematically lost after growth in

366 stressful conditions, not the complete plasmid,[94] as had been suspected.[89] Finally, it was shown

367 by another study that the TTSS deletion was caused by the recombination of insertion sequences

368 (ISs) IS*AS11*s at a temperature of 25°C or above.[95] Three different IS*AS11*s could be implicated,

giving the possibility of two different recombination events. Since then, other cases have been reported by the sequencing of pAsa5 using NGS.[15,96] In fact, pAsa5 is known to harbor various ISs,[27,97] as well as the chromosome.[27]

With the objective of resolving the inconsistencies described in 2012 concerning certain rearrangements of the plasmid pAsa5 of the strain 01-B526,[95] Tanaka et al. used long-read sequencing to close the sequence of the plasmid pAsa5 from this strain.[98] This revealed the existence of an additional IS*AS5* in plasmid pAsa5 of 01-B526 compared to the one in strain A449. This additional IS*AS5* appeared to be responsible for rearrangements that occured with another IS*AS5* also present on pAsa5 which explained the previously unresolved TTSS loss seen for this strain.

Long-read sequencing also helped to reveal that 01-B526 bears a second large plasmid, named pAsa9, which shares 40 kbp of highly similar sequences with pAsa5, but that are not related to the TTSS region. The pAsa9 plasmid contains replication-associated genes, conjugation genes, ORFs coding for hypothetical proteins, and transposase genes. This plasmid was always found in

385 isolates with another element, the genomic island *AsaGEI1a*.[98,99] As for pAsa5 that has variants

386 based on the presence or absence of ISs, pAsa9 also shares this characteristic. Indeed, the

387 SHY16-3432 strain bears a pAsa9 variant (pAsa9b) with one fewer IS*AS5*.[76]

388

389 As mentioned elsewhere, the pAsa5 plasmid is typically part of the classical plasmid set in

390 *A. salmonicida* subsp. *salmonicida* strains, and it is interesting to find traces of this plasmid in

391 other subspecies of *A. salmonicida*.[27,46,100] In fact, some strains of *A. salmonicida* subsp.

392 *masoucida* contain highly similar smaller plasmids (Ps68-2, pS121-3, pS44-3 RFAS1 unnamed

393 2), and in which TTSS genes can be found (Table S1). On the other hand, the same

394 *A. salmonicida* subsp. *masoucida* strains also harbor similar small plasmids (pS68-1, pS44-2,

395 pS121-2, RFAS1 unnamed 1) with *tra* genes (Table S1), that are necessary for plasmid transfer

396 through conjugation and these genes are pretty identical to the *tra* genes found in pAsa5.[98] It is

397 interesting to find traces of derivatives of pAsa5 in *A. salmonicida* subsp. *salmonicida* strains in

398 other subspecies of *A. salmonicida*. In fact, the two plasmids found in the four *A. salmonicida*

399 subsp. *masoucida* strains are likely the result of the split of a former pAsa5 variant or possibly

400 primitive versions which merged to give pAsa5 as known in *A. salmonicida* subsp. *salmonicida*.

401 Other pAsa5 variants of various sizes have also been sequenced from *A. salmonicida* subsp.

402 *salmonicida* strains isolated in Canada, Chile and Poland (Table S1).

403

404 Another story about putative rearrangements of pAsa5 was published in 2009.[101] This paper

405 showed the presence of a new 18 kb plasmid, named pAsa6, in the *A. salmonicida* subsp.

406 *salmonicida* RSP74.1 strain isolated from a turbot (*Psetta maxima*) in Portugal in 2002.[102] Even

407 if pAsa6 is very similar to pAsa5; it is still unclear if pAsa6 is a direct derivative of pAsa5 by a

408 process of gene reduction, or if it is a chimeric fusion between pAsa5 and a pAsa6-like

409 plasmid.[101] The profile of small plasmids of the strain RSP74.1 was much different than expected

410 for a member of the subspecies *salmonicida*,[62,102] meaning that either (1) the strain is in fact not a

411 subspecies of *salmonicida* and thus evolved under different mutational pressures or (2) the strain

412 is indeed of the subspecies *salmonicida* but experienced an unusual selection, for example to

413 adapt to turbot, which is an atypical host for this bacterial subspecies. All these pAsa5 variants

414 suggest that pAsa5 plays a central role in the plasmidome of *A. salmonicida* subsp. *salmonicida.*

415

As discussed above, pAsa5 harbors the genes to encode a functional TTSS, including three effectors: AopH, Ati2 and AopO. However, another plasmid, pAsal1, also bears a TTSS effector: AopP.[92] The plasmid pAsal1 is a ColE2-type replicon of 6 371 bp with high structural similarity with other plasmids that have the same replicon in *A. salmonicida* subsp. *salmonicida*, such as pAsa1 and pAsa3 (see below).[62] However, compared to other known ColE1-type replicon plasmids in this bacterium, pAsal1 bears the insertion sequence IS*AS11*, the same IS-type which promotes the loss of the TTSS due to the rearrangements in pAsa5.[95] The same study suggested that the IS*AS11* of pAsal1 might also be activated under stressful conditions such as growth at or above 25°C, resulting in the loss of the plasmid.[95] This hypothesis was reinforced by another study, which found non-systematic correlation between the loss of the TTSS and pAsal1.[62]

Three other variants of pAsal1 were presented in detail: pAsal1B,[103] pAsal1C,[62] and pAsal1D,[62] all of them encoding likely functional AopP. They all have an additional IS*AS5*, in common. The IS*AS5* was inserted in *mobA* for pAsal1B while it was inserted in the IS*AS11* for pAsal1C and pAsal1D. Interestingly, other pAsal1 variants from China, named pS44-5, pS68-4 and pS121-5,

have been added to the databases recently. They are almost identical to pAsal1 but without the IS*AS11* (Table S1).

Several of the variants of pAsa5, pAsa9 and pAsal1 exist by the simple presence or absence of an IS*AS5*. This suggests that this IS has an important role in the evolution of the genome of *A. salmonicida* subsp. *salmonicida*.[76,98,103,104] In addition, IS*AS5* has a major role in its heat instability.[98] It is interesting to note that IS*AS5* is larger than the other ISs found in *A. salmonicida* subsp. *salmonicida*.[27] This probably makes it a better substrate for recombinases. It has even been suggested that ISs in general had a role in the dichotomy observed between the psychrophilic and mesophilic strains of *A. salmonicida*.[15]

All the plasmids discussed above were found in *A. salmonicida* subsp. *salmonicida*, commonly known as "typical *salmonicida*". Little is actually known about virulence-causing plasmids of the other subspecies (atypical *A. salmonicida*). The complete sequencing of the JF4097 strain of *A. salmonicida* subsp. *smithia* revealed a new ColE1-like plasmid, pJF4097.[15] This plasmid, as

446 for the ColE2-like pAsal1, bears an IS*AS11* and has a gene encoding for a putative TTSS

447 effector, ExoY-like in the case of pJF4097.

448

449 **The cryptic plasmids and their variants**

450 Many plasmids in *A. salmonicida* are known to provide antibiotic resistance, and some plasmids

451 confer virulence factors. However, there are also plasmids with no biological function, which are

452 consequently known as "cryptic" plasmids. These plasmids were investigated during the pre-

453 sequencing era (Table 1). Typically, *A. salmonicida* subsp. *salmonicida* strains have three small

454 cryptic plasmids ranging from 5.2 to 5.6 kbp which bear either a ColE1- or a ColE2-type

455 replicon: pAsa1(ColE2), pAsa2(ColE1) and pAsa3(ColE2). With pAsal1 and pAsa5, these

456 plasmids constitute the core plasmidome typically carried by *A. salmonicida* subsp. *salmonicida*

457 strains (Figure 2). Plasmids carrying antibiotic resistance are usually found in one strain or a few

458 isolates. The main exception to this rule is the plasmid pSN254b which was found in

459 approximately 25% of the tested isolates from Canada in the study by Trudel et al.[69] In addition,

460 it has been shown that it is very rare to find two different antibiotic resistance plasmids in the

same strain. The most frequent case is the co-occurrence of pRAS3 and pAB5S9b, which was identified in two strains out of 36 tested as resistant to antibiotics.[69]

Even if the presence of cryptic plasmids in *A. salmonicida* subsp. *salmonicida* isolates has been known since 1983,[105] it was in 1989 that they were named,[46] and their sequences were completely sequenced in 2003.[100] Many studies have investigated these plasmids' presence or absence in *A. salmonicida* strains, and have concluded that their repertoire was highly conserved and consequently without any or limited potential to be used as an epidemiologic marker.[45–48] A recent large-scale study based on 153 *A. salmonicida* subsp. *salmonicida* isolates revealed that those from Europe do not bear pAsa3 or pAsal1 more frequently than the Canadian isolates.[62] However, these variations in plasmid content revealed in this systematic study are still not sufficiently constant or systematic to be adequate epidemiological markers. The *AsaGEIs*, a group of genomic islands, represent much more promising geographic markers than plasmids for *A. salmonicida* subsp. *salmonicida* isolates.[96,99,106]

476 It was showed that DNA sequences of pAsa3 evolve more rapidly than the DNA of other small
477 plasmids.[62] In fact, cryptic plasmids have demonstrated their role in the appearance of plasmid
478 variants possessing new characteristics useful for the bacterium. The small plasmids pAsaXI and
479 pAsaXII found in *A. salmonicida* subsp. *salmonicida* isolates are derivatives of the cryptic
480 plasmids pAsa3 and pAsa2, respectively. The discovery of modified cryptic plasmids with a
481 variety of mobile genetic elements demonstrates the fact that these plasmid derivatives actually
482 can confer benefits to this species, including potentially increasing the virulence, and at the same
483 time, making it more adaptable to a greater variety of environments.[82]
484
485 pAsaXI bears a Tn3-family like transposon. This transposon is significant because there is a high
486 homology among one of its genes and a virulent gene found in other bacteria, like *Vibrio*
487 *cholerae* and *Aeromonas hydrophila*. Also, according to BLAST analysis, the presence of the
488 complete transposon in plasmids of the other aquatic species like *Shewanella baltica* and
489 *Shewanella putrefaciens* puts the *A. salmonicida* subsp. *salmonicida* strains one more time under
490 the spotlight for being capable of gene transfers both with other strains in the same species, and
491 other bacterial species.[82]

492

493 Production of a new derivative may be a fruitful procedure for *A. salmonicida* subsp. *salmonicida*

494 strains to prevent cell death caused by the rush of the toxin counterpart of the toxin-antitoxin

495 (TA) system. At the same time, this new derivative may provide a region to acquire new

496 resistance genes without interfering with the main functions of the plasmid, like replication or

497 mobilization. This is the case for pAsaXI, in which the gene *relE* that codes the toxin counterpart

498 of a TA system is being lost due to transposon interruption.[82] The interruption of the toxin gene

499 of the TA system is not limited to the pAsaXI plasmid. A similar event happened for the

500 derivative of pAsa2 cryptic plasmid, pAsaXII, since the gene for the toxin ParE from the *parED*

501 TA system is interrupted by MIC, which is a putative mobile insertion system.[82]

502

503 pAsaXII also increases the potential of *A. salmonicida* subsp. *salmonicida* strains to receive

504 mobile genetic elements, since the same plasmid as pAsaXII can be found in the mesophilic

505 bacterium *Aeromonas bivalvium*. The *fmrAB* gene is present and provides a formaldehyde

506 detoxification system in the pAsaXII plasmid in the HER1084 strain. It can be inferred that the

adaptation of *A. salmonicida* subsp. *salmonicida* to its environment goes beyond receiving antibiotic resistance genes.[82]

The small cryptic plasmids found in *A. salmonicida* subsp. *salmonicida* are well studied, but, again, little is known about the other subspecies, though they are suspected to contain a highly diverse plasmidome.[49] Only three small cryptic plasmids were identified in the mesophilic *A. salmonicida* Y47 : pY47-1 (12 495 bp), pY47-2 (6 042 bp) and pY47-3 (5 104 bp).[15] The pY47-2 and pY47-3 plasmids have a ColE2-like replicon. Of the three plasmids, only pY47-3 was also found in another isolate, Y577, which is also a mesophilic Indian *A. salmonicida*.[15] Interestingly, pY47-3 shows a structural similarity with the ColE2-type replicon R-plasmids pAQ2-1 and pAQ2-2, found in *Aeromonas sobria* and *Aeromonas hydrophila*, respectively.[107] However, unlike these plasmids, pY47-3 does not bear the *qnrS2* quinolone resistance gene.

Conclusion

In this review we explored the plasmidome of *A. salmonicida*. Knowledge has accumulated about this diverse plasmidome, which can be separated into three categories (R-plasmids, virulence

plasmids and cryptic plasmids). The advance of DNA sequencing technology has played an important role in the elucidation of complete sequences, giving us greater clarity to study the roles and importance of plasmids in *A. salmonicida*. However, there is a clear bias in the sequencing effort towards the subspecies *salmonicida*, while at least four other subspecies exist (and maybe more). Though new plasmids certainly remain to be discovered in the subspecies *salmonicida*, especially with the high rate of horizontal gene transfers, it is important to shed light on the plasmidome of the other *A. salmonicida* subspecies as well.

In addition to providing important phenotypic characteristics, such as virulence or resistance to antibiotics, the plasmids in *A. salmonicida* tell us more about its ecology, its microbial network, and its adaptability. We have seen that several important plasmids, such as pSN254b and pAB5S9b, can originate from other bacteria, such as *S. enterica* and *A. bestiarum*. Evolution is a slow phenomenon, which usually requires many generations. Plasmids, by changing the gene repertoire, give the bacterial genome modularity and the ability to rapidly adapt. Although several plasmids have been found and characterized in *A. salmonicida*, other bacteria, for example the species of the genera *Borrelia* (relapsing fever) and *Borreliella* (Lyme

539 disease), have evolved in such a way that the plasmids can represent up to 40% of the total

540 genome.[108,109] This example illustrates the importance that plasmids can have on the evolutionary

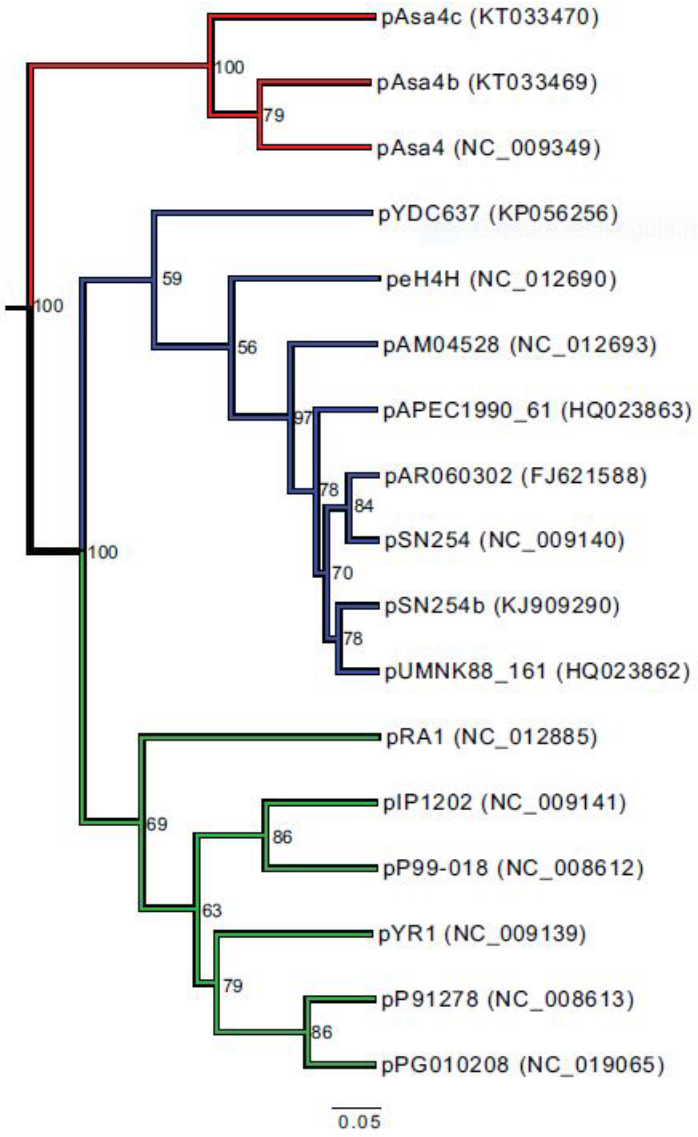
541 trajectory of bacteria, and suggests that the study of plasmids is essential in order to see the

542 panorama of bacterial adaptive mechanisms.

543

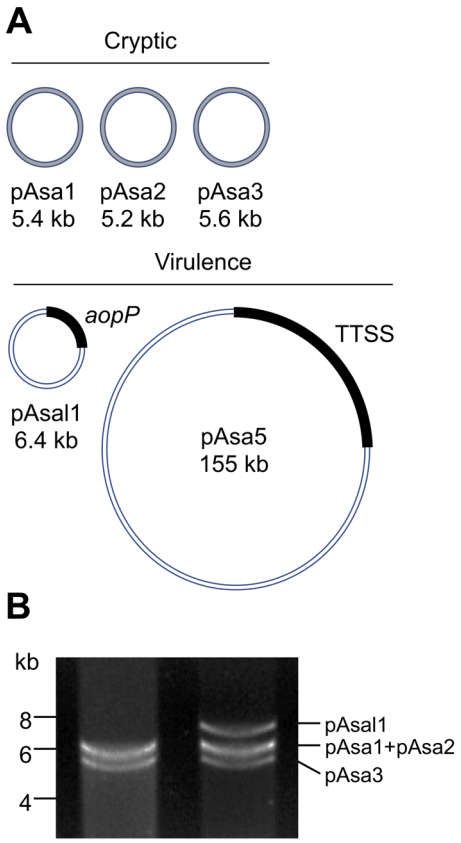
Acknowledgements

We would like to thank A. Schneider for her assistance. S.J.C. is a research scholar from the Fonds de Recherche du Québec en Santé.



548

549 **Figure 1. Clustering of 14 IncA/C plasmids in addition to 3 pAsa4s.** The accession number of
550 each plasmid is indicated between brackets. The pAsa4s are in red, and the plasmids that have or
551 lack the *bla*$_{CMY-2}$ region are in blue and green, respectively.[72] Homologous genes between
552 plasmids were found using GET_HOMOLOGUES.[113] The resulting matrix was binary encoded
553 and the distance was evaluated with the "binary" model in R.[114] Finally, hierarchical clustering
554 was performed using Pvclust with 10 000 bootstrap replicates.[115]



555

556 **Figure 2. Core plasmidome of *A. salmonicida* subsp. *salmonicida* strains.** A. Usually, pAsa1,
557 pAsa2, pAsa3, pAsal1 and pAsa5 are found in the bacterium. This is a schematic representation
558 where the dimensions are not to scale. B. It is possible to easily observe the small plasmids
559 (pAsa1, pAsa2, pAsa3, pAsal1) on an electrophoresis agarose gel. On this picture, electrophoretic
560 separation of small plasmid DNA extraction of two strains are shown with one strain bearing the
561 small plasmids of the classical core plasmidome and another without pAsal1.
562

563 **Table 1**. Timeline of the literature concerning the plasmidome of *A. salmonicida* in the pre-NGS
564 era.

Years	Content	Reference
1890	Furunculosis reported	[28]
1952	"Plasmid" was first mentioned	[30]
1971	R factors found in *A. salmonicida*	[29]
1972	R factors found in *A. salmonicida*	[31]
1981	A high growth temperature attenuates the virulence of *A. salmonicida*. Plasmids did not seem to be involved in virulence.	[41]
1983	Transferable R plasmids found in *A. salmonicida*.	[33]
1983	*A. salmonicida* bears multiple small plasmids.	[105]
1984	*A. salmonicida* usually has four small plasmids and may also contain larger ones. Impossible to clearly verify if plasmids are involved in virulence.	[42]
1986	Characterization of two R-plasmids: pAr32 and pJA8102-1.	[34]
1988	Many isolates have 4 small plasmids and one large (which seems to change in length). However, even if stable, the repertoire may change. The plasmidome changes between subspecies.	[45]
1989	The small plasmid pAsa1, 2 and 3 were named. In addition, the large R-plasmid pAsa4 was also named. Plasmids of atypical *A. salmonicida* may be used as epidemiological markers.	[46]
1991	The repertoire in plasmids is stable, even from isolate of diverse geographical origins.	[47]
1993	The plasmidome of 40 oxytetracycline-resistant *A. salmonicida* was analysed. They found that 11 of the 40 isolates had transferable R plasmids. Moreover, they tested the co-occurrence of drug resistance.	[35]
1993	The plasmid profile of 124 *A. salmonicida* subsp. *salmonicida* isolates was assessed. A large panel of plasmids of diverse sizes exists. No correlation between the plasmid repertoire and the host or the years of the isolation. However, some trends were shown between plasmid profiles and the geographical area.	[48]
1994	Description of the conjugal transfer of pRAS1 from *A. salmonicida* to bacteria in marine sediments.	[36]
1995	Analysis of Finnish *A. salmonicida* subsp. *salmonicida* isolates revealed that many of them usually have small plasmids and one or two large plasmids. Other uncommon plasmids were also reported.	[110]
1995	The plasmidome of the *A. salmonicida* isolates from the Pacific coast (variable) were found to vary somewhat from those of the Atlantic coast (constant).	[111]
1996	The R-plasmid pRAS1 was demonstrated to have a broad host range.	[37]
1996	Differences were found between the plasmidome of "atypical" and "typical" *salmonicida*. The plasmidome of the "atypical" isolate is usually diverse and sometimes correlated with the fish host.	[49]
1997	First time that pulsed field gel electrophoresis (PFGE) was used to characterize the plasmidome of *A. salmonicida*. Found no correlation between drug resistance and plasmid profiles, which suggests the resistance is not associated with either one plasmid or a particular profile.	[112]
1998	Four R-plasmids were found in Norwegian isolates.	[38]
1998	The pASOT plasmid-group mediated oxytetracycline resistance in *A. salmonicida*.	[39]
2000	A transposon, Tn5393c, was found in the R-plasmid pRAS2.	[40]
2002	Complete sequencing achieved for the R-plasmids pRAS3.1 and pRAS3.2.	[32]

565

Table 2. *A. salmonicida* sequenced plasmids. This table presents only the plasmids that have been described and characterized in a peer review article before February 2020. The other plasmids for which the sequence is available in GenBank but not described in a publication are presented in Table S1.

Plasmid	Length (bp)	GC%	CDS[a]	CDS/kb	Average CDS length	Toxin-antitoxin system	Virulence	Antibiotic resistance gene	Reference
pY47-3	5104	54.68	9	1.763	568	CcdA/CcdB	N/A[b]	N/A	15
pAsa2	5247	52.20	7	1.334	588	N/A	N/A	N/A	100
pAsa7	5276	53.39	6	1.137	578	N/A	N/A	*cat*	61
pAsa1	5424	57.02	10	1.843	542	RelB/RelE	N/A	N/A	100
pAsa3	5616	54.52	9	1.602	615	RelB/RelE	N/A	N/A	100
pY47-2	6042	57.91	9	1.489	537	RelB/RelE	N/A	N/A	15
pJF4097	6231	57.05	8	1.283	685	N/A	ExoY	N/A	15
pAsal1	6371	55.03	6	0.941	688	N/A	AopP	N/A	92
pAsaXII	7700	54.61	13	1.688	485	ParE/ParD, phd antitoxin	N/A	N/A	82
pAsal1B	8989	55.63	8	0.889	830	N/A	AopP	N/A	103
pAsal1C	8989	55.87	7	0.778	746	N/A	AopP	N/A	62
pAsa1lD	9138	55.72	8	0.875	804	N/A	AopP	N/A	62
pAsa10	9995	60.68	8	0.800	677	N/A	N/A	*tetA, tetC*	82
pRAS3.2	11 823	59.01	14	1.184	711	PemI/PemK	VirB4	*tetA*	32
pRAS3.3	11 845	58.89	14	1.181	712	PemI/PemK	VirB4	*tetA*	68
pRAS3.1	11 851	58.91	14	1.181	711	PemI/PemK	VirB4	*tetA*	32

pY47-1	12 495	53.39	19	1.520	462	ParD/ParE	N/A	N/A	[15]
pAsaXI	12 536	54.62	11	0.877	1033	RelB/StbD replicon stabilization protein)	N/A	N/A	[82]
pRAS3-3432	13 912	57.06	18	1.293	703	PemK/MazF	N/A	*tetC*	[76]
pAsa6	18 536	53.85	16	0.863	853	N/A	AopH	N/A	[101]
pAB5S9b	25 540	52.27	28	1.096	798	N/A	VirD2	*tetH, floR, sul2, strA, strB*	[68]
pAsa9-like	74 314	52.63	71	0.955	803	N/A	N/A	H-NS	[76]
pAsa9	76 724	52.76	86	1.12	736	N/A	N/A	H-NS	[98]
pAsa8	110 577	54.87	132	1.193	709	N/A	N/A	*tetA, tetR, floR, tetG, sul1, blaPSE-1, aadA*	[69]
pSN254b	152 216	52.47	180	1.182	762	RelB/RelE?	N/A	*tetA, floR, sul1, sul2, blaCMY, aadA, strA, strB*	[68]
pAsa5	155 098	54.33	157	1.012	747	N/A	TTSS	N/A	[27]
pAsa5 variant (one additional IS*AS5*)	157 744	54.38	177	1.09	729	N/A	TTSS (*ascV, pscS, ascC, pscR, yscR, yscN*), a*opH, VPA0450*	H-NS	[98]
pAsa4c	163 022	53.42	155	0.95	851	HipA/HipB	N/A	*sul1, aadA, cat*	[64]
pAsa4	166 749	52.80	164	0.983	779	HipA/HipB	N/A	*tetA*(E), *sul1, aadA, cat*	[27]
pAsa5-3432	180 043	55.29	172	0.96	771	N/A	*aopH, VPA0450, ascC, pscR,, ascV, yscN, yscR, pscS,*	*sul1, tetG, tetC, tetA,* H-NS, *aadA3, CARB/PSE* family, *qacE, CARB-3, tetG, floR, FloR family*	[76]
pAsa4b	181 933	52.48	174	0.956	840	HipA/HipB	N/A	*tetA*(E), *sul1*	[64]

a: CDS means coding sequences

b: N/A means not applicable

571 **REFERENCES**

572 1. Land M., L. Hauser, S.-R. Jun, *et al.* 2015. Insights from 20 years of bacterial genome
573 sequencing. *Funct. Integr. Genomics* **15**: 141–161.
574 2. Vincent A.T., N. Derome, B. Boyle, *et al.* 2017. Next-generation sequencing (NGS) in the
575 microbiological world: How to make the most of your money. *J. Microbiol. Methods* **138**:
576 60–71.
577 3. Douglas G.M. & M.G.I. Langille. 2019. Current and promising approaches to identify
578 horizontal gene transfer events in metagenomes. *Genome Biol. Evol.* **11**: 2750–2766.
579 4. Actis L.A., M.E. Tolmasky & J.H. Crosa. 1999. Bacterial plasmids: replication of
580 extrachromosomal genetic elements encoding resistance to antimicrobial compounds.
581 *Front. Biosci.* **4**: D43–D62.
582 5. Srivastava S. 2013. Plasmids: Their Biology and Functions. In *Genetics of Bacteria* 125–
583 151. India: Springer India.
584 6. Brooks L., M. Kaze & M. Sistrom. 2019. A curated, comprehensive database of plasmid
585 sequences. *Microbiol. Resour. Announc.* **8**: e01325-18.
586 7. Lanza V.F., M. de Toro, M.P. Garcillán-Barcia, *et al.* 2014. Plasmid flux in *Escherichia*
587 *coli* ST131 sublineages, analyzed by plasmid constellation network (PLACNET), a new
588 method for plasmid reconstruction from whole genome sequences. *PLoS Genet* **10**:
589 e1004766.
590 8. Toro M.D.E., M.P. Garcillán-barcia & F.D.E.L. a Cruz. 2014. Plasmid diversity and
591 adaptation analyzed by massive sequencing of *Escherichia coli* plasmids. *Microbiol.*
592 *Spectr.* **2**: 1–16.
593 9. Pavan M.E., S.L. Abbott, J. Zorzópulos, *et al.* 2000. *Aeromonas salmonicida* subsp.

pectinolytica subsp. nov., a new pectinase- positive subspecies isolated from a heavily polluted river. *Int. J. Syst. Evol. Microbiol.* **50**: 1119–1124.

10. Kimura T. 1969. A new subspecies of *Aeromonas salmonicida* as an etiological agent of furunculosis on "sakuramasu" (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) rearing for maturity. *Fish Pathol.* **3**: 34–44.

11. Kimura T. 1969. A new subspecies of *Aeromonas salmonicida* as an etiological agent of furunculosis on "sakuramasu" (*Oncorhynchus masou*) and pink salmon(*O. gorbuscha*) rearing for maturity. *Fish Pathol.* **3**: 45–52.

12. Smith I.W. 1963. The classification of "*Bacterium salmonicida*." *J. Gen. Microbiol.* **33**: 263–274.

13. Austin D.A., D. McIntosh & B. Austin. 1989. Taxonomy of fish associated *Aeromonas* spp., with the description of *Aeromonas salmonicida* subsp. *smithia* subsp. nov. *Syst. Appl. Microbiol.* **11**: 277–290.

14. Griffin P.J., S.F. Snieszko & S.B. Friddle. 1953. Pigment formation by *Bacterium salmonicida*. *J. Bacteriol.* **65**: 652 LP – 659.

15. Vincent A.T., M.V Trudel, L. Freschi, *et al.* 2016. Increasing genomic diversity and evidence of constrained lifestyle evolution due to insertion sequences in *Aeromonas salmonicida*. *BMC Genomics* **17**: 44.

16. Vincent A.T., F.D. Rouleau, S. Moineau, *et al.* 2017. Study of mesophilic *Aeromonas salmonicida* A527 strain sheds light on the species' lifestyles and taxonomic dilemma. *FEMS Microbiol. Lett.* **364**: fnx239.

17. Rouleau F.D., A.T. Vincent & S.J. Charette. 2018. Genomic and phenotypic characterization of an atypical *Aeromonas salmonicida* strain isolated from a lumpfish and producing unusual granular structures. *J. Fish Dis.* **41**: 673–681.

18. Vincent A.T., A. Fernández-Bravo, M. Sanchis, *et al.* 2019. Investigation of the virulence and genomics of *Aeromonas salmonicida* strains isolated from human patients. *Infect. Genet. Evol.* **68**: 1–9.

19. Vincent A.T., A. Bernatchez, J. Frey, *et al.* 2019. A mesophilic *Aeromonas salmonicida* strain isolated from an unsuspected host, the migratory bird pied avocet. *Microorganisms* **7**: 592.

20. Burr S.E. & J. Frey. 2007. Analysis of type III effector genes in typical and atypical *Aeromonas salmonicida*. *J. Fish Dis.* **30**: 711–714.

21. Dallaire-Dufresne S., K.H. Tanaka, M.V Trudel, *et al.* 2014. Virulence, genomic features, and plasticity of *Aeromonas salmonicida* subsp. *salmonicida*, the causative agent of fish furunculosis. *Vet. Microbiol.* **169**: 1–7.

22. Kamble R. 2015. *Aeromonas salmonicida* furunculosis in an adult male. *Int.J.Curr.Microbiol.App.Sci.* **4**: 59–63.

23. Ruppé E., A. Cherkaoui, N. Wagner, *et al.* 2017. *In vivo* selection of a multidrug-resistant *Aeromonas salmonicida* during medicinal leech therapy. *New Microbes New Infect.* **21**: 23–27.

24. Tewari R., M. Dudeja, S. Nandy, *et al.* 2014. Isolation of *Aeromonas salmonicida* from human blood sample: A case report. *J. Clin. Diagnostic Res.* **8**: 139–140.

25. Varshney A., M. Das, P. Chaudhary, *et al.* 2017. *Aeromonas salmonicida* as a causative agent for postoperative endophthalmitis. *Middle East Afr. J. Ophthalmol.* **24**: 213–215.

26. Yang X., Q.Q. Yang, Q.Y. Guo, *et al.* 2008. *Aeromonas salmonicida* peritonitis after eating fish in a patient undergoing CAPD. *Perit. Dial. Int.* **28**: 316–317.

27. Reith M.E., R.K. Singh, B. Curtis, *et al.* 2008. The genome of *Aeromonas salmonicida* subsp. *salmonicida* A449: insights into the evolution of a fish pathogen. *BMC Genomics* **9**:

427.

28. Emmerich R. & E. Weibel. 1890. Über eine durch Bakterien verursachte Infektionskrankheit der Forellen. *Allg. Fischerei-Zeitung* **15**: 73-77;85-92.

29. Aoki T., S. Egusa, T. Kimura, *et al.* 1971. Detection of R factors in naturally occurring *Aeromonas salmonicida* strains. *Appl. Microbiol.* **22**: 716–7.

30. Lederberg J. 1952. Cell genetics and hereditary symbiosis. *Physiol. Rev.* **32**: 403–430.

31. Aoki T., S. Egusa, C. Yada, *et al.* 1972. Studies of drug resistance and R factors in bacteria from pond-cultured salmonids. *Jpn. J. Microbiol.* **16**: 233–238.

32. L'Abée-Lund T.M. & H. Sørum. 2002. A global non-conjugative Tet C plasmid, pRAS3, from *Aeromonas salmonicida*. *Plasmid* **47**: 172–181.

33. Aoki T., T. Kitao, N. Iemura, *et al.* 1983. The susceptibility of *Aeromonas salmonicida* strains isolated in cultured and wild salmonids to various chemotherapeutics. *Bull. Japan. Soc. Sci. Fish* **49**: 17–22.

34. Aoki T., Y. Mitoma & J.H. Crosa. 1986. The characterization of a conjugative R-plasmid isolated from *Aeromonas salmonicida*. *Plasmid* **16**: 213–218.

35. Inglis V., E. Yimer, E.J. Bacon, *et al.* 1993. Plasmid-mediated antibiotic resistance in *Aeromonas salmonicida* isolated from Atlantic salmon, *Salmo salar* L., in Scotland. *J. Fish Dis.* **16**: 593–599.

36. Sandaa R.A. & O. Enger. 1994. Transfer in marine sediments of the naturally occurring plasmid pRAS1 encoding multiple antibiotic resistance. *Appl. Environ. Microbiol.* **60**: 4234–4238.

37. Sandaa R.A. & Ø. Enger. 1996. High frequency transfer of a broad host range plasmid present in an atypical strain of the fish pathogen *Aeromonas salmonicida*. *Dis. Aquat. Organ.* **24**: 71–75.

666 38. Sorum H. 1998. Mobile drug resistance genes among fish bacteria. *APMIS* **106**: 74–76.
667 39. Adams C., B. Austin, P. Meaden, *et al.* 1998. Molecular characterization of plasmid-
668 mediated oxytetracycline resistance in *Aeromonas salmonicida*. *Appl. Environ. Microbiol.*
669 **64**: 4194–201.
670 40. L'Abée-Lund T.M. & H. Sørum. 2000. Functional Tn*5393*-like transposon in the R
671 plasmid pRAS2 from the fish pathogen *Aeromonas salmonicida* subspecies *salmonicida*
672 isolated in Norway. *Appl. Environ. Microbiol.* **66**: 5533–5535.
673 41. Ishiguro E.E., W.W. Kay, T. Ainsworth, *et al.* 1981. Loss of virulence during culture of
674 *Aeromonas salmonicida* at high temperature. *J. Bacteriol.* **148**: 333–340.
675 42. Hackett J.L., W.H. Lynch, W.D. Paterson, *et al.* 1984. Extracellular protease, extracellular
676 haemolysin, and virulence in *Aeromonas salmonicida*. *Can. J. Fish. Aquat. Sci.* **41**: 1354–
677 1360.
678 43. Li W., D. Raoult & P.-E. Fournier. 2009. Bacterial strain typing in the genomic era. *FEMS*
679 *Microbiol. Rev.* **33**: 892–916.
680 44. Ochoa-Díaz M.M., S. Daza-Giovannetty & D. Gómez-Camargo. 2018. Bacterial
681 Genotyping Methods: From the Basics to Modern. In *Host-Pathogen Interactions:*
682 *Methods and Protocols* Medina C. & López-Baena F.J., Eds. 13–20. New York, NY:
683 Springer New York.
684 45. Bast L., J.G. Daly, S.A. Degrandis, *et al.* 1988. Evaluation of profiles of *Aeromonas*
685 *salmonicida* as epidemiological markers of furunculosis infections in fish. *J. Fish Dis.* **11**:
686 133–145.
687 46. Belland R.J. & T.J. Trust. 1989. *Aeromonas salmonicida* plasmids: Plasmid-directed
688 synthesis of proteins in vitro and in *Escherichia coli* minicells. *Microbiology* **135**: 513–
689 524.

690 47. Toranzo A.E., Y. Santos, S. Nunez, *et al.* 1991. Biochemical and serological
691 characteristics, drug resistance and plasmid profiles of spanish isolates of *Aeromonas*
692 *salmonicida*. *Fish Pathol.* **26**: 55–60.
693 48. Nielsen B., J. Olsen & J.L. Larsen. 1993. Plasmid profiling as an epidemiological marker
694 within *Aeromonas salmonicida*. *Dis. Aquat. Organ.* **15**: 129–135.
695 49. Pedersen K., I. Dalsgaard & J.L. Larsen. 1996. Characterization of atypical *Aeromonas*
696 *salmonicida* isolates by ribotyping and plasmid profiling. *J. Appl. Bacteriol.* **80**: 37–44.
697 50. Sanger F., G.M. Air, B.G. Barrell, *et al.* 1977. Nucleotide sequence of bacteriophage phi
698 X174 DNA. *Nature* **265**: 687–695.
699 51. Sutcliffe J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid
700 pBR322. *Cold Spring Harb. Symp. Quant. Biol.* **43**: 77–90.
701 52. Reardon S. 2014. Antibiotic resistance sweeping developing world. *Nat. News* **509**: 141–
702 142.
703 53. Morin R. 2010. L'utilisation des antibiotiques pour combattre la furonculose chez l'omble
704 de fontaine génère de l'antibiorésistance chez *Aeromonas salmonicida*. *L'aquicole* **15**: 1.
705 54. Cabello F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing
706 problem for human and animal health and for the environment. *Environ. Microbiol.* **8**:
707 1137–1144.
708 55. Gordon L., A. Cloeckaert, B. Doublet, *et al.* 2008. Complete sequence of the *floR*-carrying
709 multiresistance plasmid pAB5S9 from freshwater *Aeromonas bestiarum*. *J. Antimicrob.*
710 *Chemother.* **62**: 65–71.
711 56. Bradley D.E., T. Aoki, T. Kitao, *et al.* 1982. Specification of characteristics for the
712 classification of plasmids in incompatibility group U. *Plasmid* **8**: 89–93.
713 57. Watanabe T., C. Furuse & S. Sakaizumi. 1968. Transduction of various R factors by phage

714 P1 in *Escherichia coli* and by phage P22 in *Salmonella typhimurium*. *J. Bacteriol.* **96**:
715 1791–1795.
716 58. Sandaa R.A. & O. Enger. 1994. Transfer in marine sediments of the naturally occurring
717 plasmid pRAS1 encoding multiple antibiotic resistance. *Appl. Environ. Microbiol.* **60**:
718 4234–4238.
719 59. Sørum H., T.M. L'Abée-Lund, A. Solberg, *et al.* 2003. Integron-containing IncU R
720 plasmids pRAS1 and pAr-32 from the fish pathogen *Aeromonas salmonicida*. *Antimicrob.*
721 *Agents Chemother.* **47**: 1285–1290.
722 60. L'Abée-Lund T.M. & H. Sørum. 2001. Class 1 integrons mediate antibiotic resistance in
723 the fish pathogen *Aeromonas salmonicida* worldwide. *Microb. Drug Resist.* **7**: 263–72.
724 61. Vincent A.T., J.-G. Emond-Rheault, X. Barbeau, *et al.* 2016. Antibiotic resistance due to
725 an unusual ColE1- type replicon plasmid in *Aeromonas salmonicida*. *Microbiology* **162**:
726 942–53.
727 62. Attéré S.A., A.T. Vincent, M.V Trudel, *et al.* 2015. Diversity and homogeneity among
728 small plasmids of *Aeromonas salmonicida* subsp. *salmonicida* linked with geographical
729 origin. *Front. Microbiol.* **6**: 1274.
730 63. Vincent A.T., J.-G. Emond-Rheault, M. Frenette, *et al.* 2016. Antibiotic resistance due to
731 an unusual ColE1-type replicon plasmid in *Aeromonas salmonicida*. *Microbiology* **162**:
732 942–53.
733 64. Tanaka K.H., A.T. Vincent, M.V Trudel, *et al.* 2016. The mosaic architecture of
734 *Aeromonas salmonicida* subsp. *salmonicida* pAsa4 plasmid and its consequences on
735 antibiotic resistance. *PeerJ* **4**: e2595.
736 65. Welch T.J., W.F. Fricke, P.F. McDermott, *et al.* 2007. Multiple antimicrobial resistance in
737 plague: An emerging public health risk. *PLoS One* **2**: e309.

66. Johnson T.J. & K.S. Lang. 2012. IncA/C plasmids: An emerging threat to human and animal health? *Mob. Genet. Elements* **2**: 55–58.

67. McIntosh D., M. Cunningham, B. Ji, *et al.* 2008. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida* is associated with carriage of an IncA/C plasmid similar to the *Salmonella enterica* plasmid pSN254. *J. Antimicrob. Chemother.* **61**: 1221–8.

68. Vincent A.T., M.V Trudel, V.E. Paquet, *et al.* 2014. Detection of variants of the pRAS3, pAB5S9, and pSN254 plasmids in *Aeromonas salmonicida* subsp. *salmonicida*: multidrug-resistance, interspecies exchanges, and plasmid reshaping. *Antimicrob. Agents Chemother.* **58**: 7367–7374.

69. Trudel M.V., A.T. Vincent, S.A. Attéré, *et al.* 2016. Diversity of antibiotic-resistance genes in Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida*: dominance of pSN254b and discovery of pAsa8. *Sci. Rep.* **6**: 35617.

70. Call D.R., R.S. Singer, D. Meng, *et al.* 2010. blaCMY-2-positive IncA/C plasmids from *Escherichia coli* and *Salmonella enterica* are a distinct component of a larger lineage of plasmids. *Antimicrob. Agents Chemother.* **54**: 590–596.

71. Fricke W.F., T.J. Welch, P.F. McDermott, *et al.* 2009. Comparative genomics of the IncA/C multidrug resistance plasmid family. *J. Bacteriol.* **191**: 4750–4757.

72. Fernández-Alarcón C., R.S. Singer & T.J. Johnson. 2011. Comparative genomics of multidrug resistance-encoding IncA/C plasmids from commensal and pathogenic *Escherichia coli* from multiple animal sources. *PLoS One* **6**: e23415.

73. Beaber J.W., B. Hochhut & M.K. Waldor. 2002. Genomic and functional analyses of SXT, an integrating antibiotic resistance gene transfer element derived from *Vibrio cholerae*. *J. Bacteriol.* **184**: 4259–4269.

74. Loftie-Eaton W. & D.E. Rawlings. 2009. Comparative biology of two natural variants of the IncQ-2 family plasmids, pRAS3.1 and pRAS3.2. *J. Bacteriol.* **191**: 6436–6446.

75. Loftie-Eaton W. & D.E. Rawlings. 2010. Evolutionary competitiveness of two natural variants of the IncQ-like plasmids, pRAS3.1 and pRAS3.2. *J. Bacteriol.* **192**: 6182–6190.

76. Massicotte M.A., A.T. Vincent, A. Schneider, *et al.* 2019. One *Aeromonas salmonicida* subsp. *salmonicida* isolate with a pAsa5 variant bearing antibiotic resistance and a pRAS3 variant making a link with a swine pathogen. *Sci. Total Environ.* **690**: 313–320.

77. De Puysseleyr L., K. De Puysseleyr, L. Braeckman, *et al.* 2017. Assessment of *Chlamydia suis* infection in pig farmers. *Transbound. Emerg. Dis.* **64**: 826–833.

78. Mulvey M.R., D.A. Boyd, A.B. Olson, *et al.* 2006. The genetics of *Salmonella* genomic island 1. *Microbes Infect.* **8**: 1915–1922.

79. Boyd D.A., G.A. Peters, L.K. Ng, *et al.* 2000. Partial characterization of a genomic island associated with the multidrug resistance region of *Salmonella enterica* Typhymurium DT104. *FEMS Microbiol. Lett.* **189**: 285–291.

80. Ahmed A.M., A.I.A. Hussein & T. Shimamoto. 2007. *Proteus mirabilis* clinical isolate harbouring a new variant of *Salmonella* genomic island 1 containing the multiple antibiotic resistance region. *J. Antimicrob. Chemother.* **59**: 184–190.

81. Schmitt R., E. Bernhard & R. Mattes. 1979. Characterisation of Tn*1721*, a new transposon containing tetracycline resistance genes capable of amplification. *MGG Mol. Gen. Genet.* **172**: 53–65.

82. Attéré S.A., A.T. Vincent, M. Paccaud, *et al.* 2017. The role for the small cryptic plasmids as moldable vectors for genetic innovation in *Aeromonas salmonicida* subsp. *salmonicida*. *Front. Genet.* **8**: 211.

83. Chen L., D. Zheng, B. Liu, *et al.* 2016. VFDB 2016: hierarchical and refined dataset for

786 big data analysis-10 years on. *Nucleic Acids Res.* **44**: D694-7.
787 84. Wu H.J., A.H.J. Wang & M.P. Jennings. 2008. Discovery of virulence factors of
788 pathogenic bacteria. *Curr. Opin. Chem. Biol.* **12**: 93–101.
789 85. Costa T.R.D., C. Felisberto-Rodrigues, A. Meir, *et al.* 2015. Secretion systems in Gram-
790 negative bacteria: structural and mechanistic insights. *Nat. Rev. Microbiol.* **13**: 343–359.
791 86. Tomás J.M. 2012. The main *Aeromonas* pathogenic factors. *ISRN Microbiol.* **2012**: 1–22.
792 87. Fernández-Bravo A. & M.J. Figueras. 2020. An update on the genus *Aeromonas*:
793 Taxonomy, epidemiology, and pathogenicity. *Microorganisms* **8**: 129.
794 88. Vanden Bergh P. & J. Frey. 2014. *Aeromonas salmonicida* subsp. *salmonicida* in the light
795 of its type-three secretion system. *Microb. Biotechnol.* **7**: 381–400.
796 89. Stuber K., S.E. Burr, M. Braun, *et al.* 2003. Type III secretion genes in *Aeromonas*
797 *salmonicida* subsp. *salmonicida* are located on a large thermolabile virulence plasmid. *J.*
798 *Clin. Microbiol.* **41**: 3854–3856.
799 90. Dallaire-Dufresne S., X. Barbeau, D. Sarty, *et al.* 2013. *Aeromonas salmonicida* Ati2 is an
800 effector protein of the type three secretion system. *Microbiology* **159**: 1937–1945.
801 91. Burr S.E., K. Stuber & J. Frey. 2003. The ADP-ribosylating toxin, AexT, from *Aeromonas*
802 *salmonicida* subsp. *salmonicida* is translocated via a type III secretion pathway. *J.*
803 *Bacteriol.* **185**: 6583–6591.
804 92. Fehr D., C. Casanova, A. Liverman, *et al.* 2006. AopP, a type III effector protein of
805 *Aeromonas salmonicida*, inhibits the NF-kappaB signalling pathway. *Microbiology* **152**:
806 2809–2818.
807 93. Dacanay A., L. Knickle, K.S. Solanky, *et al.* 2006. Contribution of the type III secretion
808 system (TTSS) to virulence of *Aeromonas salmonicida* subsp. *salmonicida*. *Microbiology*
809 **152**: 1847–1856.

94. Daher R.K., G. Filion, S.G.E. Tan, *et al.* 2011. Alteration of virulence factors and rearrangement of pAsa5 plasmid caused by the growth of *Aeromonas salmonicida* in stressful conditions. *Vet. Microbiol.* **152**: 353–60.

95. Tanaka K.H., S. Dallaire-Dufresne, R.K. Daher, *et al.* 2012. An insertion sequence-dependent plasmid rearrangement in *Aeromonas salmonicida* causes the loss of the type three secretion system. *PLoS One* **7**: e33725.

96. Emond-Rheault J.-G., A.T. Vincent, M.V Trudel, *et al.* 2015. AsaGEI2b: a new variant of a genomic island identified in the *Aeromonas salmonicida* subsp. *salmonicida* JF3224 strain isolated from a wild fish in Switzerland. *FEMS Microbiol. Lett.* **362**: fnv093.

97. Vincent A.T., K.H. Tanaka, M.V Trudel, *et al.* 2015. Draft genome sequences of two *Aeromonas salmonicida* subsp. *salmonicida* isolates harboring plasmids conferring antibiotic resistance. *FEMS Microbiol. Lett.* **362**: fnv002.

98. Tanaka K.H., A.T. Vincent, J.-G. Emond-Rheault, *et al.* 2017. Plasmid composition in *Aeromonas salmonicida* subsp. *salmonicida* 01-B526 unravels unsuspected type three secretion system loss patterns. *BMC Genomics* **18**: 528.

99. Emond-Rheault J.-G., A.T. Vincent, M.V Trudel, *et al.* 2015. Variants of a genomic island in *Aeromonas salmonicida* subsp. *salmonicida* link isolates with their geographical origins. *Vet. Microbiol.* **175**: 68–76.

100. Boyd J., J. Williams, B. Curtis, *et al.* 2003. Three small, cryptic plasmids from *Aeromonas salmonicida* subsp. *salmonicida* A449. *Plasmid* **50**: 131–144.

101. Najimi M., M. Balado, M.L. Lemos, *et al.* 2009. Genetic characterization of pAsa6, a new plasmid from *Aeromonas salmonicida* subsp. *salmonicida* that encodes a type III effector protein AopH homolog. *Plasmid* **61**: 176–181.

102. Najimi M., M.L. Lemos & C.R. Osorio. 2008. Distribution of small plasmids in

Aeromonas salmonicida subsp. *salmonicida* strains isolated from NW Spain and Portugal: evidence of clonality in strains isolated from turbot, *Psetta maxima* (L.). *J. Fish Dis.* **31**: 469–472.

103. Trudel M.V, K.H. Tanaka, G. Filion, *et al.* 2013. Insertion sequence *AS5* (IS*AS5*) is involved in the genomic plasticity of *Aeromonas salmonicida*. *Mob. Genet. Elements* **5**: 1–7.

104. Attéré S.A., A.T. Vincent, M.V. Trudel, *et al.* 2015. Diversity and homogeneity among small plasmids of *Aeromonas salmonicida* subsp. *salmonicida* linked with geographical origin. *Front. Microbiol.* **6**:1274.

105. Toranzo A.E., J.L. Barja, R.R. Colwell, *et al.* 1983. Characterization of plasmids in bacterial fish pathogens. *Infect. Immun.* **39**: 184–192.

106. Long M., T.K. Nielsen, J.J. Leisner, *et al.* 2016. *Aeromonas salmonicida* subsp. *salmonicida* strains isolated from Chinese freshwater fish contain a novel genomic island and possible regional-specific mobile genetic elements profiles. *FEMS Microbiol. Lett.* **363**: fnw190.

107. Han J.E., J.H. Kim, J.H. Choresca, *et al.* 2012. First description of ColE-type plasmid in *Aeromonas* spp. carrying quinolone resistance (*qnrS2*) gene. *Lett. Appl. Microbiol.* **55**: 290–294.

108. Casjens S.R., E.F. Mongodin, W.G. Qiu, *et al.* 2012. Genome stability of lyme disease spirochetes: Comparative genomics of *borrelia burgdorferi* plasmids. *PLoS One* **7**: e33280.

109. Casjens S.R., E.B. Gilcrease, M. Vujadinovic, *et al.* 2017. Plasmid diversity and phylogenetic consistency in the Lyme disease agent *Borrelia burgdorferi*. *BMC Genomics* **18**: 165.

110. Hänninen M.L., J. Ridell & V. Hirvelä-Koski. 1995. Phenotypic and molecular characteristics of *Aeromonas salmonicida* subsp. *salmonicida* isolated in southern and northern Finland. *J. Appl. Bacteriol.* **79**: 12–21.

111. Giles J.S., H. Hariharan & S.B. Heaney. 1995. The plasmid profiles of fish pathogenic isolates of *Aeromonas salmonicida*, *Vibrio anguillarum*, and *Vibrio ordalii* from the Atlantic and Pacific coasts of Canada. *Can. J. Microbiol.* **41**: 209–216.

112. Livesley M.A., S.N. Smith, R.A. Armstrong, *et al.* 1997. Analysis of plasmid profiles of *Aeromonas salmonicida* isolates by pulsed field gel electrophoresis. *FEMS Microbiol. Lett.* **146**: 297–301.

113. Contreras-Moreira B. & P. Vinuesa. 2013. GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. *Appl. Environ. Microbiol.* **79**: 7696–7701.

114. Team R.C. 2013. R: A Language and Environment for Statistical Computing. *R Found. Stat. Comput. Vienna, Austria*.

115. Suzuki R. & H. Shimodaira. 2006. Pvclust: An R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* **22**: 1540–1542.