

Insight into the genetic diversity and population structure of *Flavobacterium psychrophilum* in France

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Abstract

Flavobacterium psychrophilum is the causative agent of bacterial cold water disease and rainbow trout fry syndrome in salmonid farming worldwide. These diseases are among the main hazards for French aquaculture [1]. In this study, a multilocus sequence typing (MLST) approach described previously [2] was used to evaluate the genetic diversity of this bacterium, and gain insight into its population structure.

MLST analysis concerned a set of 66 isolates, recently collected from rainbow trout during clinical episodes in French farms located in the main geographical areas of production. Sequences of 7 housekeeping genes (*trpB*, *gyrB*, *dnaK*, *tuf*, *fumC*, *murG* and *atpA*) were investigated and a total of 5808 bp of the sequence were analyzed for each isolate. The analysis showed 81 SNPs (1.4% of total length) and low levels of both gene ($H = 0.4313$) and nucleotide diversity ($\pi \times 100 = 0.31\%$). MLST identified numerous allele types (ATs) for each locus and sequence types (STs) resulting from the combination of these ATs (Tab. 1). A total of 15 STs were retrieved, 14 of which have never been described.

eBURST analysis was used to infer evolutionary pattern between STs and to discriminate the 15 STs in one clonal complex of 4 genetically related STs and 11 singletons, as presented in Fig. 1. ST2 and the clonal complex (as ST2 is its primary founder) were previously described [2].

Phenomena responsible for genetic diversity have been sought and we found that the diversity was largely due to recombination, as demonstrated by a pairwise homoplasy index (PHI) significantly different from zero ($p < 0.05$). Standardized association index (I_A^S) that investigates the random association of genes in a population was also used to infer recombination among the *F. psychrophilum* isolates. For the entire sample, I_A^S was 0.6088 ($p < 0.05$) and for the set of one single representative of each ST, I_A^S was 0.4761. Those two values showed statistically significant linkage disequilibrium ($p < 0.05$), indicating a possible clonal structure of the population. When we divided all STs in clusters of closely related ones for a phylogenetic analysis [3], the linkage disequilibrium disappeared, indicating an epidemic type of the population structure.

Table 1. ATs, STs (and their frequencies) retrieved from 66 French isolates sampled during outbreaks. New ATs and STs are highlighted.

ATs							ST	Frequency
<i>trpB</i>	<i>gyrB</i>	<i>dnak</i>	<i>fumC</i>	<i>murG</i>	<i>tuf</i>	<i>atpA</i>		
2	2	2	2	2	2	2	2	47%
1	1	1	1	1	2	1	<u>90</u>	15%
2	2	2	2	2	<u>47</u>	2	<u>89</u>	13%
3	2	2	2	2	<u>41</u>	2	<u>92</u>	7,50%
2	2	2	2	2	<u>41</u>	2	<u>91</u>	1,50%
4	2	2	2	2	2	2	<u>93</u>	1,50%
4	2	2	2	2	<u>41</u>	2	<u>95</u>	1,50%
<u>29</u>	2	2	2	2	2	2	<u>97</u>	1,50%
2	2	2	2	2	<u>48</u>	2	<u>98</u>	1,50%
4	<u>42</u>	<u>21</u>	5	2	<u>39</u>	<u>40</u>	<u>99</u>	1,50%
2	<u>44</u>	<u>16</u>	3	3	3	3	<u>102</u>	1,50%
1	<u>49</u>	10	7	<u>20</u>	<u>25</u>	<u>25</u>	<u>104</u>	1,50%
<u>21</u>	3	<u>22</u>	3	3	2	2	<u>105</u>	1,50%
1	1	1	2	1	<u>41</u>	1	<u>108</u>	1,50%
4	<u>29</u>	<u>24</u>	5	6	<u>49</u>	<u>40</u>	<u>113</u>	1,50%

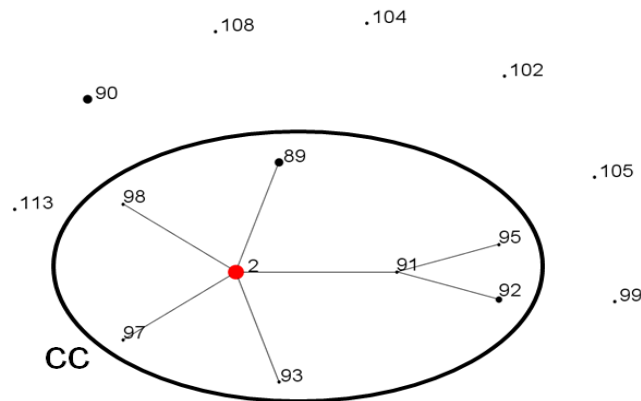


Figure 1. e-BURST analysis of STs from French isolates. Dot sizes representing STs are proportional to their occurrence frequencies. The clonal complex (CC) is circled and its primary founder is indicated by a red dot.

A neighbor-joining tree (Fig. 2) was generated from concatenated loci sequences for each isolate. This tree has internal branches with relatively high bootstrap values (50 to 100%), indicating the value of the tree in terms of genealogy of sequences. Proximity of *F. psychrophilum* isolates whose STs were grouped within the clonal complex by e-BURST analysis can be observed.

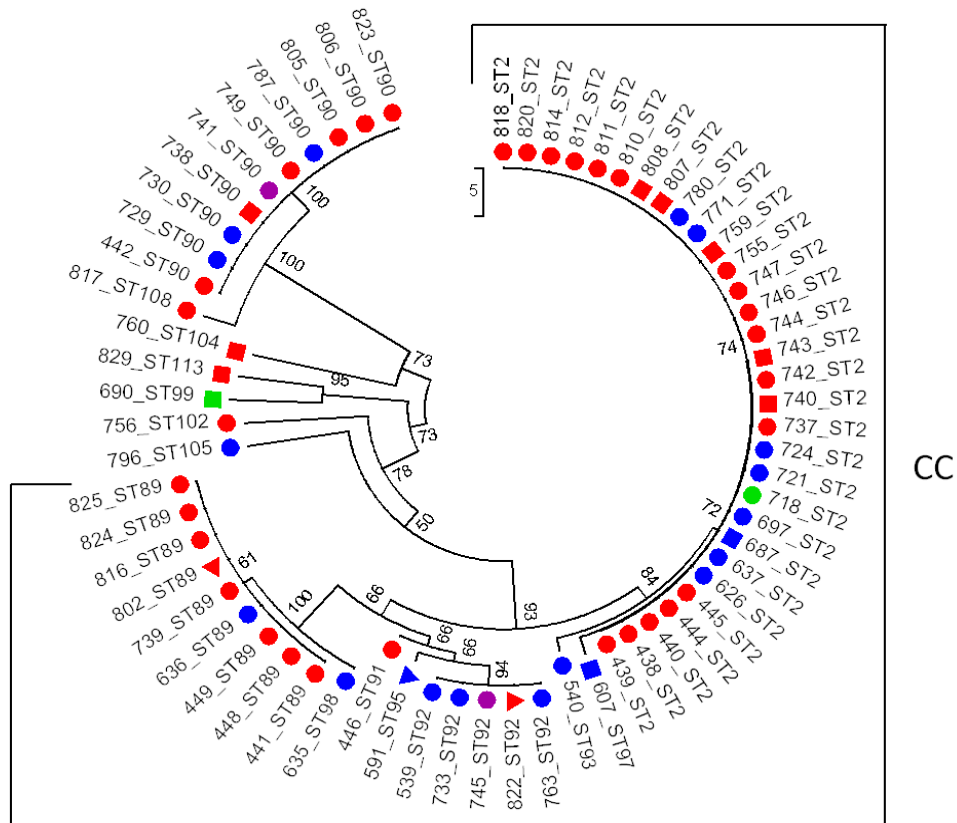


Figure 2. Neighbor-joining unrooted tree of 66 French *F. psychrophilum* isolates, including *F. psychrophilum* reference strain JIP 02/86 and NCIMB 1947^T. For each isolate, the ST identity, the French region origin encoded by color (Aquitaine, Bretagne, Poitou Charente and Pays de la Loire) and the tissue location represented by different forms (● for internal, ■ for external and ▲ for unknown tissue) are indicated. The evolutionary distances were computed using the number of differences method and are in the units of the number of base substitutions per site.

This tree shows also that very diverse STs can coexist in a same region as the same ST can be found in diverse location. Because the Fisher exact test pointed the absence of a geographic structure ($p < 0.05$), this observation was statistically confirmed for ST2, the most represented ST.

These results emphasized the expansion of a limited number of dominant genetic variants in French clinical *F. psychrophilum* isolates from a single host species (rainbow trout), with no geographic relationship. This suggests a likely role of brood fish, egg and fish trading in the dissemination of *F. psychrophilum*, at least for some specific STs. Moreover, ST2 seems to be strongly associated to rainbow trout as it has only been found in this fish species. However, many singletons STs have also been characterized in rainbow trout both worldwide (8 in the study of Nicolas et al. [2]) and in France (7 in the present study). One explanatory hypothesis for the presence of these singletons STs might be that these STs are not linked to the trading of salmonid products but are related to endemic isolates. Those endemic isolates might occur rarely and/or could be less virulent than the others or less well adapted to the rainbow trout species.

Additional analyses are now required to complete our knowledge of the genetic variants present at an even smaller geographical scale (a farm for example), to examine if singleton STs represent local and environmentally-associated types with a local clonal structure. Looking at possible links between particular STs and internal location on fish (potentially virulent) would constitute an interesting indication for vaccine research in the future.

References

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