

# Genome sequence of *Pseudoalteromonas* sp. D41, a pioneer bacterium in marine biofouling.

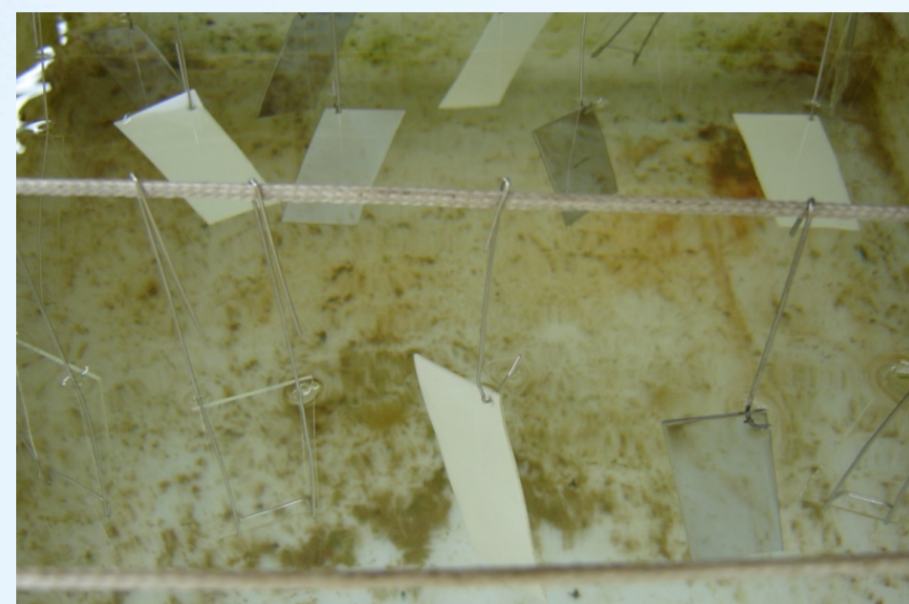


Bienvenu B.<sup>1,2</sup>, Goudenège D.<sup>2</sup>, Le Roux F.<sup>2</sup>, Quintric L.<sup>3</sup>, Compère C.<sup>1</sup>, Dreanno C.<sup>1</sup>

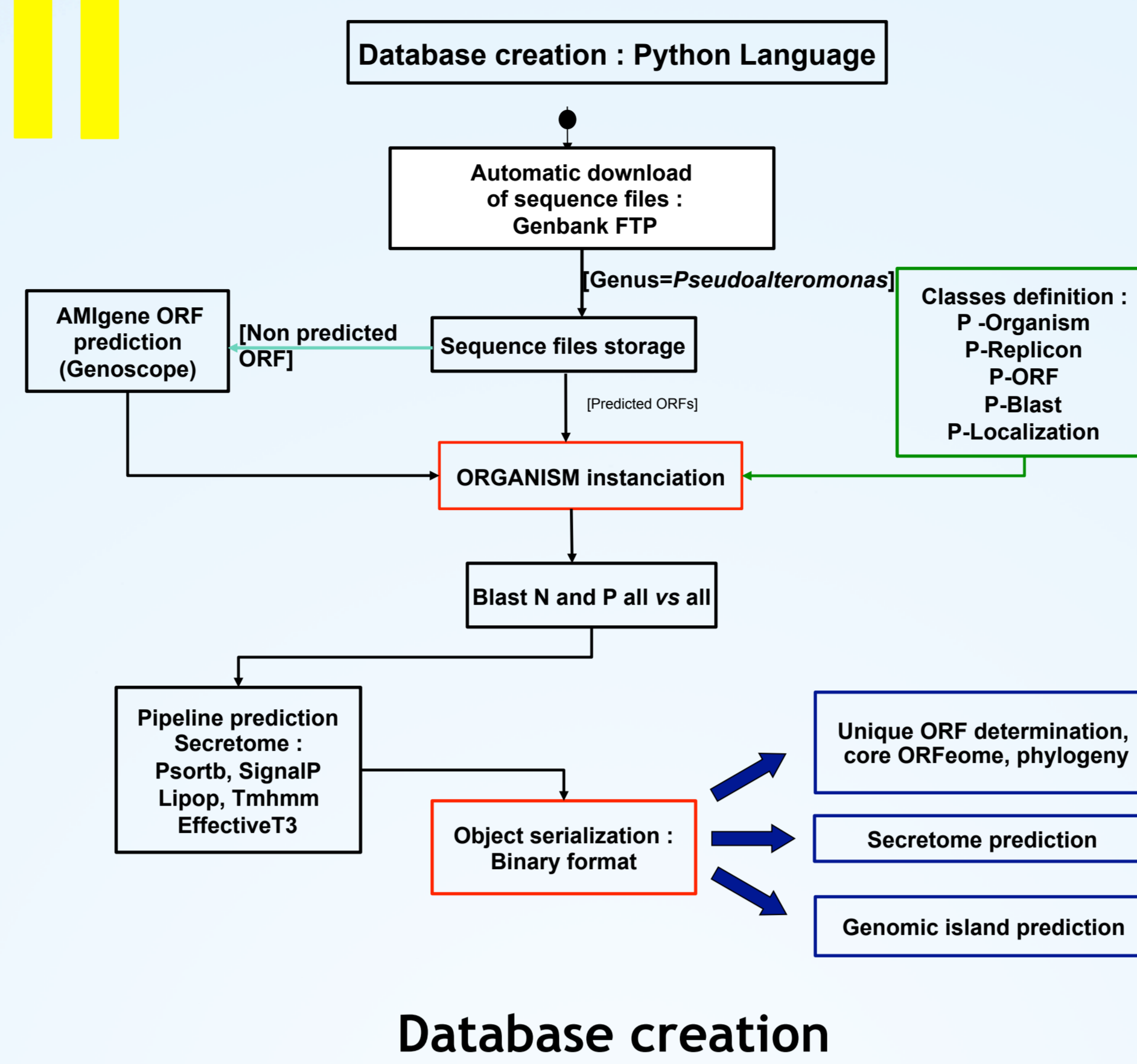
<sup>1</sup>IFREMER, RDT/LDCM, France, <sup>2</sup>IFREMER-UPMC, Vibrio Genomics, Station Biologique de Roscoff, France, <sup>3</sup>IFREMER, RIC, France

## Introduction

Surface colonization by microorganisms is one of the first steps in marine fouling. A long term biofilm formation study in a marine environment allowed the isolation of several species from stainless, steel, and glass slides immersed in natural seawater in the bay of Brest, France. A novel pioneer species, *Pseudoalteromonas* sp. D41, was selected for its high adhesion capacity on any type of material and its ability to inhibit biofilm formation of competitive bacteria. In order to investigate the singularities of this new species its genome was sequenced and compare to those of other *Pseudoalteromonas* species.



## Materials and Methods



The *P. sp. D41* strain was isolated from a Teflon slide immersed in coastal water for 24h. Genome sequence determination used a hybrid strategy (454 pyrosequencing and illumina) with 20X coverage.

**Database creation:** database scripts manage the sequence download from Genbank, ORF prediction, instantiation and serialization of the organism objects.

**Software execution:** the software scripts run the programs to assign organism attributes, perform automatically blast analyses and run several softwares to predict ORF and protein localization.

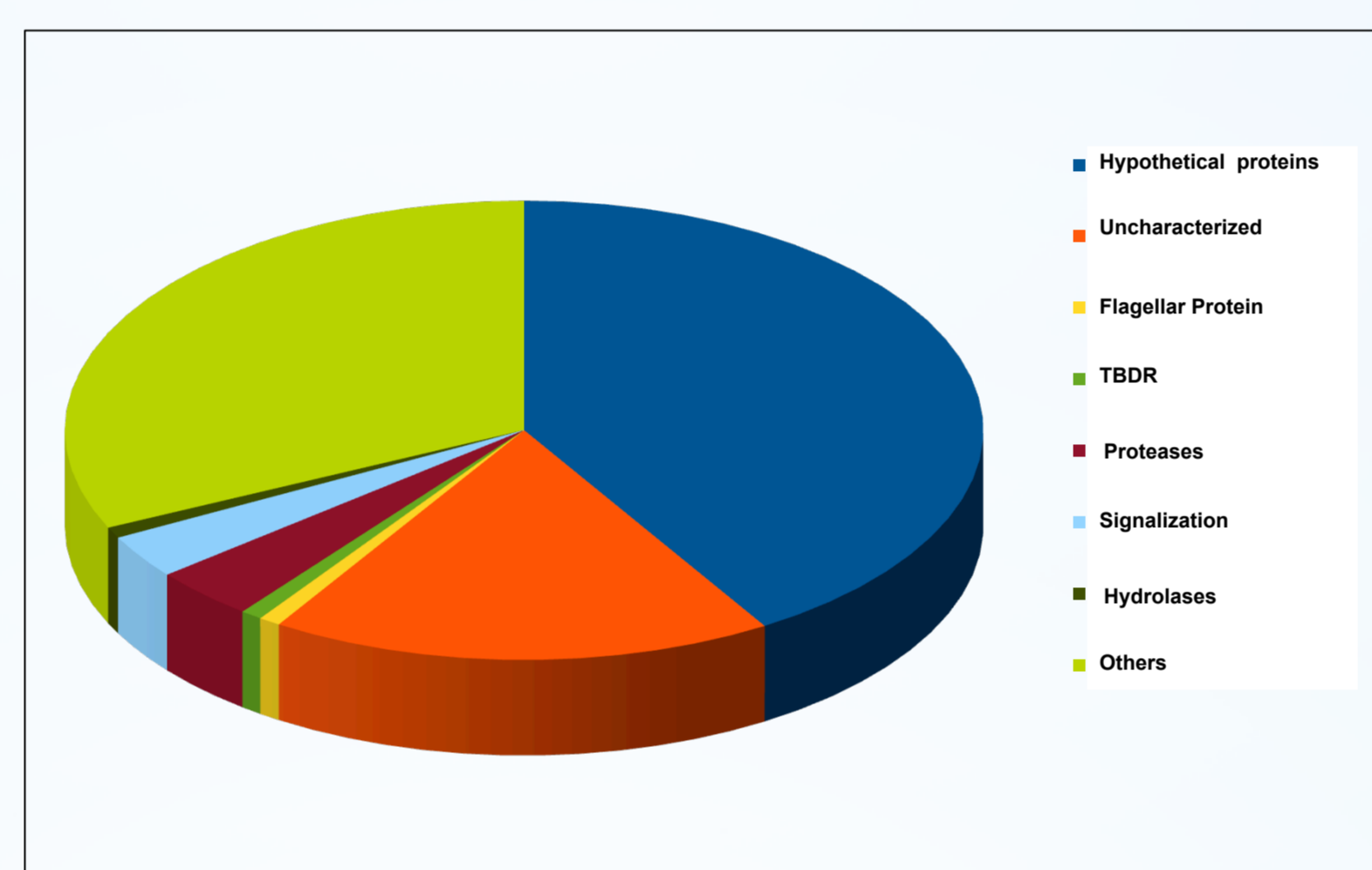
**Analysis scripts:** The analysis scripts use all the information collected to compare the organisms.

## Results

### General features of *Pseudoalteromonas* sp. D41

Organisms	size (pb)	GC (%)	Numbers of ORFs	Average size of ORFs (bp)	Percentage coding	Unique ORFs	Secreted ORFs	Unique & secreted ORFs
<i>A. bacterium</i> TW 7	4104952	39.66	3723	969	89.51	337	540	34
<i>P. arctica</i> A.37.1.2	4628018	39.03	4143	954	88.17	391	596	45
<i>P. atlantica</i> T6c	5187005	44.61	4281	1046	86.39	1271	683	258
<i>P. Bsi20311</i>	3979836	40.33	3684	938	86.06	267	486	31
<i>P. BSi20429</i>	4495777	39.04	4033	972	87.23	335	605	40
<i>P. BSi20439</i>	3882800	40.22	3615	930	86.61	299	488	26
<i>P. BSi20480</i>	4149214	39.6	3971	919	87.98	550	543	53
<i>P. BSi20496</i>	4826524	39.92	4368	952	86.23	623	619	72
<i>P. BSi20652</i>	4253936	38.85	4096	885	85.02	759	549	73
<i>P. citrea</i> NCIMB 1889	5337619	41.12	4483	1051	88.28	763	628	125
<i>P. flavipulchra</i> 2ta6	5479946	42.72	5034	960	88.27	1032	659	107
<i>P. haloplanktis</i> ANT 505	4494717	39.32	4129	942	86.58	494	592	49
<i>P. haloplanktis</i> ATCC 14393	5021465	40.84	4347	1028	89.04	522	591	69
<i>P. haloplanktis</i> TAC125	3850272	40.09	3486	963	87.27	376	434	36
<i>P. luteoviolacea</i> 2ta16	4384454	42.28	4752	950	70.97	1225	700	213
<i>P. marina</i> mano4	4172200	39.64	3756	996	89.6	235	528	22
<i>P. piscicida</i> JCM 20779	5281621	43.23	4999	1022	89	577	648	69
<i>P. rubra</i> ATCC 29570	5969931	47.79	4737	1077	85.48	1149	742	173
<i>P. SM9913</i>	4037671	40.27	3712	952	87.53	304	502	17
<b><i>P. sp. D41</i></b>	<b>4518314</b>	<b>41.67</b>	<b>3865</b>	<b>993</b>	<b>84.94</b>	<b>519</b>	<b>490</b>	<b>66</b>
<i>P. sp. Tw-2</i>	3434825	39.43	3216	950	89.01	828	441	130
<i>P. spongiae</i> UST10123 006	4724454	40.8	4242	1001	89.17	811	876	130
<i>P. tuniata</i> D2	4982425	39.85	4507	973	85.05	1037	564	129
<i>P. undina</i> NCIMB 2128	4001234	39.94	3623	992	89.66	234	513	27
<i>S. baltica</i> OS155	5342896	46.22	4489	992	83.35	1675	623	262

### Specific ORFs of *Pseudoalteromonas* sp. D41



ORFs comparison was done with Blast-P. Two ORFs are considered similar if their relative identity percentage exceeds 30% and the coverage percentage is above 80%.

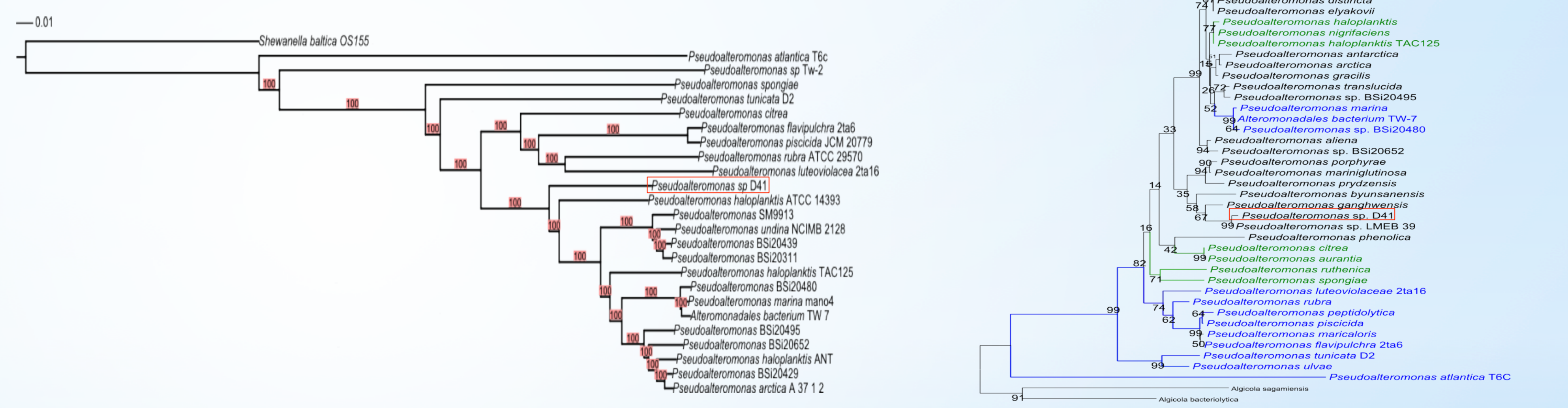
- 13.5% (519) of all ORFs were found displaying similarities

- Biofilm regulation proteins:**
- 4 TonB-dependent receptor proteins [3]
  - 24 peptidases found using blast-P and MEROPS database
  - 18 sensor proteins

### ANI values among *Pseudoalteromonas* species

To quantify intraspecies variation at the genomic level, average nucleotide identity (ANI) [1] was calculated. It detects the DNA conservation of the core genome. *P. sp. D41* does not show a particularly high ANI value (>85%) with the other strains.

### Phylogenomic and phylogenetic trees

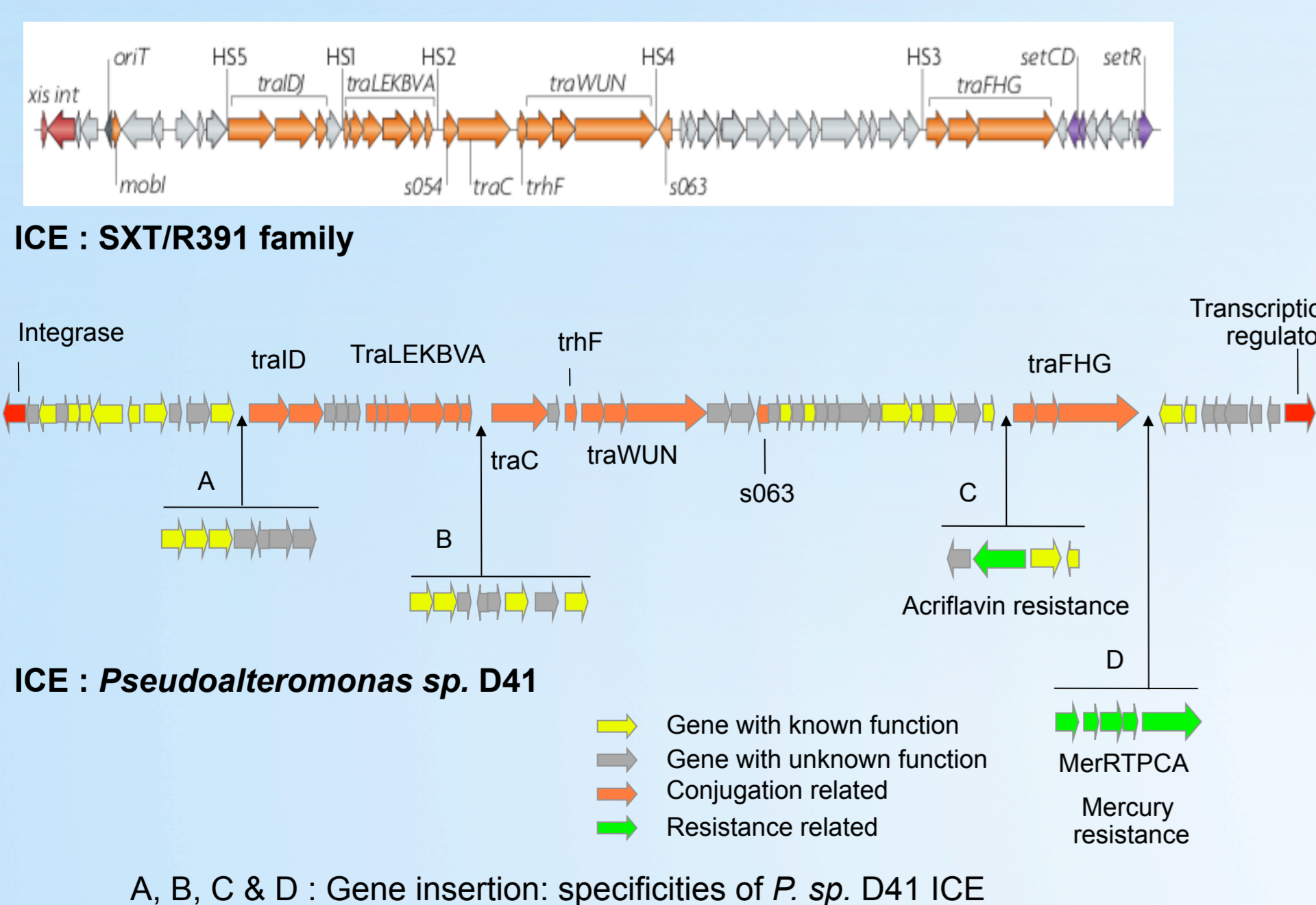


A phylogenomic tree based on core ORFeome was constructed with 578 concatenated protein sequences from *P. sp. D41* and related strains with sequenced genomes (25). The tree was generated with the neighbor-Joining algorithm (1000 bootstraps)

Consensus phylogenetic tree based on 16S rRNA gene sequences established using the neighbour-joining method (1000 Bootstraps). *Shewanella baltica* is used as outgroup.

The topology of phylogenomic tree shows few differences with the 16S rRNA tree, but it is supported by higher bootstrap values

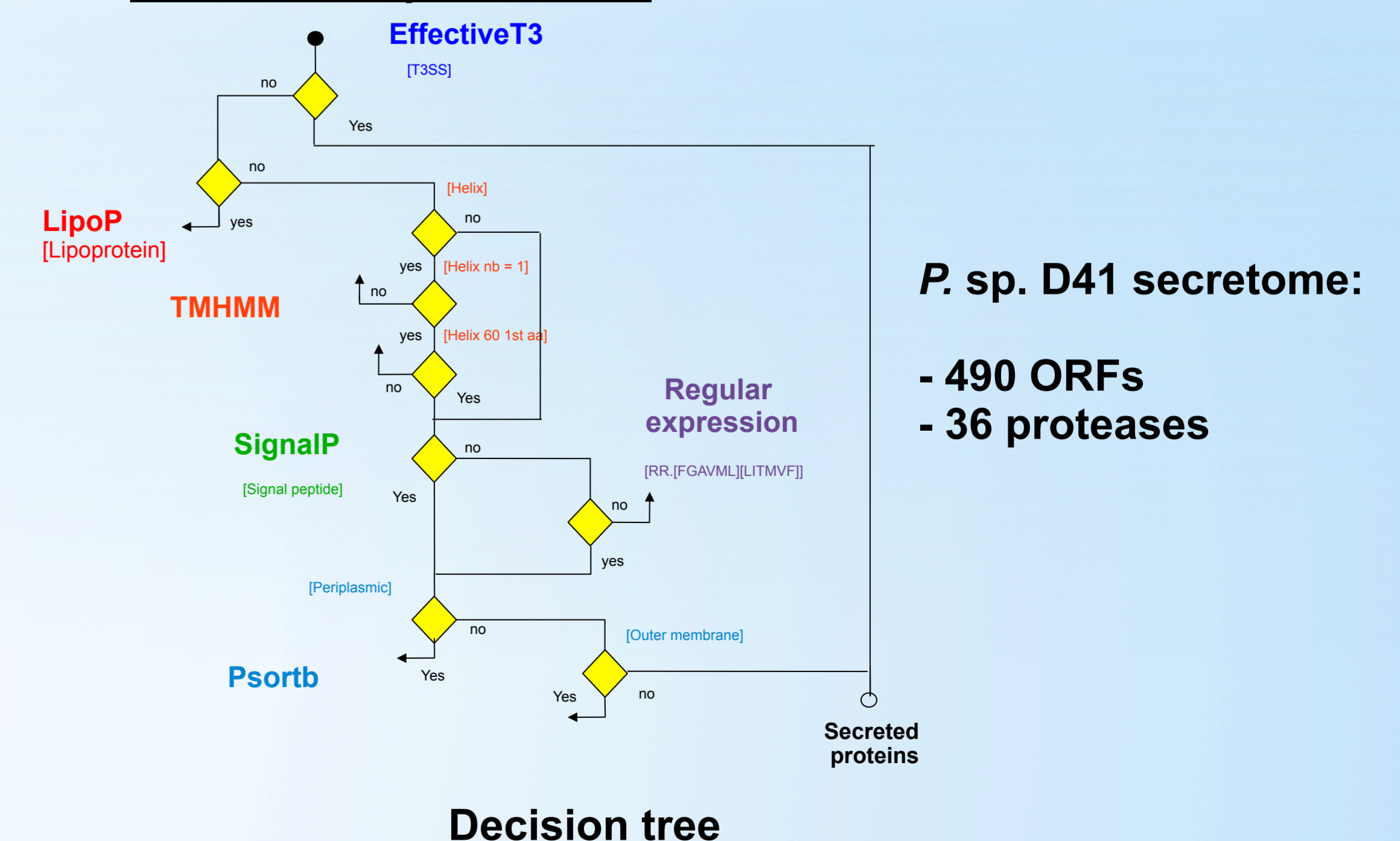
### Genomic islands



5 putative genomic islands were detected in *P. sp. D41*. An integrative and conjugative mobile genetic element belonging to the SXT/R391 ICE family was identified [2]. A similar ICE family member was found in *P. flavipulchra* 2ta6.

The *P. sp. D41* ICE carries genes conferring resistance to acriflavin and to mercury.

### Secretome prediction



***P. sp. D41* secretome:**

- 490 ORFs
- 36 proteases

## Conclusions

A bioinformatics process were successfully set up to compare and analyze automatically the different *Pseudoalteromonas* genomes. This pipeline and scripts can be used for any genome comparison study. This comparative study has enlightened many specific genomic features of this new species i.e. *P. sp. D41*. Characterization of predicted secreted proteases are currently carried out to understand their function in biofilm regulation.

## References

[1]. Konstantinidis, K. T. & Tiedje, J. M. (2005). Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci U S A 102, 2567–2572  
 [2]. Wozniak R.A.F., Waldor M.K. (2010). Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nature Reviews Microbiology 8, 552-563