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Investigative genomics on highly antibiotic resistant *Pseudomonas* isolated from rainbow trout (*Oncorhynchus mykiss*)

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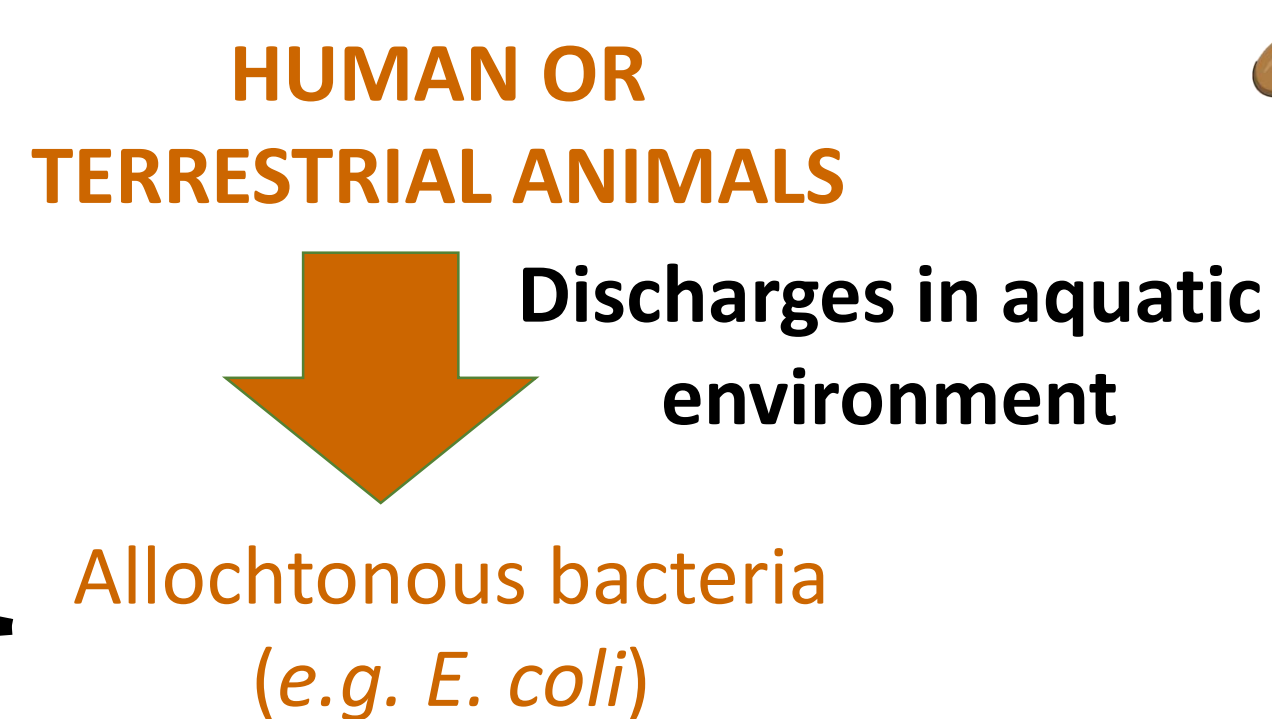
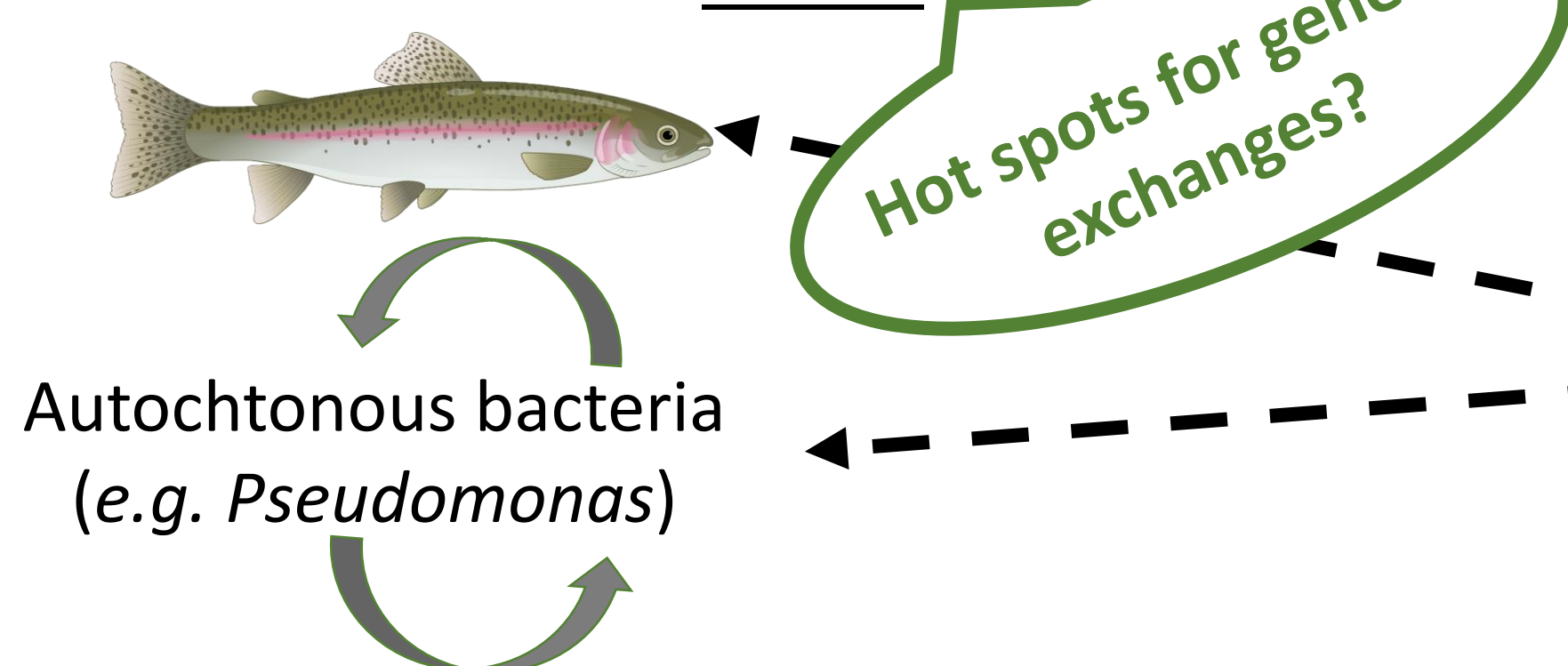
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CONTEXT AND STARTING POINT OF THIS STUDY

OPEN AQUACULTURE SYSTEMS AS RAINBOW TROUT FARMS



Minimum Inhibitory Concentrations ($\mu\text{g}\cdot\text{mL}^{-1}$) of 8 *Pseudomonas* isolates to oxytetracycline (OTC), Florfenicol (FFC), Trimethoprim/Sulfamethoxazole (TMP/SXT), colistin (COL) and ceftazidim (CAZ)

ISOLATE ID	OTC	FFC	TMP/SXT	COL	CAZ
A03RPs3-08	256	> 1,024	> 512/9,728	2	32
A12RPs2-30	64	> 1,024	256/4,864	2	8
A14RPs3-37	256	> 1,024	> 512/9,728	2	16
B16FPs1-120	4	128	1/19	16	16
B19FPs3-125	2	64	4/76	> 1,024	< 4
B21FPs3-128	64	512	128/2,432	2	8
B22FPs1-129	2	64	2/38	512	8
B26FPs3-140	64	512	128/2,432	2	8

51 *Pseudomonas* previously isolated from rainbow trout fillets directly sampled in two farms or after transformation process

Assessment of their Minimum Inhibitory Concentrations to 8 antibiotics

8 out of 51 *Pseudomonas* hosted by fillets showed resistance or non wild-type phenotypes to several antibiotics. Do you want more information? Check the table!

Non Wild-Type according to calculated ECOFF

Resistance according to clinical breakpoints (CLSI 2018)

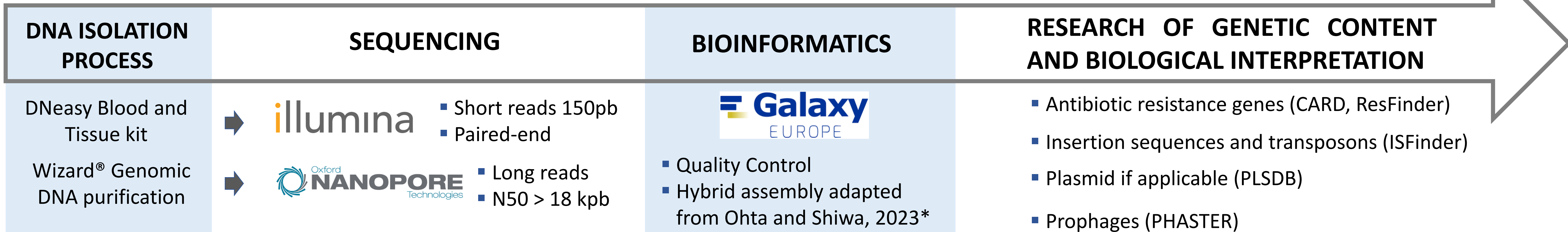
OBJECTIVES OF THIS STUDY

- Studying the relationship between *Pseudomonas* resistance phenotypes and their genetic content using whole genome sequencing
- Identify Mobile Genetic Elements (MGEs) and their carriage of antibiotic resistance genes

More details on our previous work?



MATERIAL AND METHODS



MAIN RESULTS

Genome sizes of *Pseudomonas* isolates

ISOLATE ID	SIZE AFTER ASSEMBLY (Mbp)
A03RPs3-08	6.3
A12RPs2-30	6.0
A14RPs3-37	6.4
B16FPs1-120	6.3
B19FPs3-125	6.4
B21FPs3-128	6.0
B22FPs1-129	6.8
B26FPs3-140	6.1
<i>P. fluorescens</i> *	6.7

* As reference strain SBW-25

- Genome sizes of the different strains that we sequenced ranged between 6.0 to 6.8 Mpb
- All isolates belonged to the *P. fluorescens* species according to the pubMLST typing scheme (*glnS*, *gyrB*, *ileS*, *nuoD*, *recA*, *rpoB* and *rpoD* genes)

In-depth analyses for studying the relationship between phenotypes and genotypes

ISOLATE ID	OTC	FFC	TMP/SXT	COL	CAZ
A03RPs3-08	<i>tetY</i> gene	<i>florR</i> gene	<i>sul2</i> gene	-	?
A12RPs2-30	<i>tetY</i> gene	<i>florR</i> gene	<i>sul2</i> gene	-	-
A14RPs3-37	<i>tetY</i> gene	<i>florR</i> gene	<i>sul2</i> gene	-	-
B16FPs1-120	-	-	-	?	-
B19FPs3-125	-	-	?	?	-
B21FPs3-128	<i>tetY</i> gene	<i>florR</i> gene	<i>sul2</i> gene	-	-
B22FPs1-129	-	-	-	?	-
B26FPs3-140	<i>tetY</i> gene	<i>florR</i> gene	<i>sul2</i> gene	-	-

Presence of resistance gene which can explain phenotype (Green) Phenotype not explained by a specific resistance gene (Yellow)

- Most of phenotypes (resistance or non wild-type) were explained by the presence of resistance genes (*tetY*, *florR* or *sul2*)
- Isolates with high MIC values to colistin did not carry *mcr* genes
- Additional resistance genes were identified for non tested antibiotics : quinolones (*AbaQ* efflux pump) or aminoglycosides (*aph(6)-Id*; *aph(3')-Ib*)

Mobile Genetic Elements harbored by the *Pseudomonas* isolates

- No plasmid found but *tra* genes inserted within some chromosomes: ICE?
- Several transposons or insertion sequences (Tn3 or Tn5 families) identified in all genomes
- Prophage sequences already found in other bacterial species were detected in several regions of each genome (intact or incomplete matches)

FIRST CONCLUSIONS & PROSPECTS

- Part of phenotypes can be explained by the presence of specific resistance genes (*tetY* for oxytetracycline, *sul2* for sulfamethoxazole and *florR* for florfenicol) = common ancestor?
- Further investigations of all genomes are ongoing to figure out unexplained phenotypes such as colistin = LPS?
- A deeper analyses of mobile genetic elements carrying antibiotic resistance genes must be pursued to determine the accurate genetic architecture of the 8 isolates and ability to spread towards opportunistic pathogenic bacteria of fishes or even Humans

Conjugation events? Transposition? Other HGT?

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REFERENCE

*Ohta, T., Shiwa, Y. (2023). Hybrid Genome Assembly of Short and Long Reads in Galaxy. In: Arakawa, K. (eds) Nanopore Sequencing. Methods in Molecular Biology, vol 2632. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-2996-3_2

Further discussion? Partnership?
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