Diffusion of Tn21 and Tn21-like transposons in environmental strains of *Aeromonas spp.*: preliminary results

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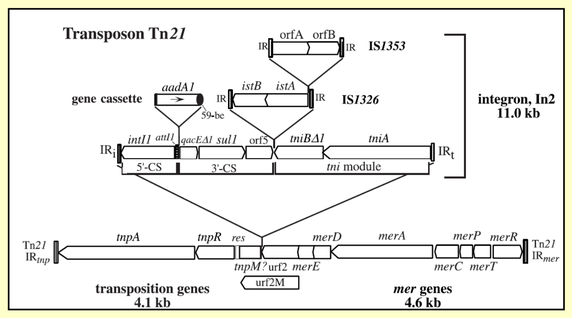
Introduction

Transposons are mobile genetic elements that randomly insert into the chromosome or into a plasmid. Transposons belonging to the Tn21 group of the Tn3 family carry an integron as well as other types of resistant determinants, such as heavy metal resistance operons (Figure). These transposons are often carried on broad-host-range conjugative plasmids, are frequently detected in gram-negative enteric bacteria, and are the most common transposons involved in the global dissemination of antibiotic resistance determinants among bacteria (1).
The aquatic environment is a natural ecosystem that act as a reservoir, but also as a medium for the spread of resistance genes and their vectors (2).
Aeromonas species are opportunistic pathogen gram-negative bacilli, which can be found in freshwater, chlorinated water, polluted water, brackish water, estuarine water, and sewage. They are associated with a wide variety of diseases in warm and cold blooded vertebrates including frogs, fishes, reptiles, snakes, birds and in humans, where they are capable of causing gastroenteritis as well as extra-intestinal infections (3).



Schematic representation of Tn21 (from (1))
The *tnp* region consist of genes for the transposase (*TnpA*) and for the resolvase (*TnpR*). The 5'CS of the integron includes integrase gene (*IntI1*), the *attI1* insertion site and the *aadA1* gene cassette conferring the resistance to aminoglycoside. The 3'CS includes gene for the resistanance to quaternary ammonium (*qacEΔ1*) and sulfonamide resistance (*sul1*)

Purpose

The aim of this work was to investigate the presence of transposons belonging to the Tn21 subgroup in *Aeromonas* strains isolated from different aquatic environments, in order to understand if these bacteria could play a role in the environmental dissemination of antibiotic resistance determinants.

Conclusions

These preliminary results show that 12.5% (29/231) of *Aeromonas spp.* can carry Tn21 and Tn21-like transposons on plasmids and/or on chromosome.
***Aeromonas* could therefore contribute to the environmental dissemination of antibiotic resistance determinants among bacteria.**

Outlook

- **To identify the plasmids carrying the transposons by Southern Blot analysis.**
- **To characterize the Tn21-like structures.**
- **To investigate if the Tn21-like transposons can be transferred by HGT from *Aeromonas spp.* to *Aeromonas spp.* or from *Aeromonas spp.* to other bacterial species.**

Results and discussion

Source	Species	Strains	Resistances	Gene Cassettes	PCR t*npA* plasmid extract	PCR t*npA* total DNA	Integrase dot-blot (total DNA)
TR-b-WWTP	*A.media*	1FT-Aer	CZ,CXM,FOX,NA,CIP(I)	ND	-	+	-
TR-b-WWTP	*A.media*	6FT-Aer	CZ,CXM,CRO,FOX,NA,CIP(I)	ND	-	+	+
TR-b-WWTP	*A.sobria*	7FT-Aer	CZ,SXT,NA,S,RL	dfrB1-aadA1b-CatB2	+	+	+
TR-b-WWTP	*A.media*	16FT-Aer	CZ,NA,S(I),RL	OXA10-aadA1	+	+	+
TR-b-WWTP	*A.caviae*	19FT-Aer	CZ,CXM,CRO,FOX,NA,S	ND	-	+	+
TR-b-WWTP	*A.media*	25FT-Aer	CZ,FOX,NA	ND	-	+	-
TR-b-WWTP	*A.sobria*	27FT-Aer	CZ,NA,S(I),RL	empty	-	+	+
TR-b-WWTP	*A.media*	34FT-Aer	CZ,FOX,NA,S(I)	ND	+	+	-
TR-b-WWTP	*A.media*	36FT-Aer	CZ(I)	ND	-	+	-
TR-b-WWTP	*A.media*	39FT-Aer	CZ,FOX,SXT,NA,S(I),TMP,RL	dfr22	+	+	+
TR-b-WWTP	*A.media*	42FT-Aer	CZ,FOX,CIP,C,NA,S(I),RL,NN	CatB3- aadA1	+	+	+
HWW-OSG	*A.hydrophila*	52SG-Aer	CZ,FOX,NA,RL	CatB8-aadA1	-	-	+
HWW-OSG	*A.hydrophila*	57SG-Aer	CZ,FOX,NA,S,RL,NN	CatB8-aadA1	-	-	+
HWW-OSG	*A.hydrophila*	77SG-Aer	CZ,CXM,CRO,GM,CIP,NA,S,RL,ATM,NN,NET	aacA4cr-Oxa1-CatB3-aar3	+	+	+
HWW-OSG	*A.hydrophila*	96SG-Aer	CZ,NA,S(I),ATM(I),RL	CatB8-aadA1	-	-	+
WWTP	*A.caviae*	101 DG-Aer	CZ,FOX,NA,S(I),RL	ND	-	+	-
WWTP	*A.caviae*	105 DG-Aer	NA,CIP(I),S,RL	CatB8-Transposase	+	+	+
WWTP	*A.caviae*	123 DG-Aer	CZ,FOX	ND	-	+	+
WWTP	*A.caviae*	128 DG-Aer B	CZ,NA,RL(I)	ND	-	+	+
WWTP	*A.media*	129 DG-Aer	CZ,NA,S(I),RL(I)	ND	-	+	+
WWTP	*A.media*	130 DG-Aer	CZ,(FOX),SXT,NA,Te(I),S,TMP,RL	drf-aadA1	-	-	+
WWTP	*A.media*	135 DG-Aer	CZ,FOX,GM(I),S(I),RL	ND	+	+	+
WWTP	*A.hydrophila*	137 DG-Aer	CZ,(FOX),CIP,NA,SXT(I)	CatB3-aadA1	-	-	+
TR-a-WWTP	*A.caviae*	152 FDD-Aer	CZ,NA,Te(I)	ND	+	+	+
TR-a-WWTP	*A.caviae*	161 FDD-Aer	CZ,FOX,NA,SXT(I),S(I),RL	CatB8-aadA1	+	+	+
TR-a-WWTP	*A.media*	167 FDD-Aer	CZ,NA,CIP(I),S(I),RL	CatB8-aadA2	+	+	+
TR-a-WWTP	*A.media*	171 FDD-Aer	CZ,FOX,NA,Te(I),RL	aacA3-Blaoxa21-CatB3-aadA16	-	-	+
TR-a-WWTP	*A.hydrophila*	180 FDD-Aer	CZ,NA	ND	-	+	+
TR-a-WWTP	*A.caviae*	187 FDD-Aer	CZ,CIP,NA	ND	+	+	+
TR-a-WWTP	*A.caviae*	188 FDD-Aer	CZ,CIP,NA,S(I)	ND	-	+	+
TR-a-WWTP	*A.punctata*	189 FDD-Aer	CZ,CIP,NA,S(I)	ND	-	+	+
TR-a-WWTP	*A.media*	190 FDD-Aer	CZ,FOX,NA	ND	-	+	-
TR-a-WWTP	*A.veronii*	195 FDD-Aer	CZ,FOX	ND	-	+	+
TR-a-WWTP	*A.media*	198 FDD-Aer	CZ,NA,S(I)	ND	-	+	+
TR-a-WWTP	*A.hydrophila*	199 FDD-Aer	NA,Te(I),S(I)	ND	-	+	+

TR-b-WWTP: river before wastewater treatment plant; HWW: Hospital WasteWater; WWTP: wastewater treatment plant; TR-a-WWTP : river after wastewater treatment plant.
CZ (Cefazoin); CXM (Cefuroxime); FOX (Cefoxitin); CRO (Ceftriaxone); NA (Nalidixic Acid); SXT (Bactrim); CIP (ciprofloxacin); GM (Gentamicin); S (Streptomycin); RL (sulfamethoxazole); TMP (Trimetroprim); ATM (Aztreonam); NN (tobramycin); NET (Netilmicin); Te (Tetracyclin).
ND: not detected.
Blue: chromosomal location of *tnpA*; **purple**: chromosomal and plasmidic location of *tnpA*; **green**: strains with gene cassettes in Class I integrons but negative for *tnpA.*

Of the 231 *Aeromonas spp.* analyzed, 29 (12.5%) carried the *tnpA* gene representative of the Tn21 and Tn21-like transposons. In 17 strains the *tnpA* gene was located only on the chromosome while in 12 strains it had also a plasmidic location.
37% of the *Aeromonas* carrying the transposase A were isolated from river before the wastewater treatment plant, 37% from river after the wastewater treatment plant,
3.4 % from hospital wastewaters, and 20% from activated sludge. No *Aeromonas* isolated from the alpine lake had a detectable *tnpA* gene.
In 8 strains it was possible to detect both *TnpA* and *IntI* as well as gene cassettes; we can therefore speculate that a Class I integron was integrated on a Tn21-like transposon. On the contrary, *IntI* and gene cassettes were detected in 5 strains which were negative for *tnpA*. This result may indicate that the resistance genes are located on integrons but not on transposons. The majority (20/29) of the *TnpA* positive isolates had no detectable gene cassettes although some strains were multiresistant. We can expect that these strains carry transposons similar to Tn501 or Tn1721, which are Tn21-like transposons that contain resistance genes but not integrons.

Material and methods

Bacterial strains: 231 *Aeromonas spp.* with different resistance and plasmidic profiles, were previously isolated from various aquatic environments (river, hospital wastewater, wastewater treatment plant, river after wastewater treatment plant and alpine lake). The strains were grown on blood agar for 24h at 30°C.
DNA extraction: chromosomal DNA was extracted from colonies using the InstaGene Matrix® (Biorad). Briefly, 2 or 3 bacterial colonies were resuspended in 200µl of InstaGene Matrix®, incubated at 95°C for 10 minutes and centrifuged at 13.000 rpm for 10 minutes.
Plasmid extraction: plasmids were purified with NucleoBond PC20® mini prep (Macherey-nagel) according to the manufacturer instructions.
Class I integrons: the presence of Class I integrons was investigated by PCR (Pant-QacΔE region) and by Dot blot analysis. Amplified gene cassettes were sequenced.
***tnpA* gene**: primers P5II (5 ' -TACTGCCGCGCATCAAGATC-3') and P2II (5'-AGAAAGTTCGTCCTGGGCTG-3') were used to amplify the transposase A, the target gene on Tn21-like transposons (4). PCR assays were set up as follows: 50µl of reaction contained 5µl of 10x PCR buffer, 1µl of dNTP mix 10mM, 2.5µl of each primers 10µM, 0.25µl of Hot start Taq polymerase 5 U/µl (Qiagen) and >1µg/50µl reaction of DNA. The DNA amplification program consisted of an initial denaturation step (95°C, 15 min) followed by 30 cycles of denaturation (95°C, 30 sec), annealing (59°C, 1 min) and extension (72°C, 1 min), and a final extension time of 10 min at 72°C. The amplification products (~400bp) were analyzed on Gel-Red™ (Biotium) stained 1% agarose (Eurobio) TBE gels with a 100bp ladder (Roche).

Reference

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