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SUPSI

COULD AEROMONAS BACTERIOPHAGES BE INVOLVED IN HORIZONTAL GENE TRANSFER?

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Introduction

The increased resistance to antibiotics of pathogenic bacteria is a world wide problem. Environmental bacteria are considered a source of antibiotic resistance genes that can be disseminated also to potentially pathogenic bacteria. Integrons and transposons are the most important mobile genetic elements that can carry antibiotic resistant genes and that can be spread by HGT, even if single genes can be transferred by this mechanism (1). HGT occurs by conjugation, transformation and transduction through bacteriophages which are the most abundant and persistent entities in the environment. Bacteriophages seem therefore to play an important role in the spread of resistance genes (2).

We have chosen *Aeromonas spp.* and their bacteriophages as a model to investigate the transmission of resistance genes in the aquatic environment.
The aims of this study were to investigate the presence of resistance genes in lytic and lysogenic *Aeromonas* bacteriophages and to verify if these genes could be transduced to host bacteria.

Material and methods

231 *Aeromonas spp*. were isolated from different aquatic environments and their resistance profiles were determined by disc diffusion test. The presence of resistance genes and of transposon 21 (*tn21*) was screened by PCR and dot blot. Lysogenic bacteriophages were induced by adding mitomycin C (0.5 mg/ml) to an over night culture of bacteria diluted 1:100. Lytic phages were isolated from water samples using the double agar layer method (3). All the phages suspensions were purified and concentrated by PEG/NaCl; host range was determined by lysotyping experiments and DNA was extracted from phages using a phenol-chloroform method prior to perform specific PCR amplifications of resistance genes. Transduction experiments were performed as follows: some colonies from recipient were resuspended in 200 ul of phage buffer, 200 ul of phage solution were added and the suspension was incubated for 15 min at 30°C. The mixture was plated on selective agar and incubated for 24 to 48h at 30°C.

Conclusion

Bacteriophages of *Aeromonas* were ubiquitous in the aquatic environment and were able to infect different species of *Aeromonas*. Most of the lytic phages isolated could acquire genetic determinants of antibiotic resistance when infecting an host that carried these genes. With mitomycin C treatment we obtained lysogenic phages that also integrated resistance determinants from their host. So far we were not able to transfer these genes by transduction.
These results suggest that *Aeromonas* bacteriophages could be involved in the spread of resistance genes in the aquatic environment.

Outlooks

To isolate other bacteriophages from different aquatic environments in order to confirm the potential role of phages in the dissemination of resistance. To improve transduction experiments.

Results and discussion

Twenty-one lytic bacteriophages were previously isolated from water. The ability to infect our 231 *Aeromonas* strains was tested for all the phages and 16% (36 of 231) of *Aeromonas spp*. could be lysed by one or more bacteriophages. The table shows that five bacteriophages were able to acquire genetic determinants from their bacterial hosts: *CatB3* gene that confers resistance to chloramphenicol, *sul I* gene that confers resistance to sulfamethoxazole, *aadA* gene that confer resistance to streptomycin, and *tnpA* gene that codify for the transposase of transposon tn21. None of the phages acquired all gene cassettes.

Lytic bacteriophages						
Bacteriophages name	***Aeromonas* host**	**Source**	**Species**	**Resistances**	**Genetic determinants of resistance**	**Genetic determinants in bacteriophages**
5	171 FDD-Aer	TR-a-WWTP	*A.media*	CZ,FOX,NA,Te(I),RL	**aacA3-Blaoxa21-CatB3-aadA16, sulI**	**CatB3, sulI**
5	123 DG-Aer	WWTP	*A.caviae*	CZ,FOX	**tn21**	**tn21**
43	34FT-Aer	TR-b-WWTP	*A.media*	CZ,FOX,NA,S(I)	**tn21**	**tn21**
43-L	34FT-Aer	TR-b-WWTP	*A.media*	CZ,FOX,NA,S(I)	**tn21**	**tn21**
18	34FT-Aer	TR-b-WWTP	*A.media*	CZ,FOX,NA,S(I)	**tn21**	**tn21**
45-L	105 DG-Aer	WWTP	*A.caviae*	NA,CIP(I),S,RL	**CatB8-Transposase, aadA, sulI, tn21**	**aadA, sulI, tn21**
45-L	34FT-Aer	TR-b-WWTP	*A.media*	CZ,FOX,NA,S(I)	**tn21**	**tn21**
45-L	123 DG-Aer	WWTP	*A.caviae*	CZ,FOX	**tn21**	**tn21**

TR-b-WWTP: river before wastewater treatment plant; WWTP: wastewater treatment plant; TR-a-WWTP : river after wastewater treatment plant. CZ (Cefazoin); FOX (Cefoxitin); NA (Nalidixic Acid); CIP (ciprofloxacin); S (Streptomycin); RL (Sulfamethoxazole).

By induction of all *Aeromonas* isolates with mitomycin C, 17 lysogenic bacteriophages were recovered. Three lysogenized *Aeromonas* strains carried resistance genes and all their lysogenic bacteriophages carried the same genetic determinants (tn21 and *dfr22* gene that confer resistance to trimethoprim).

Lysogenic bacteriophage						
Source	***Aeromonas* species**	***Aeromonas* hosts**	**Resistances**	**Genetic determinants of resistance**	**Bacteriophages name**	**Genetic determinants in bacteriophages**
TR-b-WWTP	*A.caviae*	19FT-Aer	CZ,CXM,CRO,FOX,NA,S	tn21	19 lisogeno	tn21
TR-b-WWTP	*A.media*	34FT-Aer	CZ,FOX,NA,S(I)	tn21	34 lisogeno	tn21
TR-b-WWTP	*A.media*	39FT-Aer	CZ,FOX,SXT,NA,S(I),TMP,RL	tn21, dfr22	39 lisogeno	dfr22, tn21

TR-b-WWTP: river before wastewater treatment plant. CZ (Cefazoin); CXM (Cefuroxime); FOX (Cefoxitin); CRO (Ceftriaxone); NA (Nalidixic Acid); SXT (Bactrim); S (Streptomycin); RL (Sulfamethoxazole); TMP (Trimetroprim);

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