Intragenomic heterogeneity and intergenomic recombination among *Vibrio parahaemolyticus* 16S rRNA genes

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Vibrio parahaemolyticus is a marine bacterium bearing 11 copies of ribosomal operons. In some strains, such as RIMD2210633, the genome includes identical copies of 16S rRNA genes (*rrs*). However, it is known that other strains of the species, such as strains ATCC 17802 and RIMD2210856, show conspicuous intragenomic *rrs* heterogeneity. The extent and diversity of the *rrs* heterogeneity in *V. parahaemolyticus* were studied in further detail by characterization of the *rrs* copies in environmental isolates belonging to 21 different genotype groups. Thirteen of these groups showed intragenomic heterogeneity, containing altogether 16 sequences differing within a 25 bp segment of their *rrs*. These sequences grouped into four clusters differing in at least four nucleotide sites. Some isolates contained *rrs* alleles from up to three different clusters. Each segment sequence conserved the stem-loop characteristic of the 16S rRNA structure of this 25 bp sequence. The double-stranded stem sequence was quite variable, but almost every variation had a compensatory change to maintain seven to eight paired bases. Conversely, the single-strand loop sequence was conserved. The results may be explained as a consequence of recombination among *rrs* evolving in different bacteria. The results suggest that intergenomic *rrs* recombination is very high in *V. parahaemolyticus* and that it occurs solely among *Vibrio* species. This high *rrs* homologous intergenomic recombination could be an effective mechanism to maintain intragenomic *rrs* cohesion, mediating the dispersal of the most abundant *rrs* version among the 11 intragenomic loci.

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INTRODUCTION

Many bacteria possess more than one and up to 15 copies of the 16S rRNA gene (*rrs*) (Acinas *et al.*, 2004). It is believed that the different copies do not differentiate and evolve in concert, from the existence of a mechanism called gene conversion (Liao, 2000). However, in some isolates from several species, one or more of the multiple intragenomic *rrs* loci may differ substantially from the rest, and their presence is considered to be due to homologous recombination with related bacteria (Morandi *et al.*, 2005; Sneath, 1993; Ueda *et al.*, 1999; Yap *et al.*, 1999). *Vibrio parahaemolyticus* is one of the bacterial species with a larger number of rRNA operons (*rrn*). This species encompasses different marine bacterial groups, including some human pathogens responsible for outbreaks of diarrhoea related to seafood consumption. The genome of strain RIMD2210633 of *V. parahaemolyticus*

(VpKX) consists of two circular chromosomes containing 11 *rrn* copies: 10 on chromosome 1 and one on chromosome 2 (Makino *et al.*, 2003). Examination of the sequence of each operon shows that they contain almost identical *rrs* alleles. However, other strains of *V. parahaemolyticus*, strains ATCC 17802 (VpD) and RIMD2210856 (VpAQ), show *rrs* intragenomic heterogeneity (González-Escalona *et al.*, 2005b; Moreno *et al.*, 2002). Strain VpD contains two classes of *rrs* (Moreno *et al.*, 2002), differing at 10 nucleotide sites within a 25 bp segment. This segment encodes a hypervariable stem-loop of the 16S rRNA which includes nucleotides 455–479 (*Escherichia coli* numbering). Seven operons contain *rrs* alleles with one of the sequences and four contain the other (González-Escalona *et al.*, 2005b). One of these segments differs at seven nucleotide sites from the corresponding segment in strain VpKX and the other differs at 10. Strain VpAQ contains some *rrs* alleles with the sequence observed in VpKX, and others with a unique sequence that differs from the two found in VpKX and VpD (González-Escalona *et al.*, 2005b; Moreno *et al.*, 2002). However, despite the large sequence differences, every segment conserves the stem-loop structure

Abbreviations: DGREA, direct genome restriction enzyme analysis; RDP, Ribosomal Database Project.

The GenBank/EMBL/DDBJ accession numbers for the *rrs* sequences of *V. parahaemolyticus* are DQ 868329–DQ 868341.

characteristic of this region in the rRNA. The large number of mismatches, together with compensating changes to conserve the stem structure in the rRNA, implies that their divergence is relatively ancient, and hence it is difficult to accept that they evolved in the same cell over such a long period. The most plausible explanation is that the differences were caused by recombination with *rrs* alleles that evolved in different bacterial strains, as originally proposed by Sneath for bacteria of the genus *Aeromonas* (Morandi *et al.*, 2005; Sneath, 1993). Lateral gene transfer in *V. parahaemolyticus* may be augmented by the high levels of bacteriophage transduction that occur in seawater (Beumer & Robinson, 2005; Jiang & Paul, 1998). Here, we report the extent and diversity of the intragenomic *rrs* heterogeneity in different groups of *V. parahaemolyticus* obtained from seafood samples during outbreaks in the austral summers of 1998, 2004 and 2005 in Chile (Fuenzalida *et al.*, 2006; González-Escalona *et al.*, 2005a). Our data suggest high intergenomic *rrs* recombination between *Vibrio* species that could have important consequences for the evolution of the species.

METHODS

Bacterial strains. *V. parahaemolyticus* RIMD2210633 (VpKX) was obtained from the Research Institute for Microbial Diseases, Osaka University, Japan. *V. parahaemolyticus* ATCC 17802 (VpD) was obtained from the American Type Culture Collection. The Chilean non-pandemic strains PMA37.5, PMA112, PMA3.5, PMA339, PMA337, PMA189, PMA16.5, PMA2.5, PMA79, PMA3316, PMA45.5, PMA27.5, PMA1.5, PMA19.5, PMA22.5 and PMA109.5 were obtained from shellfish samples taken during outbreaks that occurred in 2004 and 2005. Each of these isolates corresponds to the type isolate of the 16 groups differentiated by direct genome restriction enzyme analysis (DGREA), as previously described (Fuenzalida *et al.*, 2006). Nine isolates from the outbreaks that occurred in 1998 (González-Escalona *et al.*, 2005b) were analysed by DGREA and divided into five groups. Representative isolates from each of these groups were chosen and they corresponded to isolates ATC230, ATC297, COA62, ATA65 and LIA138. The patterns of the 21 type isolates from each of the DGREA groups were analysed with GelCompar II (Applied Math), and their genetic distances were calculated on the basis of the number of bands shared between isolates. Similarity matrices were calculated by using the Dice coefficient, and clustering was achieved by using the unweighted pair group method with arithmetic mean.

***rrs* analyses.** Bacterial DNA was extracted from overnight cultures in Luria-Bertani broth/3 % NaCl using the Wizard Genomic DNA Purification kit (Promega). The DNA concentration was estimated visually by comparison of the ethidium bromide-stained bands with a standard. PCR amplification of *rrs* was performed using approximately 10 ng per reaction tube, as previously described (Moreno *et al.*, 2002), and universal primers Eubac27F (5'-AGAGTTTGATC-CTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') to amplify 16S rDNA, and 357F (5'-CTCTACGGGAGGCAGCA-3') and 518R (5'-CGTATTACCGCGGCTGCTGG-3') to amplify the shorter fragments containing the variable region of the *rrs*. PCR products were assayed by electrophoresis and visualized as previously described (Moreno *et al.*, 2002).

Heteroduplex assay for *rrs* heterogeneity. Intragenomic *rrs* heterogeneity was tested by examining the formation of heteroduplexes after PCR amplification of the 16S rDNA, as described

previously (Espejo *et al.*, 1998). Formation of heteroduplexes occurs when the amplicons contain mismatches within a short segment. In this case, heteroduplexes were formed between *rrs* amplicons from different *rrs* alleles that differed in sequence. These amplicons hybridized when the temperature was decreased for primer annealing after melting during the last PCR cycle. As a consequence of the mismatches, these hybrids showed retarded electrophoretic migration in polyacrylamide gels.

***rrs* sequencing.** For direct sequencing of the products, these were purified with the Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced at Macrogen, Korea. DNA sequences were analysed individually and manually assembled. The alignments and sequence similarities were obtained using BioEdit (Hall, 1999). For sequencing of individual *rrs* alleles, the *rrs* in different restriction fragments were separated by PFGE, as described previously (González-Escalona *et al.*, 2005b), before PCR amplification. Sequencing took place as described for the whole PCR product. Briefly, bacterial genomic DNA in agarose plugs was prepared as described by Iida *et al.* (1997) and digested with the restriction enzyme *I-CeuI* (New England Biolabs) for 16 h at 37 °C, using 50 U enzyme per plug. Electrophoresis was performed on a CHEF-DRII system (Bio-Rad Laboratories) using a 1 % low-melting-point agarose (Promega) gel in 0.5 × TBE buffer (0.45 mM Tris-borate, 1 mM sodium EDTA, pH 8.0). The pulsed time employed was 1–80 s ramp time at 200 V for 20 h at a constant temperature of 14 °C. After electrophoresis, the gel was stained with ethidium bromide for 30 min and photographed. Five or six of the observed bands with larger migration distances were then excised from the gel with sterile razors, and a slice of each band was then melted at 65 °C in twice its volume of 1 × TE buffer (10 mM Tris/HCl, 1 mM EDTA-NaCl, pH 8.0). A 10 µl sample of the solution containing DNA from each band was then used for PCR, as described above. The products of the PCR were sequenced at Macrogen. For construction of the RFLP-PFGE dendrogram, bands with similar and different migration patterns were distinguished and identified by their relative migration in the gel. The generated data were used to construct a similarity matrix calculated using the Nei-Li coefficient (Nei & Li, 1979). Finally, this matrix was used to obtain the dendrogram by applying WPGM in Treecom (Van de Peer & De Wachter, 1994).

The presence of the different sequences in different bacterial taxonomic groups was tested using the probe match of the Ribosomal Database Project (RDP; <http://rdp.cme.msu.edu/>) database and by restricting the search to sequences of isolates with more than 1200 sequenced nucleotides and sequence data containing the nucleotides 400–500 (*E. coli* numbering) (Cole *et al.*, 2005).

The model of 16S rRNA was obtained from Cannone *et al.* (2002) (<http://www.rna.cccb.utexas.edu/>).

RESULTS

Intragenomic *rrs* heterogeneity in *V. parahaemolyticus*

Intragenomic heterogeneity in the multiple *rrs* alleles of different *V. parahaemolyticus* isolates obtained from shellfish was analysed. These isolates have been classified in 21 groups by comparison of their DNA restriction fragment length patterns by DGREA (Fuenzalida *et al.*, 2006). Fig. 1 shows the DGREA patterns and the dendrogram illustrating the similarity of the clusters. Intragenomic heterogeneity was initially tested by examining the formation of heteroduplexes after PCR amplification of the 16S rRNA genes, as

Fig. 1. DGREA of *V. parahaemolyticus* isolate types for every DGREA group observed in both clinical and shellfish samples collected in the summers of 1998, 2004, and 2005. Left of figure, dendrogram illustrating the clusters of the patterns by similarity. KX corresponds to the south-east pandemic strain RIMD2210633.

described in Methods. Only the type isolate from each of the 21 DGREA groups was tested at first. The formation of heteroduplexes was initially tested by PCR amplification of almost the complete *rrs* using the universal primers 27F and 1492R. Thirteen of the 21 isolates showed the characteristic heteroduplexes (results not shown). To determine if the heterogeneity occurred in nucleotides 455–479 (*E. coli* numbering) as observed for VpD (Moreno *et al.*, 2002), a shorter fragment of 161 bp encompassing this variable segment was amplified by PCR with primers 357F and 518R, and subsequently checked for formation of heteroduplexes (Fig. 2)

Fig. 2. PCR amplification products of the variable segment of the *rrs* of different *V. parahaemolyticus* isolates. The variable segments of a representative isolate from each DGREA group were PCR-amplified and separated by PAGE. VpKX, south-east pandemic strain RIMD2210633; VpD, species type strain ATCC 17802. All the other strains correspond to isolates from Chile. MW, BenchTop 100 bp Ladder (Promega).

(González-Escalona *et al.*, 2005b). By amplification of almost the whole *rrs*, formation of heteroduplexes was observed in the 13 isolates showing intragenomic heterogeneity, indicating that the heterogeneity was located in the same segment as that found in the heterogeneous strain VpD. The number of bands above the main lower band, corresponding to the homoduplex, reflected to some degree the extent of polymorphism and the number of different intragenomic *rrs* alleles (Espejo *et al.*, 1998). Examination of further isolates belonging to the more numerous DGREA groups showed that isolates from the same group shared the same heteroduplex pattern, suggesting that isolates from the same group had identical *rrs* heterogeneity. This analysis included seven isolates from group 2.5 and six from each of the groups 112 and 45 (results not shown). The high incidence of *rrs* polymorphism in isolates from shellfish was in agreement with our earlier observation that most strains of the genus *Vibrio* show intragenomic *rrs* polymorphism (Moreno *et al.*, 2002).

rrs sequences in *V. parahaemolyticus*

The sequences of heterogeneous segments were determined in each of the isolates representing each DGREA group. The sequences in isolates without *rrs* heterogeneity were directly determined in the PCR amplification product obtained from the bacterial DNA. To determine the sequences in isolates containing different *rrs* alleles, the different *rrn* operons were separated by cleavage of their

Fig. 3. PFGE-RFLP of DNA from VpKX and type isolates of the 13 *V. parahaemolyticus* DGREA groups with heterogeneous *rrs* digested with *I*-*CeuI*. The gel shows the same type isolates as those shown in Fig. 2. The dendrogram illustrating the PFGE-RFLP pattern clusters by dissimilarity is shown on the right. MW, genomic DNA of *Saccharomyces cerevisiae*.

genomic DNA with the restriction enzyme *I*-*CeuI* and subsequent PFGE (Fig. 3). *I*-*CeuI* cleaves a 19 bp sequence in the 23S rRNA gene (Liu *et al.*, 1993), generating fragments with a single *rrs*, except in those containing two adjacent *rrn* operons with opposite orientations. According to the genome sequence of VpKX (Makino *et al.*, 2003), *I*-*CeuI* generates 11 fragments. However, only six bands were sufficiently clear to be excised from the gel under the conditions employed. The expected sizes of these six fragments are indicated in Fig. 3 (bands marked by asterisks). The two smallest expected fragments of 5.2 and 5.6 kb ran off the gel. The two largest fragments of ~2 Mb were barely detectable in the upper part of the gel. The two expected fragments of 59 and 60 kb co-migrated in the gel. Due to the opposite orientations of two neighbouring operons (Makino *et al.*, 2003), one of the fragments of 2 Mb lacked *rrs*, while the fragment of 89 kb contained two *rrs*. Strain VpKX, isolates PMA19.5 and PMA27.5, which were readily discriminated by DGREA, showed identical *I*-*CeuI* restriction fragment patterns. Following separation of the fragments, the bands from 40 to 680 kb in size were excised from the gel, the *rrs* alleles were amplified, and the PCR products were sequenced. The sequences obtained for the eight monomorphic isolates (PMA37.5, PMA3.5, PMA79, PMA1.5, PMA109.5, ATC230, ATC297 and COA62) were identical to those found in the 11 *rrs* alleles of VpKX. According to their DGREA patterns, some of these isolates correspond to distant clades (Fig. 1). Interestingly, some monomorphic clades seemed to be closely related to clades with heterogeneous *rrs* (e.g. see clades PMA3.5 and PMA189). Sixteen different sequences or alleles were observed in the 13 isolates with heterogeneous *rrs* (Table 1). These 16

sequences clustered into four groups differing in at least four nucleotide sites. One group corresponded to sequences similar to those in VpKX. The other two groups showed sequences similar to those found in VpD, D1 and D2. The fourth group (ATA65-B2) contained a new set of sequences. Every sequence conserved the stem-loop structure characteristic of this 25 bp segment. The sequence forming the stem was very variable, but every variation had a compensatory change to maintain seven to eight paired bases (Fig. 4). Conversely, the sequence of the single-strand loop was conserved in every segment, except in segments PMA3316-B1 and LIA138-B6, in which it contained an additional nucleotide. Inclusion of this additional nucleotide in the single-stranded region was necessary to maintain the double-stranded secondary structure of the stem. Although these sequences differed in up to 12 of the 25 nucleotide positions, they were almost exclusively found in isolates of the genus *Vibrio* when searched in the RDP 16S rRNA database (Cole *et al.*, 2005). Table 1 also shows the number of hits obtained after searching each of the 25 bp sequences in the RDP database using the ProbeMatch tool. The percentage of the total hits corresponding to either bacteria of the family *Vibrionaceae* or of the genus *Vibrio* are shown in separate columns of Table 1.

Table 2 shows the distribution of the *rrs* alleles in VpKX and each of the 13 strains with *rrs* heterogeneity. To facilitate scrutiny, entries in Table 2 that contain sequences of different clusters have been differentiated by different typefaces. The degree of *rrs* intragenomic heterogeneity was variable, with some isolates containing *rrs* alleles from at least three different clusters (e.g. PMA189 and PMA112). Most polymorphic isolates contained *rrs* alleles differing

Table 1. *rrs* segment sequences in reference strains and isolates with heterogeneous *rrs*, and their frequencies in the RDP database

Segment sequences are identified by the name of the isolate in which they were found in greatest abundance; followed by the number assigned to the band containing that sequence. AB, all bands; Y, C/T; M, A/C; R, A/G.

Isolate-PFGE band*	Sequence of <i>rrs</i> variable segment	No. of hits†	Percentage hits	
			<i>Vibrionaceae</i> ‡	<i>Vibrio</i> §
VpKX-AB	GTAGTGTAGTTAATAG-CTGCATTAT	82	100	95
ATA65-B1	-A.....	1	100	100
PMA22.5-B1	..G.....-.....	4	100	100
PMA337-B1	-.....C	2	100	100
PMA19.5-B3	C.....-.....	1	100	100
PMA3316-B1	G.....	82 (1)	100	95
LIA138-B6	C.....	82 (1)	100	95
PMA189-B3	..G.....-...Y..C..	8 (1)	100	75
PMA45.5-B3	-...MT.YR.	85 (1)	100	92
VpD2-B1	.CG.GTAC.....-GTAT.CG.	4	100	100
ATA65-B2	.CG.C..C.....-G..G..G.	3	100	100
PMA339-B1	.CG....C.....-.....G.	31 (2)	100	6.5
PMA189-B6	.CG.....-.....CG.	1	100	0¶
VpD1-B4	.AG..AGT.....-ACT..C..	22 (1)	100	95.5
PMA16.5-B3	..G..AGT.....-ACT..C..	22 (1)	100	95.5
PMA2.5-B6	..G..AGT.....-ACT.....	22 (1)	100	95.5

*Designations in bold are the names assigned to the sequence group.

†Number of hits obtained after searching each of the 25 bp sequences over the RDP database using the ProbeMatch tool. Numbers in parentheses indicate the errors allowed in probe matching of the RDP. This error was only allowed when no match was obtained with the exact sequence.

‡Percentage of the total hits corresponding to bacteria of the family *Vibrionaceae*.

§Percentage of the total hits corresponding to bacteria of the genus *Vibrio*.

||*Photobacterium*=90 %.

¶Unclassified *Vibrionaceae*=100 %.

from four to 12 sites. In some cases, the sequence obtained from the amplicon of the PFGE band showed polymorphism for some sites, suggesting that it contained more than one sequence, probably two. Two *rrs* alleles in the same band could appear as a result either of the existence of adjacent *rrn* operons of opposite orientation in the genome or of co-migration of two restriction fragments. In these cases, only two bases were observed at each polymorphic site, and their presence could be explained by the occurrence of two *rrs* segment sequences, which is shown in Table 1. For example, the polymorphism found for band B1(40–50) of isolate PMA19.5 was explained by the presence of the two sequences ATA65B2 and D2.

To explore the homogeneity among isolates of the same DGREA cluster, a similar sequence-based analysis was done for two strains of clusters PMA2.5 and PMA45.5, and for one additional strain of cluster PMA112, all isolated from different samples. Only two fragments were sequenced on this occasion. This analysis showed that strains from the same DGREA cluster contained the same sequences as those found in the group type strain, but that these

sequences were not always present in the same restriction fragment (results not shown).

DISCUSSION

Our results show that the intragenomic *rrs* heterogeneity found in *V. parahaemolyticus* strains within the 25 bp stem-loop structure of the 16S rRNA is a characteristic of this species, as it was found in more than one-half of the genotypes examined. Two features of this 25 bp intragenomic and intergenomic variable segment, already reported in two other strains (VpD and VpAQ) (González-Escalona *et al.*, 2005b; Moreno *et al.*, 2002), were shown to be retained in two respects: (i) a highly variable sequence maintaining the secondary structure of the stem; and (ii) a conserved sequence of the single stranded loop. This overall conservation suggests that the segment may have an essential role in the functioning of the ribosome. One function reported for this segment is the requirement for a specific interaction between nucleotides 456–476 of the 16S rRNA of *E. coli* and protein S4, a

Fig. 4. 16S rRNA secondary structure of *V. parahaemolyticus* X56580 and secondary structures of the four main *rrs* sequences found in different environmental isolates of *V. parahaemolyticus*. Nucleotide changes accompanied by compensatory changes are shown in grey boxes and nucleotide changes without compensatory change are shown in black boxes.

ribosomal protein essential for ribosome assembly (Sapag *et al.*, 1990). Altogether, our results suggest that the stem sequence might be considered a neutral allele as long as the double-stranded structure is conserved. As previously stated for some *V. parahaemolyticus* strains and for other bacterial species, the accumulation of so many substitutions with compensating changes implies that the divergence of the different versions of these *rrs* segments is relatively ancient. It is likely that each version evolved in different bacteria and that assortment then took place by lateral gene transfer (González-Escalona *et al.*, 2005b; Morandi *et al.*, 2005; Moreno *et al.*, 2002; Sneath, 1993; Yap *et al.*, 1999). The results reported here show that some *V. parahaemolyticus* strains can contain up to three *rrs* alleles differing in at least four nucleotide sites. The presence of several *rrs* alleles with so many differences in single isolates reinforces the idea that the assortment took place by acquisition from different bacterial clones. If *rrs* intragenomic heterogeneity is indeed generated by recombination, the high proportion of environmental

isolates with heterogeneity (62%) suggests that lateral transfer and recombination are frequent among *V. parahaemolyticus* bacteria. However, lateral transfer may have different effects on *rrs* intragenomic heterogeneity. The *rrs* composition observed within each DGREA cluster will be maintained as long as intergenomic recombination occurs among members of the same DGREA group. This is highly likely because bacteria in close proximity probably belong to the same clone and also because recombination is higher among identical genes (Majewski & Cohan, 1999). In this situation, lateral gene transfer could be a mechanism of concerted evolution of the 11 intragenomic *rrs* alleles if intergenomic recombination in *V. parahaemolyticus* is more frequent than mutation, as occurs in some other bacterial species (Feil *et al.*, 1999, 2000, 2001). An example is what could happen in a DGREA cluster with identical intragenomic *rrs*. In this case, intergenomic recombination with bacteria of the same clone would replace *rrs* containing mismatches generated by mutation with those containing the sequence prevailing in the population. However, there will be

Table 2. Sequences of *rrs* variable segments in different PFGE bands observed after hydrolysis of isolate DNA with restriction enzyme I-CeuI

KX corresponds to the sequence of the south-east pandemic strain RIMD2210633. The three groups of sequences differing from KX are indicated as follows: bold type, ATA65B2; italic bold type, VpD1; underlined bold type, VpD2.

Isolate	PFGE band (kb)					
	B1 (40–50)	B2 (51–85)	B3 (86–120)	B4 (104–170)	B5 (180–610)	B6 (540–630)
VpKX	KX	KX	KX	KX	KX	KX
PMA45.5	KX	KX	45.5B3	KX	KX	KX
PMA3316	3316B1	KX	LIA138B6	KX	KX	ND
PMA2.5	KX	KX	KX	KX	KX	2.5B6
PMA337	337B1	3316B1	337B1	KX	KX	16.5B3
LIA138	KX	KX	D2	KX	KX	LIA138B6
PMA22.5	22.5B1	KX	22.5B1	D2	22.5B1	ND
PMA27.5	KX	KX	KX	16.5B3	KX	LIA138B6
PMA189	KX/22.5B1	16.5B3	189B3	16.5B3	KX	189B6
PMA19.5	ATA65B2/D2	KX	19.5B3	KX	ATA65B2	ND
PMA112	16.5B3	16.5B3	KX	16.5B3	LIA138B6	189B6
PMA339	339B1	339B1	KX/339B1	KX	16.5B3	339B1
ATA65	ATA65B1	ATA65B2	ATA65B2	ATA65B2	ATA65B2	ND
PMA16.5	ATA65B2	KX	16.5B3	16.5B3	ATA65B2	16.5B3
VpD	D2	D2	D1/D2	D1	D1	D1

instances of intergenomic recombination between bacteria of different clones containing different *rrs* that will cause the emergence of variants. These variants would destroy the coherence of the DGREA clusters unless these clusters correspond to ecotypes and coherence is recovered by periodic selection.

Our results also reinforce previous observations with the *rrs* sequences of two *V. parahaemolyticus* strains; this putative intergenomic *rrs* recombination seems to occur almost exclusively between bacteria of the same genus, since all the sequences observed have rarely been reported in bacteria outside the genus *Vibrio*. Although other mechanisms cannot be rejected, it is likely that intergenomic *rrs* recombination is mediated by bacteriophages. Indeed, bacteriophages that transduce genes to *V. parahaemolyticus* have been isolated from the sea (Chang *et al.*, 1998), in which transduction seems to occur abundantly (Jiang & Paul, 1998). Additionally, it has been demonstrated that some marine phages can harbour 16S rRNA genes (Beumer & Robinson, 2005).

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