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Comparison of Genome Structures of Vibrios, Bacteria Possessing Two Chromosomes

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Vibrios are gram-negative γ-proteobacteria which are ubiquitous in marine and estuarine environments. Recently, we demonstrated that some, if not all, *Vibrio* species have two circular chromosomes. The whole genome sequence of *Vibrio cholerae* N16961 has been reported. In this study, we constructed a physical and genetic map of the genome of Kanagawa phenomenon-positive *Vibrio parahaemolyticus* strain KX-V237 and compared it with those of *V. parahaemolyticus* AQ4673 and *V. cholerae* N16961. The genome of KX-V237 comprised two circular chromosomes (3.3 and 1.9 Mb), similar to the structure of the AQ4673 genome. The relative positions of the genes on the genomes were well conserved in the two strains, but a large inversion on the large chromosomes, probably symmetric around the replication origin, was suggested. Although the sizes of the large chromosomes of KX-V237 and *V. cholerae* N16961 were similar, the sizes of the small chromosomes were very different. Unlike N16961, the superintegron of KX-V237 was located on the large chromosome. Comparison of the genetic maps of the chromosomes of KX-V237 and *V. cholerae* N16961 revealed that most of the open reading frames (ORFs) present on the large chromosome of the *V. cholerae* strain had homologues on the large chromosome of the *V. parahaemolyticus* strain and that most of the ORFs on the small chromosome of N16961 were present on the small chromosome of KX-V237. The difference in the orders of the ORFs on the chromosomes of N16961 and KX-V237 implies that numerous and frequent genetic exchanges have occurred intrachromosomally rather than interchromosomally.

Vibrios are gram-negative γ-proteobacteria which are ubiquitous in marine and estuarine environments. *Vibrio cholerae* (32) is the most clinically important vibrio, because it is the etiological agent of cholera, a severe diarrheal disease that is highly lethal unless it is properly treated. This organism has multiple lifestyles, including a planktonic, free-swimming form, a sessile form attached to zooplankton and other aquatic flora and fauna (6), and a pathogen of host organisms (humans). It also enters a viable but nonculturable state (24) under certain conditions. Recently, it was revealed that some, if not all, *Vibrio* species, including *V. cholerae*, have two circular chromosomes (10, 31, 33). Determination of the whole genome sequence of *V. cholerae* strain N16961 (10) demonstrated that the vast majority of recognizable genes for essential cell functions (such as DNA replication, transcription, translation, and cell wall biosynthesis) and for pathogenicity (for example, genes encoding toxins, surface antigens, and adhesins) are located on the large chromosome. In contrast, the small chromosome contains a higher proportion of hypothetical genes than the large chromosome. Comparing the bias in the gene contents of the two chromosomes, Heidelberg et al. (10) conjectured that under some environmental conditions, there might be a difference in copy number between the chromosomes and that one of the chromosomes may have accumulated genes that are better expressed at a higher or lower copy number than genes on the other chromosome. They also postulated from evidence based on genome sequence information that the small chromosome of *V. cholerae* may have originally been a megaplasmid that was captured by an ancestral *Vibrio* species.

Vibrio parahaemolyticus, another vibrio, is recognized as a major, worldwide cause of gastroenteritis, particularly in areas of the world where seafood consumption is high (16). This organism is an emerging pathogen in North America (2, 3, 7). Like *V. cholerae*, this organism has multiple lifestyles in various environments (20). Hemolysis on a special blood agar (Wagatsuma's agar), known as the Kanagawa phenomenon (KP), has been recognized to be strongly associated with human-pathogenic *V. parahaemolyticus* strains (26). The hemolysin that causes KP is called the thermostable direct hemolysin (TDH) (11). Although much less frequently, KP-negative *V. parahaemolyticus* strains that produce a toxin named TDH-related hemolysin have also been reported to cause gastroenteritis similar to that caused by TDH-producing isolates (12, 14, 30). Both TDH and TDH-related hemolysin, encoded by the *tdh* and *trh* genes, respectively, are now recognized as important virulence factors in the pathogenesis of *V. parahaemolyticus* (11, 22).

We have previously presented a physical and genetic map of the genome of *V. parahaemolyticus* strain AQ4673 and demonstrated that it has two circular chromosomes (33). AQ4673 is a KP-negative strain, a comparative rarity among clinical isolates of *V. parahaemolyticus.* In this study, we analyzed a physical and genetic map of the genome of the KP-positive strain *V. parahaemolyticus* KX-V237 and compared it with that of AQ4673. We also compared the map with that of *V. cholerae* N16961 to determine the similarities and differences in the genome structures of two species in the genus *Vibrio.*

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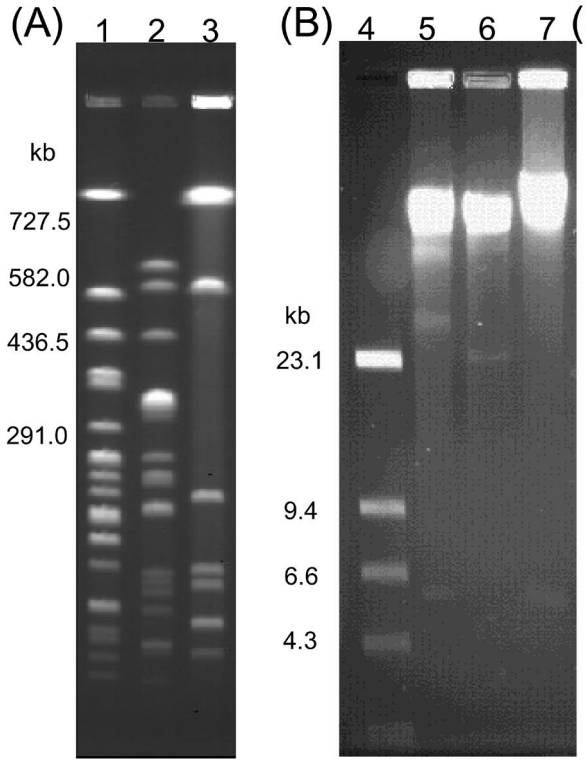

(C)

*Not*I digests (kb)		*Sfi*I digests (kb)		I-*Ceu*I digests (kb)	
NA	1,080	SA	620	CA	2,250
NB	560	SB	570	CB	1,900
NC	440	SC	440	CC	570
ND	360	SD(5)	320	CD	160
NE	340	SE	300	CE	100
NF	280	SF	240	CF	90
NG(2)	240	SG(2)	220	CG(2)	55
NH	220	SH(3)	160	CH	40
NI	190	SI	100	CI	6
NJ(3)	150	SJ	95	total size	5.23 Mb
NK(2)	130	SK	90		
NL	110	SL	85		
NM(3)	80	SM(2)	45		
NN	60	SN	40		
NO	55	SO	23		
NP	40	total size	5.17 Mb		
NQ	27				
NR	6				
total size	5.20 Mb				

FIG. 1. Restriction fragments of the genomic DNA of KX-V237. (A and B) Genomic DNA of KX-V237 was digested with *Not*I (lanes 1 and 5), *Sfi*I (lanes 2 and 6), or I-*Ceu*I (lanes 3 and 7) and then analyzed by PFGE (A) or FIGE (B). Lane 4 contained λ *Hin*dIII molecular weight markers. (C) Size of each restriction fragment.

MATERIALS AND METHODS

Bacterial strains. *V. parahaemolyticus* strain RIMD2210633 (referred to in this paper as KX-V237) used in this study was isolated at the Kansai International Airport quarantine station in 1996 from a patient with traveler's diarrhea (21). This strain is KP positive and possesses two copies of the *tdh* gene.

PFGE and FIGE. Samples of bacterial genomic DNA for pulsed-field gel electrophoresis (PFGE) or field inversion gel electrophoresis (FIGE) were prepared by a previously described method (15). PFGE was carried out with a CHEF DRIII or CHEF MAPPER system (Bio-Rad). FIGE was performed with a CHEF MAPPER system. The agarose gels used for PFGE or FIGE were 1% pulsed-field-certified agarose (Bio-Rad) or 0.8% chromosomal grade agarose (Bio-Rad) gels.

DNA techniques. After electrophoresis, nicking of the DNA fragments, transfer of the DNA fragments onto a nylon membrane, and hybridization were done by using previously described procedures (15). The nylon membrane used for DNA transfer was a GeneScreen membrane (NEN Life Science Products). A UV chamber GS Gene Linker (Bio-Rad) was used for nicking DNA fragments in the gels after electrophoresis and for cross-linking the DNA to nylon membranes. The hybridization temperature was 42°C. General DNA techniques, such as digestion of DNA with restriction enzymes and ligation, were performed as previously described (27). Preparation of plasmids from bacterial cells was performed by the alkaline lysis methods (1).

Preparation of linking clones. Two methods were used in combination to obtain linking clones to construct the physical map of the chromosomes of the *V. parahaemolyticus* KX-V237 strain. One procedure was a previously described procedure (33). Briefly, to isolate *Not*I-linking clones, the genomic DNA of the KX-V237 strain was completely digested with the enzyme *Eco*RI, *Pst*I, *Sal*I, *Eco*T22I, *Ban*III, or *Mlu* I. Each digest was then self-ligated. The resulting circular molecules were linearized by *Not*I digestion. The linear DNA fragments were cloned into the *Not*I site of the pBluescript II vector (Stratagene). For use in *Not*I-linking probes, we characterized the plasmids that were obtained on the basis of the sizes of the inserts and the specificity for hybridizing to the *Not*I fragments of KX-V237. In the second method, a lambda phage library of genomic DNA of KX-V237 (unpublished data) was used. The insert of each clone in the library (a total of 2,500 clones) was amplified by PCR by using LA *Taq* DNA polymerase (Takara), and the products were treated with restriction enzyme *Not*I. Inserts possessing a *Not*I site were used as linking clones.

Preparation of DIG-labeled DNA probe. The DNA fragments used as *Not*I-linking probes were labeled with digoxigenin (DIG) by using a random primer extension method and a DIG DNA labeling kit (Roche Molecular Biochemicals). The probes used for open reading frames (ORFs) of *V. parahaemolyticus* and *V. cholerae* were prepared by using a PCR DIG labeling kit (Roche Molecular Biochemicals). Sequence data required for PCR primer design were obtained from the DNA Database of Japan and the Comprehensive Microbial Resource of the Institute for Genomic Research. For comparison of the genome structures of *V. cholerae* N16961 and *V. parahaemolyticus* KX-V237, DNA probes were obtained as follows. A number of ORFs present on the N16961 genome were selected, and PCR primer sets were designed for these ORFs. By using these primer sets, PCR was carried out with the genomic DNA of KX-V237. From the primer sets with which DNA fragments of the expected sizes were amplified, we selected 42 sets that showed the most even overall distribution across the N16961 genome (see Fig. 4). The amplified PCR products were used to prepare DIG-labeled probes by the method described above.

RESULTS

Construction of a physical map of *V. parahaemolyticus* KX-V237 chromosomes. Digestion of the genomic DNA of *V. parahaemolyticus* KX-V237 with restriction enzymes *Not*I, *Sfi*I, and I-*Ceu*I yielded 24, 23, and 10 DNA fragments, respectively (Fig. 1). The sums of the sizes of the *Not*I, *Sfi*I, or I-*Ceu*I fragments were approximately 5.2 Mb in each case (Fig. 1C); thus, the total genome size of KX-V237 was estimated to be approximately 5.2 Mb. We constructed a physical map of this genome by linking the 24 *Not*I fragments using linking clones

TABLE 1. Linking clones used and *Not*I fragments linked by the linking clones

*Not*I fragments linked	*Not*I-linking clone(s)	*Sfi*I fragment hybridized	I-*Ceu*I fragment hybridized
NI-NJ	#12-10-H	SD	CA
NG-NM	P106, T104	SB	CB
ND-NN	E103, S111, #13-6-A	SE	CD
NB-NF	E105, #12-6-C	SH	CA
NE-NG	E009	SG	CC
NJ-NM	E107, #14-2-E	SD	CB
NL-NM	B103	SE	CG
NM-NN	P102	SE	CG
NC-NM	P103, #13-12-B	SB	CB
NI-NK	P107	SD	CA
NA-NR	#14-5-A	SA	CA
NQ-NR	P109	SA	CA
NH-NL	M104, #13-2-C	SD	CF
NK-NQ	S111, #14-4-E	SA	CA
NG-NH	P108, #13-3-A	SD	CC
NC-NF	T106, #12-5-A	SD	CB
NE-NJ	E104	SD	CA
NO-NP	S104, #11-12-B	SD	CA
NA-NJ	E101	SC	CA
NB-NJ	S114	SD	CB
NG-NK	T101	SB	CB
NJ-NK	B102	SD	CB
NM-NP	E102	SD	CA
ND-NO	M101	SD	CA

prepared as described in Materials and Methods. Table 1 contains a list of the linking clones that we used and the *Not*I fragments linked by these linking clones. The physical map of the KX-V237 genome which we constructed is shown in Fig. 2. The genome of KX-V237 comprised two separate circular chromosomes, similar to the structure that we reported for *V. parahaemolyticus* strain AQ4673 (33). The size of the large chromosome was 3.3 Mb, and the size of the small chromosome was 1.9 Mb. These sizes conformed well with those found for the two chromosomes of AQ4673 (Fig. 3).

Comparison of genetic maps of the chromosomes of KX-V237 and AQ4673. Localizing the genes of *V. parahaemolyticus* reported so far by using Southern hybridization, we constructed a genetic map of the KX-V237 chromosomes (Fig. 3). Our results indicate that the small chromosome possesses at least one *rrn* operon that encodes rRNA. Since digestion of the KX-V237 genome with I-*Ceu*I, which cleaves a specific sequence present only in *rrn*, yielded 10 DNA fragments (Fig. 1), it is likely that the genome possesses at least 10 *rrn* operons. Similar analysis with I-*Ceu*I digestion suggested that AQ4673 possesses nine *rrn* operons (data not shown). Comparing the genetic maps of the large chromosomes of KX-V237 and AQ4673, we found an apparent difference in gene order in the circular chromosome. This difference can be plausibly interpreted as resulting from a genetic inversion event occurring as indicated in Fig. 3. On the small chromosome, both of the copies of the *tdh* gene, a major virulence factor gene of *V. parahaemolyticus*, were located on a single *Not*I fragment, fragment NC (Fig. 3). Compared with the positions of *rrn* and *ldh*, the relative position of the *tdh* gene on the small chromosome of KX-V237 was similar to that of *tdh* and *trh* on the small chromosome of AQ4673 (Fig. 3).

Comparison of genetic maps of the chromosomes of KX-V237 and *V. cholerae* N16961. To ascertain the differences in the genome structures of two species of *Vibrio*, the genetic map of the chromosomes of KX-V237 was compared with that of *V. cholerae* N16961. Since the whole genome sequence of N16961 was available from the Institute for Genomic Research database, we obtained 27 probes (probes L1 to L27) for the ORFs present on chromosome 1 (large chromosome) and 15 probes

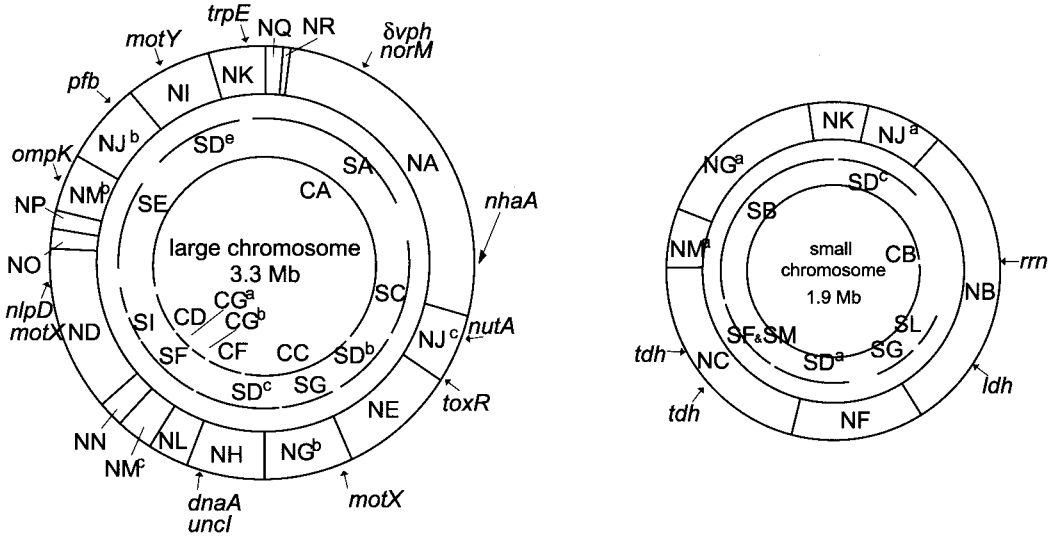

FIG. 2. Physical map of the genome of *V. parahaemolyticus* KX-V237. The linkage of *Not*I fragments and the relative locations of *Sfi*I and I-*Ceu*I fragments are shown. In this study, we constructed the map of *Not*I restriction fragments. The *Sfi*I or I-*Ceu*I fragments are not linked by, for example, linking clones. This is why the arcs that indicate the *Sfi*I or I-*Ceu*I fragments have gaps. The positions of the *Sfi*I or I-*Ceu*I fragments were determined from the results of hybridization with the *Not*I-linking clones or probes for the genes indicated. The restriction fragments whose designations have a superscript (e.g., SD^b) are the fragments for which multiple fragments having indistinguishable sizes were found (Fig. 1); we could not identify which fragment was which.

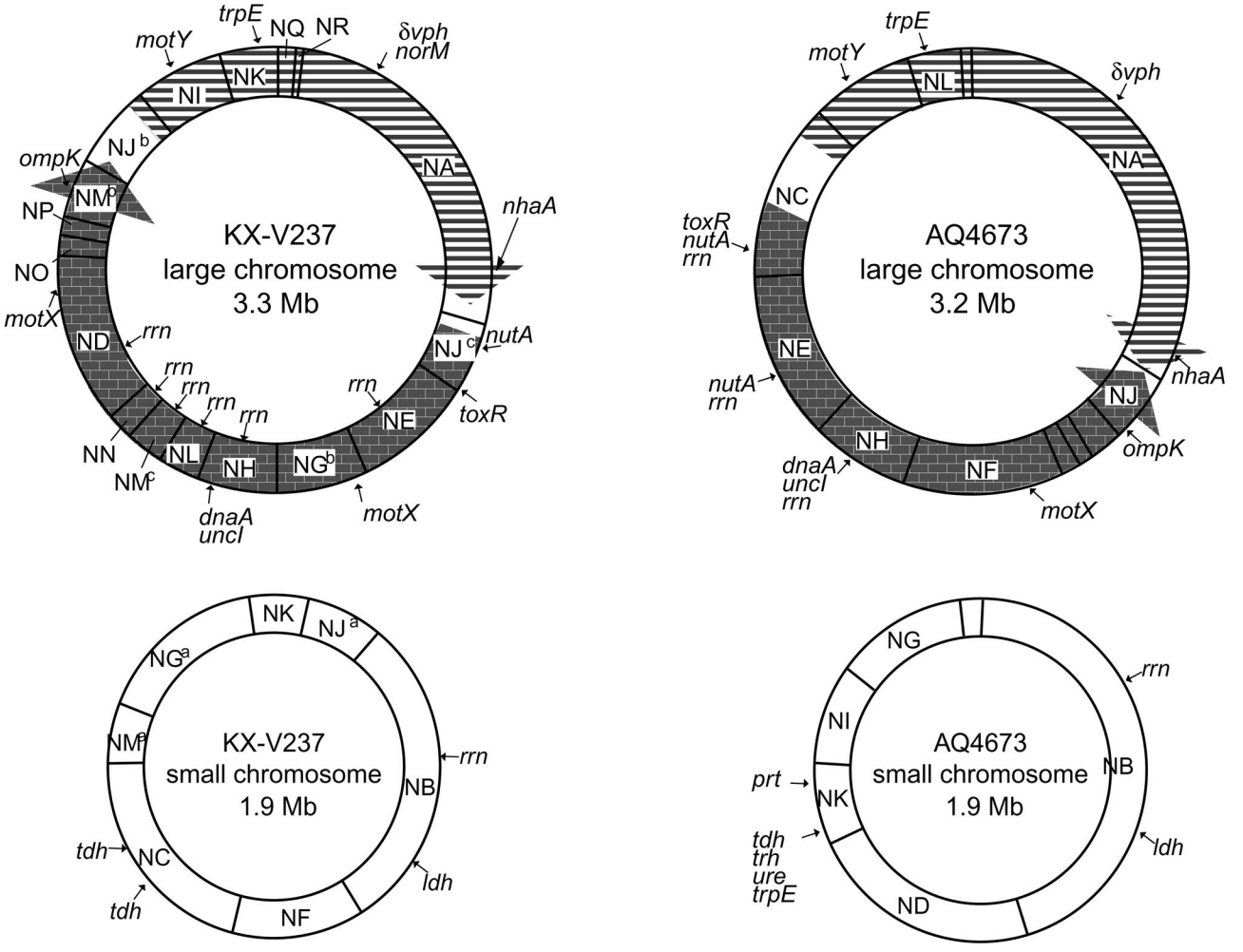

FIG. 3. Comparison of genetic maps of KX-V237 and AQ4673. The arrows on the large chromosomes indicate a putative genetic inversion between the two strains. Although I-*Ceu*I digestion of the genomic DNA of KX-V237 and AQ4673 suggested the presence of 10 and 9 *rrn* operons, respectively, fewer *rrn* operons are indicated because a single *Not*I fragment can have more than one *rrn* operon.

(probes S1 to S15) for ORFs on chromosome 2 (small chromosome) of N16961, as described in Materials and Methods; the probes are described in Table 2. Positions corresponding to these probes were distributed in almost all the regions of the N16961 genome, as shown in Fig 4. The locations of homologues for the ORFs on the KX-V237 genome were analyzed by Southern hybridization with the prepared probes. The results are summarized in Fig. 4 and 5. The data revealed that most of the ORFs present on chromosome 1 of N16961 had a homologue on the large chromosome of KX-V237. The findings were similar for ORFs present on the small chromosome. Most of the ORFs present on chromosome 2 of N16961 had a homologue on the small chromosome of KX-V237. These results suggest that the bias of distribution of the ORFs on the large and small chromosomes has been well conserved in the two species. Nevertheless, it is curious that the orders of the locations of the homologous sequences in the chromosomes of the two species have diverged so much (Fig. 5).

In previous studies (10, 31) workers reported that the *rrn* genes were present only on the large chromosome of *V. cholerae* and that no *rrn* genes were present on the small chromosome. In *V. parahaemolyticus* AQ4673, however, *rrn* was found on both the large and small chromosomes (33). In this study, we found that KX-V237 possessed at least 10 *rrn* operons and that at least one of them was on the small chromosome (Fig. 3). Thus, we suggest that even though *V. cholerae* and *V. parahaemolyticus* belong to the same genus, the presence of *rrn* on the small chromosome varies.

Heidelberg et al. (10) reported that the superintegron and the gene encoding its integrase (IntI4) (5, 19) were located on the small chromosome in *V. cholerae* N16961. The presence of a similar superintegron was also demonstrated for *V. parahaemolyticus* (25). Therefore, we investigated on which chromosome the superintegron was present in KX-V237. The probe which we prepared for *intI4* (probe S5) hybridized with the NA fragment of the KX-V237 genome (Fig. 4). We also prepared a probe for VPR, a repeat sequence that constitutes the superintegron of *V. parahaemolyticus* (25), and analyzed the location of VPR in the KX-V237 genome. The probe hybridized only with the NA fragment (data not shown), thus confirming that the superintegron is located on the NA fragment. These results suggest that the superintegron of *V. parahaemolyticus* is

TABLE 2. Probes and ORF of *V. cholerae* N16961 corresponding to each probe

Probe	ORF	Description
L1	VC0003	Thiophene and furan oxidation protein ThdF
L2	VC0012	Chromosomal DNA replication initiator DnaA
L3	VC0134	Conserved hypothetical protein
L4	VC0275	Phosphoribosylamine-glycine ligase PurD
L5	VC0533	Lipoprotein NlpD
L6	VC0543	RecA protein
L6	VC0717	Putative protease
L8	VC0852	DNA repair protein RecN
L9	VC0984	Cholera toxin transcriptional activator ToxR
L10	VC1008	Sodium-type flagellar protein MotY
L11	VC1091	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA
L12	VC1174	Anthranilate synthase component I TrpE
L13	VC1220	Phenylalanyl-tRNA synthetase, beta chain PheT
L14	VC1354	Conserved hypothetical protein
L15	VC1486	ABC transporter, ATP-binding protein
L16	VC1540	Multidrug resistance protein NorM, putative
L17	VC1623	Carboxynorspermidine decarboxylase NspC
L18	VC1627	Na^+/H^+ antiporter protein NhaA
L19	VC1751	Conserved hypothetical protein
L20	VC1903	Cell division protein FtsK, putative
L21	VC2030	RNase E Rne
L22	VC2162	Permease PerM, putative
L23	VC2174	UDP-sugar hydrolase UshA
L24	VC2290	NADH:ubiquinone oxidoreductase, Na translocating, beta subunit NqrF
L25	VC2305	Outer membrane protein OmpK
L26	VC2431	Topoisomerase IV, subunit B ParE
L27	VC2560	Sulfate adenylate transferase, subunit 2 CysD
S1	VCA0011	Regulatory protein MalT
S2	VCA0103	Sulfate permease family protein
S3	VCA0204	ATP-dependent RNA helicase RhlE
S4	VCA0276	Glycine cleavage system P protein GcvP, authentic frameshift
S5	VCA0291	Site-specific recombinase IntI4
S6	VCA0511	Anaerobic ribonucleoside triphosphate reductase NrdD
S7	VCA0606	Phosphonoacetaldehyde phosphonohydrolase PhnX
S8	VCA0657	Aerobic glycerol-3-phosphate dehydrogenase GlpD
S9	VCA0702	Iron-containing alcohol dehydrogenase
S10	VCA0759	Arginine ABC transporter, periplasmic arginine-binding protein ArtI
S11	VCA0805	Exoribonuclease II Rnb
S12	VCA0859	Oxidoreductase, aldo/keto reductase 2 family
S13	VCA0937	Transcriptional regulator, AraC/XylS family
S14	VCA0982	Transcriptional regulator, LysR family
S15	VCA1026	Conserved hypothetical protein

located on the large chromosome and not on the small chromosome.

DISCUSSION

In this study, we constructed a physical and genetic map of the chromosomes of KX-V237, a KP-positive *V. parahaemolyticus* strain. KX-V237 was found to possess two circular chromosomes whose sizes were similar to those previously reported for the KP-negative *V. parahaemolyticus* AQ4673 (Fig. 2 and 3) (33). The relative positions of the genes on the genomes were well conserved in the two strains, but a large inversion on the large chromosomes was suggested (Fig. 3). Taking into consideration the position of *dnaA*, which is often found near the replication origin of bacterial chromosomes, the observed inverse locations of the genes on the large chromosomes of KX-V237 and AQ4673 are likely to be due to a symmetric chromosomal inversion around the replication origin. Similar symmetric chromosomal inversions in strains belonging to a single species have been reported by several researchers (8, 18, 28); thus, the present data might be another example of such inversions.

It has been suggested that *tdh* and *trh* were introduced some time in the past from another bacterium into *V. parahaemolyticus* (22). Recently, it was reported that the DNA regions surrounding *tdh* and *trh* might form a pathogenicity island (9) in the genome of *V. parahaemolyticus* (14, 15, 23). In this study, the *tdh* gene, the major virulence factor gene of *V. parahaemolyticus*, was found on the small chromosome of KX-V237. The relative position of the *tdh* gene was similar to the position of the *tdh* and *trh* genes on the small chromosome of the KP-negative strain AQ4673 (Fig. 3). Although the presence of *tdh* and *trh* in *V. parahaemolyticus* varies depending on the strain (14, 29, 30), the present data suggest that the integrated positions of the putative pathogenicity islands of KP-positive (e.g., KX-V237) and KP-negative (e.g., AQ4673) strains might be the same.

The use of I-*Ceu*I to digest the genomic DNA of KX-V237

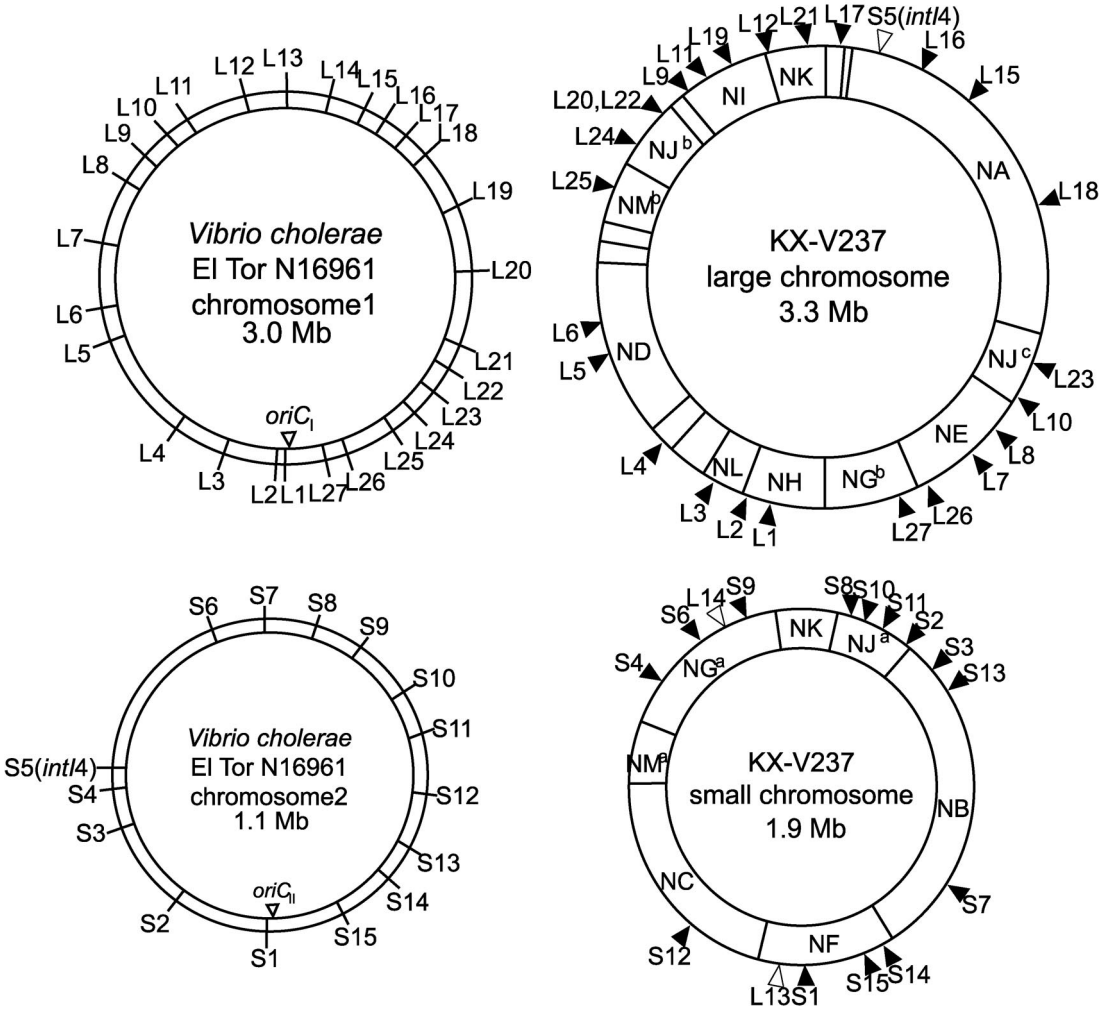

FIG. 4. Comparison of genetic maps of *V. cholerae* N16961 and *V. parahaemolyticus* KX-V237. The ORFs of N16961 corresponding to probes L1 to L27 and S1 to S15 are listed in Table 2.

and AQ4673 provided evidence that these strains possess 10 and 9 *rrn* operons, respectively. KX-V237 is one of the strains recently exhibiting pandemic spread across Asia and North America (2–4, 7, 13). Since an increase in the number of *rrn* operons may give an organism a selective advantage for survival in a continually fluctuating environment (17), the observed increase in the number of *rrn* operons could be one of the factors responsible for making certain strains of *V. parahaemolyticus* pandemic.

Recently, the whole genome sequence of another vibrio, *V. cholerae* N16961, was reported (10). Noting that the N16961 genome also has a two-chromosome structure (10), we compared the genome structures of *V. parahaemolyticus* KX-V237 and *V. cholerae* N16961 to elucidate the structural similarities and differences of these organisms. Although the sizes of the large chromosomes of *V. parahaemolyticus* and *V. cholerae* were similar, the sizes of the small chromosomes differed greatly (1.9 Mb in KX-V237 and 1.1 Mb in N16961). At present, it is unclear why the small chromosome of *V. parahaemolyticus* is 0.8 Mb bigger than that of *V. cholerae*. The toxin gene of *V. parahaemolyticus*, *tdh*, was present in the small chromosome. If the region surrounding the *tdh* gene does form a pathogenicity island (14, 15, 23), this might be one of the components which makes the small chromosome of *V. parahaemolyticus* so much bigger than that of *V. cholerae*. Detailed elucidation of the actual extra DNA sequences of the small chromosome of *V. parahaemolyticus* is a task for the future.

Comparison of the genetic maps of N16961 and KX-V237 revealed that most of the ORFs present on the large chromosome of *V. cholerae* had a homologue on the large chromosome of *V. parahaemolyticus*. We also found that most of the ORFs present on the small chromosome of N16961 had a homologue on the small chromosome of KX-V237. The difference in the orders of the homologues on the chromosomes of N16961 and KX-V237 implies that numerous frequent genetic exchanges have occurred intrachromosomally rather than interchromosomally (Fig. 4 and 5). The observed discrepancy of the frequencies of hypothetical intra- and interchromosomal genetic exchanges can be explained in several ways. It might simply be due to physical distance. Proximity itself may result in recombination events that occur more frequently within chromosomes than between chromosomes. Another interesting possi-

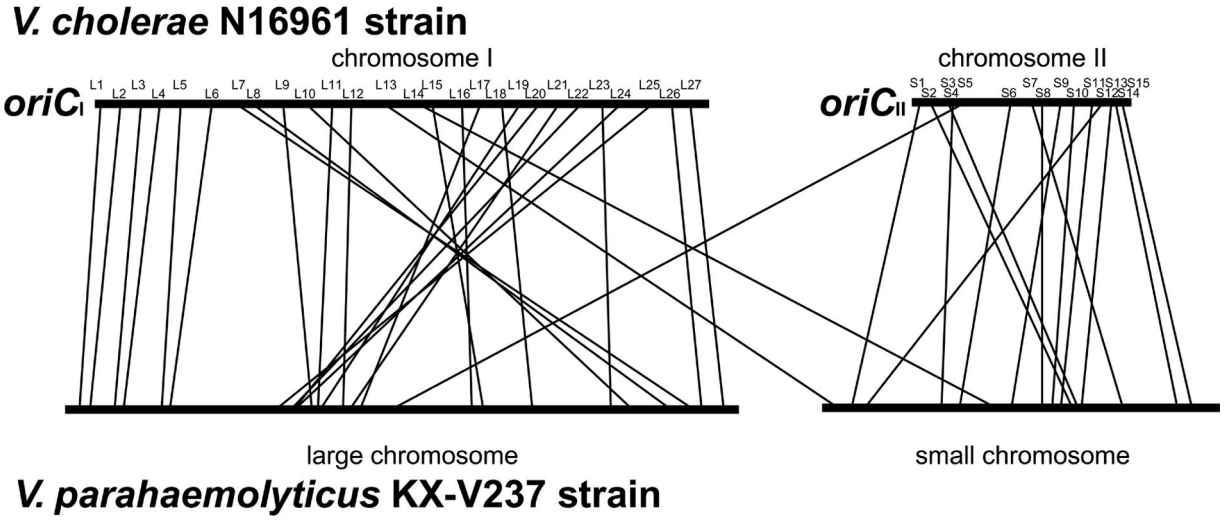

FIG. 5. Comparison of genetic maps of *V. cholerae* N16961 and *V. parahaemolyticus* KX-V237. The maps are in a linearized form, starting at the *oriC* sites of the N16961 genome.

bility is that the discrepancy is due to the differences in the functions of each chromosome. Vibrios, especially those pathogenic for humans, have to adapt to more than one environments to survive under various circumstances, such as in marine or estuarine water, on the surfaces of aquatic flora and fauna, and in human hosts. Heidelberg et al. (10) have proposed that the genes on the large and small chromosomes of *V. cholerae* function differently depending on the environments encountered by the organisms and that the organisms adapt to different situations by varying the copy number of the chromosomes. Adaptation of vibrios to different environments that is accomplished by regulating the copy number of the large and small chromosomes would explain the observed similarity of the distribution of the homologues on each chromosome. This variation in the copy number of the chromosomes in vibrios, however, has not been experimentally demonstrated yet. Further study is needed to resolve this issue.

Heidelberg et al. (10) have proposed that the small chromosome of *V. cholerae* was derived from a megaplasmid captured by an ancestral vibrio. Several lines of evidence support this hypothesis. For example, an integron, an element often found on plasmids (19), has been found on the small chromosome of N16961 (10). Evidence from the present study, however, shows that the superintegron occurs on the large chromosome rather than the small chromosome in *V. parahaemolyticus.* This difference in the location of the superintegron in the same *Vibrio* species indicates that the chromosomal location of the superintegron cannot be used as evidence to support the megaplasmid hypothesis.

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