

The Relationship Between Plasmid Fingerprints and Chromosomal Fingerprints of Fish Pathogenic Vibrios

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Abstract - It is well known that many pathogenic vibrios of marine fish possess plasmids. When plasmid DNA fingerprints of *Vibrio* strains containing plasmids were compared their corresponding chromosomal DNA fingerprints, we found that some bands of the chromosomal fingerprints originated from plasmid DNA. The connection with plasmid DNA fingerprints and chromosomal fingerprints has to be considered to characterize *Vibrio* spp. properly.

The possession of plasmids by marine vibrios is common (Hada and Sizemore 1984). Plasmid-mediated characteristics are involved in antibiotic resistance (Aoki et al. 1974; 1981; Sizemore and Colwell 1977; Toranzo et al. 1983), virulence (Wiik et al. 1989) iron limitation (Crosa et al. 1977, 1980; Crosa 1980; Crosa and Hodges 1981) and heavy metal resistance (Hada and Sizemore 1984). Characterization of plasmid DNAs to differentiate strains of bacteria at the species, serovar or biovar level has been used in studies of human Gram-negative bacteria (Schaberg et al. 1981; Holmberg et al. 1984). Fingerprints of chromosomal DNA have been used in epidemiological studies of fish pathogenic vibrios (Holm et al. 1985; Jorgensen et al. 1989; Nilsen et al. 1989; Tsai et al. 1990). In this communication, we report the connection between chromosomal and plasmid DNA fingerprints based on restriction patterns and band intensity of *Vibrio* spp. containing plasmids.

Fourteen strains of *Vibrio* spp. were collected. They were *Vibrio alginolyticus* ATCC 17749; *V. anguillarum* 860306L1 (serovar A),

PT493 (serovar B), Ital72 (serovar C), LS174 (serovar C) and NIE275 (serovar C); *V. damsela* ATCC33539; *V. harveyi* ATCC14126; *V. ordalii* V-1669; *V. parahaemolyticus* ATCC17802; *V. vulnificus* biogroup I (ATCC27562, SG716, TG617), and biogroup II (UE516). The sources and the growth conditions were described previously (Tsai et al. 1990).

On percent inocula from overnight cultures of the bacteria were grown in a 500-ml tryptic soy broth supplemented with 2.5% NaCl while undergoing vigorous shaking for 6 hours at 28°C or until mid-log phase was reached. Cells were harvested and lysed by the alkaline lysis method (Sambrook et al. 1989). Covalently closed circular plasmids were isolated by CsCl-ethidium bromide density gradient ultracentrifugation at 45,000 rpm for 36 hours at 20°C (Hitachi 55P-72 with RSP50.2 rotor) (Sambrook et al. 1989).

The procedures of chromosomal DNA preparation and agarose gel electrophoresis were performed as described by Tsai et al. (1990). Endonucleases (*Cla*I, *Eco*RI, *Hind*III and *Sac*I) used to cleave chromosomal or plasmid DNA were purchased from Boehringer Mannheim, Germany. Reaction and stop conditions were as recommended by the manufacturer.

Six of the 14 strains of *Vibrio* spp. examined contained plasmids. These strains were *V. alginolyticus* ATCC17749, *V. anguillarum* Ital72, LS174, NIE275, *V. damsela* ATCC14126, *V. ordalii* V-1669, and *V. vulnificus* UE516. The molecular size of each plasmid is listed in Table 1. The fingerprints of chromosomal DNA and plasmid DNA of three *V. anguillarum* strains, harboring 44.0 and 18.7 kilobase pairs (Kb) plasmids, are shown in Fig. 1. A

Table 1. Plasmid profile of *Vibrio* species.

Species	Strain	Plasmid contents (Kb) ^a
<i>Vibrio alginolyticus</i>	ATCC17749	16.4, 11.9, 8.3, 6.0
<i>V. anguillarum</i>	Ital72	44.0, 18.7
<i>V. anguillarum</i>	LS174	44.0, 18.7
<i>V. anguillarum</i>	NIE275	44.0, 18.7
<i>V. damsela</i>	ATCC33539	24.5, 17.8, 15.1
<i>V. ordalii</i>	V-1669	24.3, 22.4, 16.9
<i>V. vulnificus</i>	UE516	78.1, 47.3, 22.2

^a Plasmid sizes (kilobase pairs, Kb) were estimated by comparing migration distances with supercoil plasmid markers on the agarose gel and were corrected by adding all the molecular weights of the linear DNA fragments digested with the restriction enzyme.

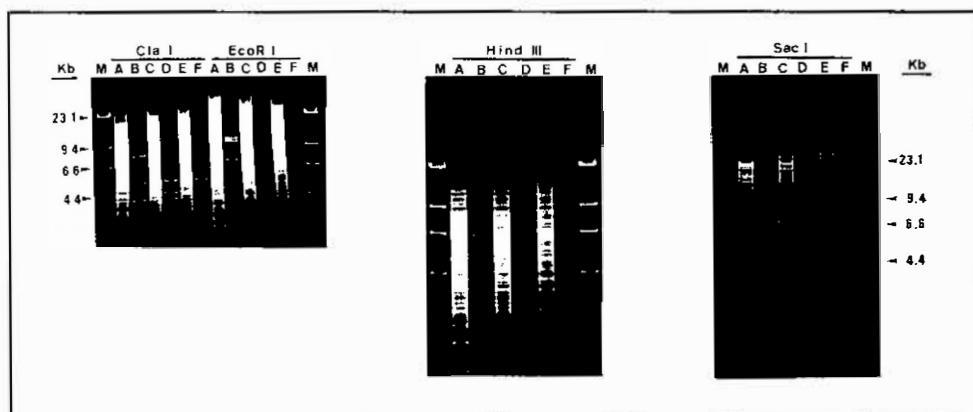


Fig. 1. Both chromosomal DNA fingerprints and plasmid DNA fingerprints of *Vibrio anguillarum*. Chromosomal DNA and plasmids were respectively obtained from strain Ital72, LS174 and NIE275, and were digested with various restriction enzymes (*ClaI*, *EcoRI*, *HindIII* and *SacI*). M, molecular marker of lambda DNA digested with *HindIII*; A and B, respectively, for chromosomal DNA fingerprint and plasmid DNA fingerprint of strain Ital72; C and D, respectively, for chromosomal DNA fingerprint and plasmid DNA fingerprint for strain LS174; E and F, respectively, for chromosomal DNA fingerprint and plasmid DNA fingerprint of strain NIE275; Kb, kilobase pairs.

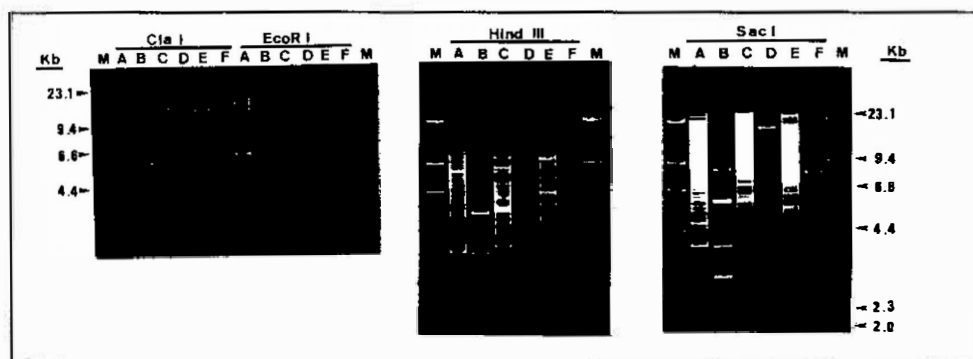


Fig. 2. Both chromosomal DNA fingerprints and plasmid DNA fingerprints of other *Vibrio* species studied. Chromosomal DNA and plasmids were obtained from vibrio strains and were digested with various restriction enzymes (*ClaI*, *EcoRI*, *HindIII* and *SacI*). M, molecular marker of lambda DNA digested with *HindIII*; A and B, respectively, for chromosomal DNA fingerprint and plasmid DNA fingerprint of *V. alginolyticus* ATCC17749; C and D, respectively, for chromosomal DNA fingerprint and plasmid DNA fingerprint of *V. damsela* ATCC33539; E and F, respectively, for chromosomal DNA fingerprint and plasmid DNA fingerprint of *V. vulnificus* UE516; Kb, kilobase pairs.

comparison of chromosomal DNA and plasmid DNA digested with *Cla*I showed that bands found in the plasmid fingerprints were located at the same position as certain bands found in the chromosomal fingerprints. Bands not corresponding to those in the chromosomal and plasmid fingerprints were rarely observed. In some cases, the intensity of the bands which corresponded to plasmid fingerprints was increased. This finding suggested that the increasing intensity of some bands was due to co-migration of corresponding chromosomal DNA fragments and plasmid DNAs. This was supported by fingerprints obtained from *Eco*R1, *Hind*III and *Sac*1.

Four restriction digestion patterns (*Cla*1, *Eco*R1, *Hind*III and *Sac*1) produced the same result for *V. alginolyticus*, *V. damsela* and *V. vulnificus* (Fig. 2). These results demonstrate that plasmid DNA might contribute to certain bands in chromosomal DNA fingerprints. The clearest example is shown by the *Sac*1 chromosomal fingerprint of *V. damsela* (Fig. 2, lane C and D). Here, a 24.5 Kb band is shown on chromosomal as well as plasmid fingerprints. It is likely that these bands originate from plasmid DNA. Thus, it is necessary to analyze whether or not certain bands originate from plasmid DNAs when DNA restriction patterns are used as fingerprints to characterize vibrios at the molecular level.

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