

## A Profile of Naturally Occurring Plasmids from Selected Strains of Vibrios

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The naturally occurring plasmids of *Vibrio* species have been isolated in part to investigate their genetic traits. Among six different *Vibrio* species tested, *Vibrio anguillarum*, *Vibrio fluvialis*, *Vibrio vulnificus*, *Vibrio mimicus* and *Vibrio furnissi* did not show any presence of plasmid. One environmental isolate of *Vibrio parahaemolyticus* harboring plasmid was observed. The isolated plasmid was 8.7 kb by analysis with restriction endonuclease digestion. No common feature was shown relationships between the presence of plasmid and resistance against commonly used antibiotic compounds from the tested *Vibrios*.

Key words : plasmid, *Vibrio anguillarum*, *Vibrio fluvialis*, *Vibrio vulnificus*, *Vibrio mimicus*, *Vibrio furnissi*, *Vibrio parahaemolyticus*.

### 1. Introduction

A marine microbe *Vibrio* is known as a pathogen to humans and fish (Baya, 1986, Tiainen, 1994) and is widely distributed in marine environment and seafood. The incidence of vibriosis varies greatly depending on various levels of fecal contamination, season, location, analytical methods, and samplings. Various methods have been adopted to identify this bacterium to overcome disease and to avoid food product contamination. Still no enough reports are available on genetic characteristics of *Vibrios*. The genetic characteristics of marine *Vibrios* have to be investigated since environmental pollution is increasing and imported sea products from other country is increasing. Quick and rapid analytical techniques have to be established to identify these bacteria and some potential pathological factors.

The presence of plasmids in most bacterial

species has been known although it is not a universal feature of the procaryotes. The possession of plasmid has been adopted for the purpose of strain typing, a wide variety of genes including those mediating pathogenicity factors (Baya, 1986, Quentmeier and Cornelius, 1994, Skerman, 1972) and characterization of species specificity. As the number of plasmids and their sizes varies from species to species, this can be a useful tool to identify a certain strain among a collection of isolates in an outbreak investigation. The plasmid in fish may spread indirectly to human and R factor from fish can transfer to human (Toranzo, 1983). There was a controversy that pathological genes could be plasmid coded in some strains and chromosomal determinant in others (Guerry and Colwell, 1977, Honda, 1993). An early report proposed that plasmid may play a role in the production of enterotoxin (Skerman, 1972) in some enteric pathogenic bacteria. It can not be denied that the plasmid profiling is of

limited value, it has been used in epidemiological studies.

Still there is only a limited research on plasmid of *Vibrios* and the function of its presence has yet to be studied. There were no many reports concerning either the biological role or epidemiological part in the plasmid of *Vibrio* except *V. parahaemolyticus* plasmid for the cryptic function (Guerry and Colwell, 1977, Twedt, 1981) and the virulence involvement of *V. anguillarum* (Trainen, 1994, Toranzo, 1983) in fish.

Therefore in this present study, the naturally occurring plasmids of *Vibrios* were studied in part to elucidate the genetic characteristics of these species.

## 2. Materials and Methods

### 2.1. Bacterial strains and culture media

*Vibrio anguillarum*, *V. vulnificus*, *V. furnissi*, *V. fluvialis*, *V. mimicus* and serologically identified *V. parahaemolyticus* strains from marine isolation were used (Table 1). Cells were grown in Luria-Bertani (LB) broth supplemented with 1-3% NaCl at 37°C for 12 hrs and then maintained in our laboratory during the process.

### 2.2. Antimicrobial susceptibility

All isolated strains were tested for antimicrobial susceptibility. Antibiotics were added from filter-sterilized stock solutions at the following concentrations (micrograms per milliliter); ampicillin 50, tetracycline 15, kanamycin 500, streptomycin 1000, gentamycin 30, respectively. The final concentrations of antibiotics ranged from 15 to 1000 µg/ml.

### 2.3. Extraction of plasmid and restriction enzyme analysis

All samples were prepared for the mini-preparation described by Kado and Liu (1981), (Maniatis, 1982). Digestion with restriction endonuclease was performed by incubating the purified plasmid for 1 hr with the relevant enzymes according to the supplier's instruction.

### 2.4. Plasmid screening

Isolated plasmid DNA was electrophoresed on a 0.8% agarose gel in a TAE buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH 8.0). The gels were stained in ethidium bromide (0.5 µg/ml final) and visualized under a UV illuminator.

**Table 1.** Selected Strains of *Vibrios*

Species	Type(serovar)	Source
<i>Vibrio anguillarum</i>	KS410	Fish
<i>Vibrio furnissi</i>	ATCC35016	Human feces
<i>Vibrio fluvialis</i>	ATCC33803	Human feces
<i>Vibrio mimicus</i>	ATCC33653	Human feces
<i>Vibrio vulnificus</i>	ATCC27562	Human blood
<i>Vibrio parahaemolyticus</i>	O1:K1	Environment
<i>Vibrio parahaemolyticus</i>	O2:K3	Environment
<i>Vibrio parahaemolyticus</i>	O3:K6	Environment
<i>Vibrio parahaemolyticus</i>	O4:K9	Environment
<i>Vibrio parahaemolyticus</i>	O4:K10	Environment
<i>Vibrio parahaemolyticus</i>	O4:K11	Environment
<i>Vibrio parahaemolyticus</i>	O4:K13	Environment
<i>Vibrio parahaemolyticus</i>	O5:K17	Environment
<i>Vibrio parahaemolyticus</i>	O8:K20	Environment
<i>Vibrio parahaemolyticus</i>	O10:K21	Environment
<i>Vibrio parahaemolyticus</i>	O4:K22	Environment
<i>Vibrio parahaemolyticus</i>	O1:K25	Environment
<i>Vibrio parahaemolyticus</i>	O1:K33	Environment
<i>Vibrio parahaemolyticus</i>	O3:K30	Environment
<i>Vibrio parahaemolyticus</i>	O1:K32	Environment
<i>Vibrio parahaemolyticus</i>	O2:K28	Environment
<i>Vibrio parahaemolyticus</i>	O10:K52	Environment
<i>Vibrio parahaemolyticus</i>	O3:K57	Environment
<i>Vibrio parahaemolyticus</i>	O4:K8	Environment

### 3. Results and Discussion

It is well known that most bacterial species harbor plasmids and it encodes a wide variety of genes, including those mediating antimicrobial resistance, virulence, and metabolism of hydrocarbons. The function of the plasmid or the genes it carries is irrelevant. In early studies, the workers have stressed that plasmids involvement in the production of pathogenic factors (Skerman, 1972) and resistance in antibiotics. And it also has been a topic that gene transfer has been occurred in the natural environment and among species. However, there were not many reports on apparent correlation between the presence of plasmid and either pathological role or resistance factor in *Vibrio* except that of deoxyribonucleic acid and the ureolytic activity in *V. parahaemolyticus* (Kim, 1996). Recent reports on the involvement of plasmid and virulence in *V. anguillarum* has been proved by Tiainen *et al.* (1994). Much work is required on the epidemiological role or biological function of plasmids on these species.

*Vibrio* plasmids were isolated for profiling by alkaline lysis as described by Kado and Liu (1981). Several methods including boiling methods have been adopted, but the former was more effective than the latter in regards to recovery. A total of five different *Vibrio* species and 18 different *V. parahaemolyticus* strains possessing different antigenic type were tested with respect to their plasmid contents.

*V. anguillarum*, which cause fish disease have been characterized with regard to plasmid contents and its virulence has been discussed. Our isolate did not possess plasmid, possibly reflecting difference in geographical distribution or a limited number of tested strains. *V. vulnificus*, *V. furnissi*, *V. mimicus* and *V. fluvialis* did not show any evidence of plasmids. (Fig. 1). Of 18 strains we tested, one antigenic type of *V. parahaemolyticus* (K1) showed to have plasmid deoxyribonucleic acid (Fig. 2) whereas the remaining K-antigen serotype did not (Fig. 1). Guerry and Colwell (1977) mentioned that all of his isolates contained covalently closed circular deoxyribonucleic acid ranging from the smallest 7

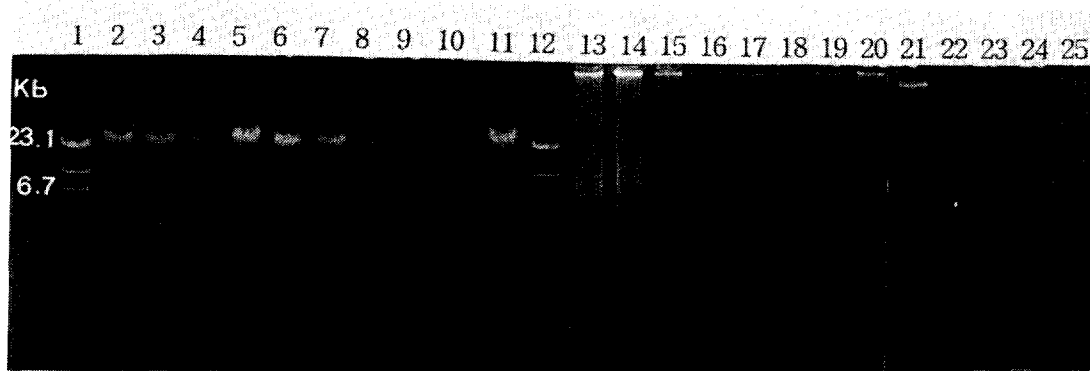
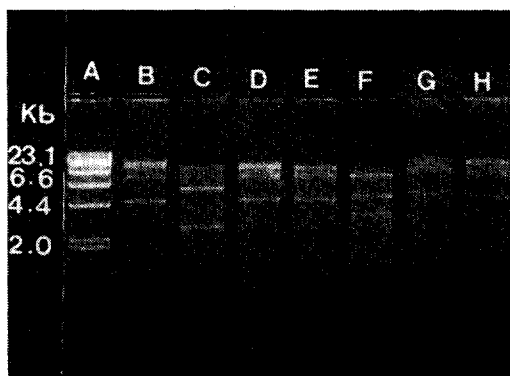


Fig. 1. Demonstration of *Vibrio* plasmids. Lane 1, Lambda DNA *Hind* III marker; Lanes 2 to 11, *V. parahaemolyticus* antigenic type of K8, K13, K21, K22, K17, K25, K30, K33, K52, K57; Lane 12, lambda marker; Lane 13, *V. vulnificus*; Lane 14, *V. mimicus*; Lane 15, *V. furnissi*; Lanes 16 to 20, *V. parahaemolyticus* K3, K6, K9, K10, K11; Lane 21, *V. anguillarum*; Lane 22, *V. fluvialis*; Lanes 23 to 25, *V. parahaemolyticus* K20, K28, K32.



**Fig. 2.** Plasmid profile of *Vibrio parahaemolyticus* K1 from marine isolate. Lanes A, lambda DNA *Hind* III marker; B, Control; C, *EcoR* I and *Hind* III digest; D, *Bam* HI; E, *Pst*; F, *Hind* III; G, *Bgl* II; H, *Xho*.

kilobase to the largest 62 kilobase in his clinical isolates of *V. parahaemolyticus*. Our isolate showed 8.7 kilobase by analysis of restriction endonuclease cleavage. The difference between his result and ours can be account for, in part, by the different origins of isolate and geographic distribution. Guerry and Colwell (1977) reported that plasmid deoxyribonucleic acid was present in four of nine patient isolates but in none of the three environmental isolates of *V. parahaemolyticus*.

According to the restriction enzyme analysis of the plasmid (Fig. 2), enzymes of *EcoR* I and *Hind* III digested deoxyribonucleic acid of *V. parahaemolyticus* K1 whereas one band pattern was not affected (Fig. 1).

In an attempt to ascribe a function to cryptic plasmid of *Vibrio*, an obvious marker to test was antibiotic resistance. Disk tests of various strains showed that several were sensitive to tetracycline, kanamycin, streptomycin and gentamycin but resistant to ampicillin, with minimum inhibitory concentrations of 50  $\mu\text{g/ml}$ . This resistance to ampicillin was found in both plasmid positive

and negative in *V. parahaemolyticus* strains according to the report (Guerry and Colwell, 1977). The relationship between the resistance to antibiotics and the presence of plasmid in *Vibrio* from our tested strains did not give any distinctive correlation. Antibiotic resistance plasmids do not seem to be common to the tested strains of *Vibrio*.

The analysis of plasmid pattern could be useful for epidemiological surveys, and identification of pathogenic bacterium since *Vibrios* are shuttle bacteria between man and environment. A considerable amount of ground work is necessary to characterize the *Vibrio* on the basis of genetic level, and for the fast change of marine and socioeconomic conditions, further studies of genetic characteristics involved in pathogenicity, metabolism and adaptability have to be explained on the molecular biological level. The author expects other workers results to support our study and to establish genetic traits of marine microorganisms. The author is still working and further result will be continued.

#### 4. Conclusion

An attempt was made to investigate the genetic traits of marine *Vibrios*. Among six different origins of *Vibrio* species tested, *Vibrio anguillarum*, *Vibrio furnissi*, *Vibrio fluvialis*, *Vibrio vulnificus* and *Vibrio mimicus* did not show any distinctive plasmids. One antigenic type of *Vibrio parahaemolyticus* out of 18 isolates from environmental sources represented typical feature of plasmid whereas the others did not. The isolated plasmid was analyzed with restriction endonucleases and determined to be 8.7 kb. The presence of plasmid and antibiotic resistance was not common in *Vibrios*.

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