ENVIRONMENTAL SCIENCES Vol. 1, No. 2, 93~97
Bulletin of the Korean Environmental Sciences Society

A Profile of Naturally Occurring Plasmids from Selected Strains of Vibrios

Younghee Kim

Department of Microbiology, Dongeui University,
Pusanjin-gu Gaya-dong, Pusan, 614-714, Korea

(Manuscript received on 18 September, 1997)

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. anguillarium* (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℃ 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
Vibrio anguillarium	KS410	Fish
Vibrio furnissi	ATCC35016	Human feces
Vibrio fluvialis	ATCC33803	Human feces
Vibrio mimicus	ATCC33653	Human feces
Vibrio vulnificus	ATCC27562	Human blood
Vibrio parahaemolyticus	O1:K1	Environment
Vibrio parahaemolyticus	O2:K3	Environment
Vibrio parahaemolyticus	O3:K6	Environment
Vibrio parahaemolyticus	O4:K9	Environment
Vibrio parahaemolyticus	O4:K10	Environment
Vibrio parahaemolyticus	O4:K11	Environment
Vibrio parahaemolyticus	O4:K13	Environment
Vibrio parahaemolyticus	O5:K17	Environment
Vibrio parahaemolyticus	O8:K20	Environment
Vibrio parahaemolyticus	O10:K21	Environment
Vibrio parahaemolyticus	O4:K22	Environment
Vibrio parahaemolyticus	O1:K25	Environment
Vibrio parahaemolyticus	O1:K33	Environment
Vibrio parahaemolyticus	O3:K30	Environment
Vibrio parahaemolyticus	O1:K32	Environment
Vibrio parahaemolyticus	O2:K28	Environment
Vibrio parahaemolyticus	O10:K52	Environment
Vibrio parahaemolyticus	O3:K57	Environment
Vibrio parahaemolyticus	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. anguillarium* 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. anguilarium, 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

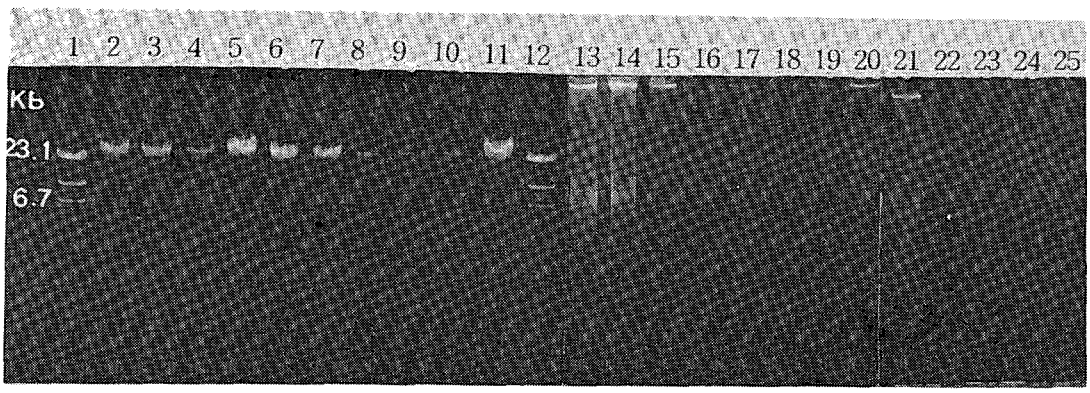

Fig. 1. Demonstration of Vibrio plasmids. Lane 1, Lambda DNA *Hind* III marker; Lanes 2 to 11, *V. parahaemolyticus* antigenic type of K8, K13, K21, K22, K17, K25, K30, K33, K52, K57; Lane 12, lambda marker; Lane 13, *V. vulnificus*; Lane 14, *V. mimicus*; Lane 15, *V. furnissi*; Lanes 16 to 20, *V.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

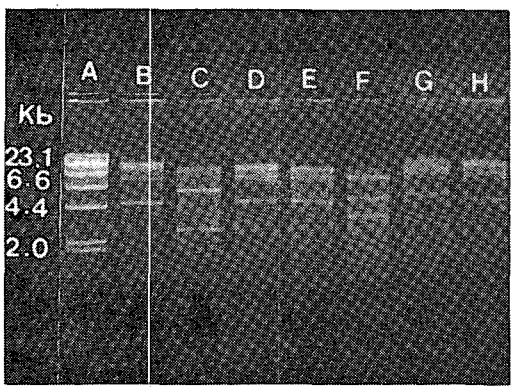

Fig. 2. Plasmid profile of *Vibrio parahaemolyticus* K1 from marine isolate. Lanes A, lambda DNA *Hind* III marker; B, Control; C, *Eco*R I and *Hind* III digest; D, *Bam* HI; E, *Pst*; F. *Hind* III; G, *Bgl* II; H, *Xho*.

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 *Eco*R 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 μg/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 endonucleses and determined to be 8.7 kb. The presence of plasmid and antibiotic resistance was not common in Vibrios.

Acknowledgement

This paper was supported by The Institute for Biological-Production Research Center (IBP) of Dongeui Univ., 1997. The author appreciates very much for this support.

REFERENCES

Baya, A. M., 1986, Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted Atlantic Ocean samples, *Appl. Environ. Microbiol.*, 51, 1285-1292.

Guerry, P. and R. Colwell, 1977, Isolation of cryptic plasmid deoxyribonucleic acid from Kanagawa-positive strains of *Vibrio parahaemolyticus, Infection and Immunity*, 16(1), 328-334,

Kado, C. I., S. T. Liu, 1981, Rapid procedure for detection and isolation of large and small plasmids, *J. Bacteriol.*, 145, 1365-1373.

Kim, Y. H., 1996, A correlation of urease production and the presence of plasmid in *Vibrio parahaemolyticus* from marine isolate, *J. Kor. Soc. Micro.*, 31(3), 373-377.

Mianitis, T. E., Fritsch, F., Sambrook, J, 1982, Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N. Y.

Olsen, J. E., 1990, An improved method for rapid isolation of plasmid DNA from wild-type Gram-negative bacteria for plasmid restriction profile analysis, *Lett. Appl. Microbiol.* 10, 209-212.

Quentimeier, A., F. Cornelius, 1994, Transfer and expression of degradative and antibiotic resistance plasmids in Acidophilic Bacteria, *Appl. Environ. Microbiol.*, 60(3), 973-978.

Sizemore, R. K., 1977, Plasmids carried by antibiotic-resistant marine bacteria, *Antimicrobe. Agents Chemother.*, 12, 373-381.

Skerman, F. J., S. B. Forml, S. B., Stanley, F., 1972, Plasmid-associated enterotoxin production in a strain of *E. coli* isolated from humans, *Infection and Immunity*, 5(4), 622-624.

Tiainen, T., J. L. Larsen, S. Pelkonen, 1994. Characterization of *Vibrio anguillarium* strains isolated from diseased fish in Finland, *Acta Vet. Scan.*, 35, 355-362.

Toranzo, A. E., 1983, Characterization of plasmids in bacterial fish pathogens, *Infection and Immunity*, 39(1), 184-192.

Twedt, R. M., O. F. Brown, D. I. Zink, 1981, Comparison of plasmid deoxyribonucleic acid contents, culture characteristics and indices of pathogenicity among selected strains of *Vibrio parahaemolyticus, Infection and Immunity*, 33(1), 322-325.