〈NOTE〉

Cloning of the *Vibrio mimicus* Hemolysin (Vm-hemolysin) Gene and Expression in *Escherichia coli.*

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Vibrio mimicus is an enteropathogenic bacterium which apparently causes diarrhea, usually after the consumption of uncooked seafood (Shandera *et al.*, 1983). *V.mimicus* produced several pathogenic factors such as cholera toxin (CT) (Chowdhury *et al.*, 1991), CT-related enterotoxin (Davis *et al.*, 1981) and *Escherichia coli* heat-stable enterotoxin (ST)-like toxins (Gyobu *et al.*, 1988; Nishibuchi *et al.*, 1983). The hemolysin is involved in the bloody diarrhea which is one particular clinical symptom of *V.mimicus* gastroenteritis (Shandera *et al.*, 1983). *V.mimicus* produced two kinds of hemolysin, Vm-hemolysin (M.W 58,000) and Vm-rTDH (MW22,000) (Honda *et al.*, 1987). Vm-hemolysin is immunologically cross-reactive with *V.cholerae* biovar El Tor hemolysin while Vm-rTDH is cross-reactive with *V.parahaemolyticus* thermostable direct hemolysin (TDH). In the present study, we have cloned the gene encoding Vm-hemolysin of *V.mimicus* and expressed in *E.coli* JM83.

For the construction of genomic library, chromosomal DNA of *Vibrio mimicus* (ATCC 33653) cultured in BHI broth was partially digested with *Eco*RI (1unit) at 37℃ for 30 min and ligated into pUC 19, digested with *Eco*RI. This ligation mixture transformed into *E.coli* JM83. To find out hemolysis positive clone, the screening was performed by observing β-hemolysis activity on TSAII medium (BBL Co., USA) containing 5% sheep blood supplemented with ampicillin (100 μg/mℓ). Among about 7,000 recombinants, a single clone was detected for hemolysis after incubation for 40 hr at 37℃ (Fig. 1). Plasmid DNA was isolated from this hemolysis-positive (hly+) clone when the isolated DNA was retransformed to *E.coli* cell, each colony produced 100% hemolysis, indicating that the isolated plasmid contains hemolysin gene. The recombinant plasmid was designated as pVMH191 and was mapped by several restriction endonucleases.

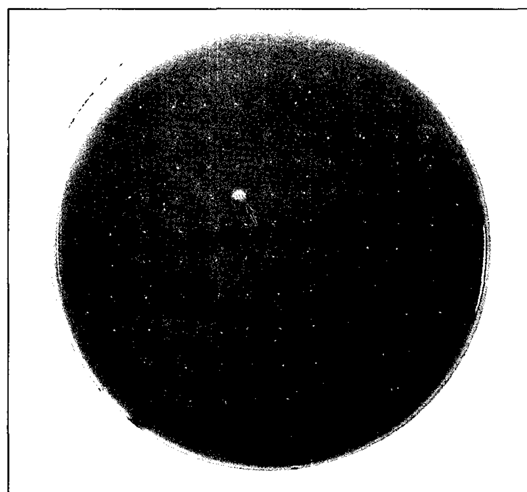

Fig. 1. Screening of *E.coli* JM83 (pVMH191) containing recombinant hemolysin gene on TSA II medium.

*Eco*RI digestion of pVMH191 yielded 2.7kb of vector and 8 kb of insert. For the subcloning, restriction endonuclease sites within the pVMH191 insert were decided. Plasmids termed as pVMH 192 and pVMH193 had the 5.3 kb-*Hin*dIII/*Eco*RI and 4 kb-*Pst*I/*Eco*RI inserts, respectively, in the same orientation of the pUC19 vector (Fig. 2). Plate assay for extracellular hemolysin production showed that these subclones have the strong hemolytic activity than that of *E. coli* JM83 (pVMH191) (Fig. 3). To further characterize pVMH193, pVMH193 insert was subcloned using other restriction endonucleases. However, as shwon in Fig 2, no hemolysis was detected in pVMH193-1 and the pVMH193-2. These result showed that 4 kb-*Pst*I/*Eco*RI insert contains entire ORF encoding Vm-hemolysin. (Fig. 2).

Expression of Vm-hemolysin gene in *E.coli* JM83 containing pVMH193 was measured by the activity against

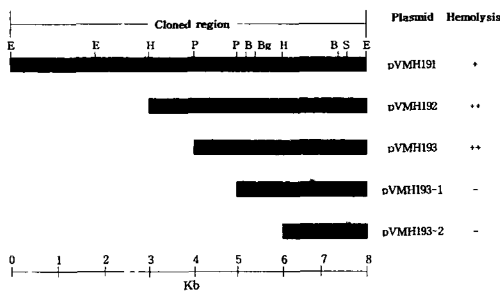

Fig. 2. Restriction map of the cloned region of Vibrio mimicus DNA containing hemolysin genes and its deletion derivatives.
Restriction enzymes; *E*, *Eco*RI; H, *Hin*dIII; P, *Pst*I; B, *Bam*HI; S, *Sph*I; Bg, *Bgl* II

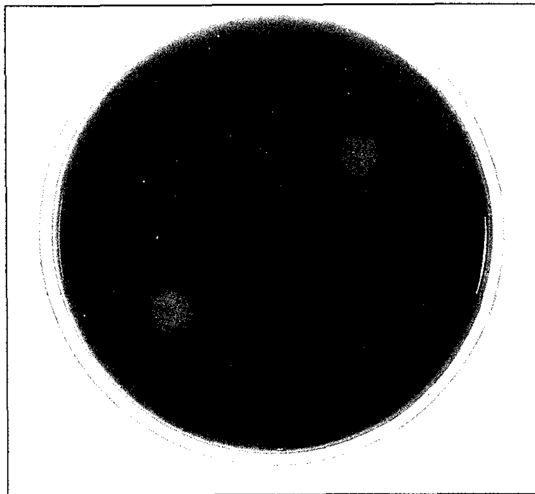

Fig. 3. Hemolysis of sheep erythrocyte by *E. coli* transformants with several derivative plasmid.
a, *E.coli* JM83 (pUC19); b, *E.coli* JM83 (pVMH 191); c, *E.coli* JM83 (pVMH192);
d, *E.coli* JM83 (pVMH193); e, *E.coli* JM83 (pVMH193-1); f, *E.coli* JM83 (pVMH193-2).

sheep RBCs with the culture supernatant, fraction of cytoplasm and periplasm. Approximately 60% of the hemolysis activity was detected in the cytoplasmic fraction and approximately 35% of it was detected in the extracellular medium. The periplasmic fraction showed little activity.

In Conclusion, Vm-hemolysin gene of *Vibrio mimicus* (ATCC33653) was cloned and the essential DNA region responsible for the expression of hemolysin gene was determined. Further studies of the biochemical action mode of hemolysin and DNA analysis will be investigated.

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