Review

_Vibrio parahaemolyticus_, enterotoxigenic _Escherichia coli_, enterohemorrhagic _Escherichia coli_ and _Vibrio cholerae_

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Abstract: This review highlighted the following: (i) pathogenic mechanism of the thermostable direct hemolysin produced by _Vibrio parahaemolyticus_, especially on its cardiotoxicity, (ii) heat-labile and heat-stable enterotoxins produced by enterotoxigenic _Escherichia coli_, especially structure–activity relationship of heat-stable enterotoxin, (iii) RNA N-glycosidase activity of Vero toxins (VT1 and VT2) produced by enterohemorrhagic _Escherichia coli O157:H7_, (iv) discovery of _Vibrio cholerae_ O1 El Tor that carries classical ctxB, and production of high concentration of cholera toxin by these strains, and (vi) conversion of viable but nonculturable (VBNC) _Vibrio cholerae_ to culturable state by co-culture with eukaryotic cells.

Keywords: thermostable direct hemolysin, heat-labile and heat-stable enterotoxins, RNA N-glycosidase activity of Vero toxins, _Vibrio cholerae_ O139, Viable but nonculturable (VBNC) _Vibrio cholerae_

Introduction

My scientific career was initiated in 1961 as a student of Dr. Tsunesaburo Fujino, Professor of Bacteriology and Serology, Research Institute for Microbial Diseases, Osaka University, who discovered _Vibrio parahaemolyticus_. During last 50 years, I have worked on enteric pathogens such as _Vibrio parahaemolyticus_, enterotoxigenic _Escherichia coli_, enterohemorrhagic _E. coli_ and _Vibrio cholerae_. In this article I will summarize what my colleagues and myself have accomplished on these enteric pathogens. Some background to understand why I have worked on the specific topics will also be discussed.

_Vibrio parahaemolyticus_

In 1950, Fujino et al.1) discovered _Vibrio parahaemolyticus_ as a causative bacterium of a case of food poisoning occurred in Osaka, Japan. It is one of the most important causative agents of bacterial food poisoning. In epidemiological studies on _V. parahaemolyticus_, Kato et al.2) found that strains isolated from patients caused hemolysis on special blood agar. The agent to cause the hemolysis was identified to be thermostable direct hemolysin (TDH).3) To study the pathogenesis of TDH, we first purified TDH from a strain isolated from a patient.1) The purified TDH is composed of two subunits of 21K dalton5) and is not inactivated by heating at 100 °C for 10 minutes. It shows various biological activities such as hemolytic activity, cytotoxic activity on various cultured cells, enterotoxicity in rabbit ileal loops and lethal toxicity in mice and rats.

We demonstrated that the lethal toxicity of the purified TDH was due to its cardiotoxicity.5) This was first suggested by a rapid death of mice and rats after intravenous injection of TDH. As shown in Table 1, more than 5 µg of TDH killed mice within 1 minute after intravenous injection. Even 1 µg of TDH killed mice within 10 minutes. Several bacterial toxins, such as streptolysin O, tetanolysin, hemolysin of _Listeria monocytogenes_, caused rapid death of animals on intravenous injection and it was demonstrated that they showed cardiotoxicity. Thus, we attempted to demonstrate the cardiotoxicity of TDH.

When ventricular tissue from the hearts of 14–16 days old mouse fetuses were cultured in Eagle's
minimum essential medium supplemented with 10% fetal bovine serum at 36 °C under an atmosphere of 5% CO₂ and 95% air, the cells beat spontaneously and regularly. When 1–1.5 × 10⁶ cells were seeded into dishes, a large cluster of 2–4 mm in diameter containing more than 10⁵ cells was obtained after cultivation for 1 day. All the myocardial cells in cell cluster beat synchronously and regularly at 100–180 beats/minute and this rate was maintained for at least 24 hours.

Figure 1 shows the effect of TDH on the beating of a cell cluster of cultured myocardial cells⁵. On addition of 0.05 µg/ml of TDH to the medium the beating increased slightly, but it returned to normal within 10 minutes (Fig. 1A). On addition of 0.1 µg of TDH, the beating was first rapidly stimulated and then stopped suddenly within one minute. Then, six minutes after the addition of TDH, the beating suddenly started again at the normal rate and remained unchanged during further observation (Fig. 1B). Similarly on addition of 0.2 µg of TDH, the beating first increased, then stopped and then started again (Fig. 1C), but interval between the time of stopping and of starting again was longer than that on addition of 0.1 µg of TDH. On addition of 1 µg or more of TDH per ml of medium, the beating also first increased and then stopped abruptly, and then almost all the cells rapidly disintegrated (Fig. 1D).

To study further the various kinds of biological activities of TDH on various kinds of cells, such as hemolysis on erythrocytes and cardiotoxicity on myocardial cells, attempts to determine the membrane receptor for TDH was carried out. Figure 2 shows the result of such an experiment.⁶ When TDH was preincubated with GT1 and GD1a gangliosides, its hemolytic activity was lost, whereas when it was preincubated with GM1 and GM2 gangliosides its hemolytic activity did not decrease. Treatment of ganglioside GT1 and GD1a with neuraminidase abolished their inhibitory effects on the hemolytic activity of TDH. It is known that TDH caused

| Amount of TDH injected (µg of protein per mouse) | Survival time after injection (mean ± S.D.) |
|-----------------------------------------------|--------------------------------------------|
| 10.0                                         | 35.5 ± 4.8 sec.                             |
| 5.0                                          | 49.0 ± 8.4                                  |
| 2.5                                          | 561.2 ± 368.8                               |
| 1.0                                          | 1121.5 ± 291.0                              |
| 0.5                                          | no death                                    |

Table 1. Lethal activity of TDH on intravenous injection into mice (from Ref. 6)

Fig. 2. Effect of various gangliosides on the hemolytic activity of TDH (from Ref. 7). The indicated amounts of various gangliosides were mixed with 5 µg (0.12 nmol) of TDH in 0.01 M Tris–HCl buffer (pH 7.2) in a volume of 0.125 ml and incubated at 37 °C for 30 min. Hemolytic activity was assayed after further incubation at 37 °C for 30 min by measuring the absorbance at 540 nm. (○) GT1, (●) GD1, (△) GM1, (▲) GM2.
hemolysis of human erythrocytes, but not of horse erythrocytes. The absence of GT1 and GD1a gangliosides on horse erythrocytes well explained the above results.

**Diarrheagenic Escherichia coli**

*Escherichia coli* is a commensal bacterium in human large intestine. However, several kinds of diarrheagenic *E. coli* that cause diarrhea in humans have been reported. They are enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC). Each of them causes diarrhea in human by different mechanisms. Among these five different kinds of diarrheagenic *E. coli*, diseases caused by ETEC and EHEC are known to be toxin-mediated. In this section, I will discuss on the toxins produced by ETEC and EHEC and the diseases caused by these diarrheagenic *E. coli*.

(i) ETEC. ETEC was first reported in 1956 by De in India. He found that *E. coli* strain isolated from stool of cholera-like patient gave a similar positive reaction in the ileal loop test as *V. cholerae* did. Later in 1971, Sack et al. demonstrated a protein toxin in the culture supernatant of ETEC, which is currently known as heat-labile enterotoxin (LT).

The importance of ETEC is highlighted in 1976 when Merson et al. reported that ETEC is a major enteropathogen that cause traveller’s diarrhea. In our country, we reported that ETEC was isolated from about 40% of diarrheal patients who just came back to Osaka (Itami) International Airport from South-East Asian countries. Smith and Gyles reported that ETEC produces heat-stable enterotoxin (ST) in addition to LT already reported by Sack et al. LT is a protein toxin inactivated by heating at 60°C for 10 minutes while ST is a peptide toxin not inactivated by heating even 100°C for 10 minutes.

Among many reports that attempted to purify LT, the most successful one is that reported by Clements and Finkelstein. Their method is quite unique: they found that LT was absorbed by Agarose A5m gel and was eluted out by a buffer containing 0.2 M galactose. Applying their method to purify ST we found that there are two immunologically distinct LTs. One is LT from ETEC isolated from human patient (LTh) and the other is that from porcine stool (LTP). At that time, it was known that LT is cross-reactive with cholera toxin (CT) produced by *V. cholerae*. We examined the immunological relationship among these three enterotoxins, LTh, LTP and CT, and found that they are immunologically related but not identical. Several other investigators confirmed our finding by nucleotide sequence analysis of the genes encoding LTh, LTP and CT.

Regarding ST, we purified ST in collaboration with Dr. Yasutugu Shimonishi and his colleagues of Protein Institute of Osaka University. Like LT, we found that there are two different kinds of ST, that is one from human patient (STh) and the other from porcine stool (STp). It was found that STh consisted of 19 amino acids while STp consisted of 18 amino acids. As shown in Table 2, primary structure of both STs is quite similar but is differ in some amino acids.

To study a structure–activity relationship of STs, we tried chemical synthesis of mature STs as well as their short analogues. It was found that chemically synthesized STh and STp showed the similar biological activity to those produced by ETEC in suckling mouse assay (accumulation of fluid in intestine). Moreover, as shown in Table 3, short analogues of both STh and STp, which have less numbers of amino acids at their N-terminus as well as C-terminus, showed positive reaction in the suckling mouse assay, thus was concluded that a peptide consist of 13 amino acids was a core to show the biological activity.

(ii) EHEC. EHEC is defined by its ability to produce Vero toxin (VT) that is cytotoxic to Vero cells. The first outbreak of food poisoning caused by EHEC was reported in 1982 in the US. In this outbreak, Riley et al. isolated an *E. coli* O157:H7 strain as a new kind of bacteria to cause diarrhea. Symptoms associated with this organism were quite severe with abdominal cramps and bloody diarrhea, which was named as hemorrhagic colitis. O’Brien et al. found that *E. coli* O157:H7 reported by Riley et al. produced VT and the cytotoxic activity was
neutralized by an antitoxin to Shiga toxin produced by Shigella dysenteriae type 1. This finding was quite unique at that time as the toxins produced by two different bacterial species were immunologically related each other. It is because of this related characteristic that EHEC is also called Shiga-toxin producing E. coli (STEC).

There are two types of VT, namely VT1 and VT2. VT1 was first reported by Konowalchuk in 1977 and several years later confirmed by others. On the other hand, VT2 that was immunological related but different to VT1 was isolated for the first time in 1986 from a patient admitted to an Infectious Disease Hospital in Tokyo. Almost the same time, Scotland et al. and Strockbine et al. reported the existence of VT2.

Several investigators including our group purified both VT1 and VT2. Although both VTs have the similar biological activities, VT2 was more potent than VT1. Both VT1 and VT2 consist of two subunits: one molecule of A subunit and five molecules of B subunit. The A subunit has an enzymatic activity as described below and the B subunit binds to the receptor molecule of cell surface. Many investigators reported the primary structure of both VTs. We determined by both amino acid and nucleotide sequencing that the primary structure of VT1 is exactly the same to that of Shiga toxin. 

Regarding the molecular mode of action of Shiga toxin, it has been known that Shiga toxin inhibits protein synthesis in eukaryotic cells by inhibiting elongation factor 1 (EF-1)-dependent aminoacyl-tRNA binding to 60S ribosomal subunits. We demonstrated that not only VT1 but also VT2 inactivated 60S ribosomal subunits and inhibited EF-1-dependent aminoacyl-tRNA binding in eukaryotic cells. To elucidate further the mode of action of VTs, we studied in conjunction with the results obtained on the mode of action of a plant rectin, ricin. Endo and his coworkers demonstrated that ricin showed RNA \( N^\text{glycosidase} \) activity and cleaved \( N^\text{glycosidic bond} \) of an adenosine at position 4,324 from the 5′ terminus of the 28S ribosomal RNA of 60S ribosomal subunit of rabbit reticulocytes (Fig. 3).

Since the molecular modes of action of Shiga toxin and VTs were found to be exactly the same as that of ricin, the amino acid sequences of the A

### Table 3. Biological activity of chemically synthesized STh, STp and their analogues (from Ref. 20)

| Amino acid sequence       | Minimum effective dose (ng/100µl) |
|---------------------------|----------------------------------|
| Asn-Ser-Asn-Tyr-Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr | STh(1–19) 0.8 |
| Asn-Ser-Asn-Tyr-Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys   | STh(1–18) 0.4 |
| Ser-Ser-Asn-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr   | STh(2–19) 0.8 |
| Ser-Asn-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr       | STh(3–19) 1.3 |
| Asn-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr           | STh(4–19) 1.1 |
| Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr               | STh(5–19) 0.8 |
| Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys                   | STh(5–18) 0.5 |
| Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr               | STh(6–19) 0.6 |
| Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys                   | STh(6–18) 0.6 |
| Asn-Thr-Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr   | STp(1–18) 1.0 |
| Asn-Thr-Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys       | STp(1–17) 1.3 |
| Thr-Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr       | STp(2–18) 0.5–2.0 |
| Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys/Tyr           | STp(3–18) 1.5–2.0 |
| Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr               | STp(4–18) 0.8–1.0 |
| Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys                   | STp(4–17) 1.2 |
| Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr               | STp(5–18) 0.8–1.0 |
| Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys                   | STp(5–17) 0.7 |
| Asn-Thr-Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr   | STp(1–18) 1.0 |
| Asn-Thr-Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys       | STp(1–17) 1.3 |
| Thr-Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr       | STp(2–18) 0.5–2.0 |
| Phe-Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys/Tyr           | STp(3–18) 1.5–2.0 |
| Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr               | STp(4–18) 0.8–1.0 |
| Tyr-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys                   | STp(4–17) 1.2 |
| Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys-Tyr               | STp(5–18) 0.8–1.0 |
| Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Ala-Gly-Cys                   | STp(5–17) 0.7 |
subunit of VT1, VT2 and ricin were compared to determine sequence homology(s). Three regions of the homology containing conserved (identical or chemically similar) amino acid residues were identified. By site-directed mutagenesis of the targeted gene to encode a specific amino acid, we constructed several mutant genes that encoded mutant VT1 with a single amino acid replacement in each of the three regions described above. It was found that among many mutant VT1s, two mutants that had the replacement of Glu 167 by glutamine (E167Q) and of Arg 170 by leucine (R170L) showed significantly reduced toxicity. The mutant VT1s were purified and their activities were examined. As shown in Table 4, both cytotoxic activity to Vero cells and inhibitory activity of protein synthesis in rabbit reticulocyte lysate were markedly decreased in the purified E167Q. It was demonstrated that the mutant VT1, E167Q, possessed similar antigenicities to that of wild type VT1, suggesting that the mutant VT1, E167Q, may be used as a candidate toxoid to protect VT1-mediated disease.

**Vibrio cholerae**

*V. cholerae* is classified into two biotypes, namely classical and El Tor. The classification is based on several phenotypes, such as susceptibility to polymixin B, chicken erythrocytes agglutination, hemolysis of sheep erythrocytes and Voges–Proskauer test which measures the production of acetylthiocholinesterase, and phage susceptibilities. The organisms of each biotype are further classified into serogroups on the basis of variations in the cell surface lipopolysaccharide (O antigen). More than 200 serogroups are so far identified. Moreover, both classical and El Tor biotypes show three different serotypes, namely Ogawa, Inaba and Hikojima. A summary of the classification is as shown in Fig. 4.

(i) **Discovery of V. cholerae O139.** Until 1992, it was known that only O1 serogroup of *V. cholerae* strain was associated with epidemic and pandemic cholera, and that strains which did not agglutinate with the O1 antiserum (collectively called non-O1 *V. cholerae*) were widely distributed in the aquatic environment and were responsible for sporadic cases of gastroenteritis.

In November 1992, non-O1 *V. cholerae* strains were isolated from patients of cholera-like disease in Chennai (then Madras), India where a large explosive outbreak of the disease occurred. Almost concur-

### Table 4. Comparison of biological activities of the purified mutant VT1s and wild-type VT1 (from Ref. 38)

| Toxin  | Cytotoxic activity CD50 (µg) | Mouse lethality LD50 (µg) | Inhibition of protein synthesis ED50 (µg) |
|--------|-----------------------------|---------------------------|-----------------------------------------|
| E167Q  | 30                          | 80                        | >100                                    |
| R170L  | 9                           | 10                        | 2                                      |
| Wild-type | 0.0001                   | 0.04                      | 0.008                                   |

Fig. 3. RNA N-glycosidase activity of VT1 (Shiga toxin), VT2 and ricin (from Ref. 37).
by the O1 to be indistinguishable from those of cholera caused by the O139 non-O1 strains of V. cholerae and found that all the isolated non-O1 strains and found that all the occurrences in the isolation rates of the dominant O1 serogroup to the non-O1 serogroup currently, an unexplained shift from the previously occurring since 1995.55) Initially it was predicted that V. cholerae O139 might spread all over the world and the eighth pandemic of cholera might be recorded, but the spread was restricted to the Indian subcontinent. Moreover, the isolation of O139 strains from cholera patients was so limited that the isolation rate in Kolkata these days has been less than 1%.48)

(ii) Emergence of V. cholerae El Tor variant and its cholera toxin production. In two biotypes of Vibrio cholerae O1, the classical biotype has been responsible for the fifth and sixth cholera pandemics, which were recorded during 1881–1896 and 1899–1923, respectively, while the El Tor biotype is responsible to the seventh pandemic which started in 1961 from Celebes (currently Sulawesi), Indonesia and is still ongoing. One of the characteristics of these two biotypes is that each biotype has unique gene sequences for cholera toxin B subunit (CTB), that is, classical ctxB and El Tor ctxB.

Nair et al.49) in 2006 in Bangladesh isolated strains that possess phenotypic El Tor biotype with classical ctxB. For this new type of strains of V. cholerae O1, we have recently proposed the designation of El Tor variants.50) Subsequent to the isolation of El Tor variant in Bangladesh,49) El Tor variant strains were isolated from several countries and areas in Asia and Africa.51)–53) In Kolkata, India, we also found that all V. cholerae O1 isolated were El Tor variant.55) Moreover, as shown in Fig. 5, El Tor variant appeared in 1990 and a complete replacement of prototype El Tor strains by El Tor variant strains occurred since 1995.55)

It has been known that clinical manifestation of cholera caused by classical strains is more severe than that caused by prototype El Tor strains. This phenomenon has been hypothetically explained due to a significant difference between the amounts of CT produced by these two biotype strains, that is, classical strains produce much more CT than prototype El Tor strains. Recently World Health Organization56) reported that V. cholerae El Tor variant causes more severe episodes of cholera with...
higher case fatality rates. This report prompted us to 
examine whether El Tor variant strains produce 
more CT than prototype El Tor strains. As shown in 
Fig. 6, it was found that the amount of CT produced 
by El Tor variant strains was more or less similar 
to that produced by classical strains and was much 
higher than that produced by prototype El Tor 
strains.\textsuperscript{57} From these data we hypothesize that 
severe symptoms of cholera caused by El Tor variant 
strains of \textit{V. cholerae} might be due to high CT 
production of the strains.

(iii) Viable but nonculturable \textit{V. cholerae}. 
The viable but nonculturable (VBNC) state in 
bacteria is defined as the bacteria remain viable but 
the cells do not grow or divide on, or in, routinely 
used bacteriological media. VBNC state of \textit{Vibrio cholerae} O1 was first reported by Colwell and her 
colleagues\textsuperscript{58} and this was subsequently confirmed by 
many groups.\textsuperscript{59)--64} In \textit{V. cholerae} O1 or O139, fresh 
culture of the bacteria was inoculated in a liquid of 
little nutrient like buffers and kept at low temper-
ature in dark, cells became VBNC state. VBNC state of 
\textit{V. cholerae} can be easily identified morphologi-
cally, as it became a round form as shown in Fig. 7.\textsuperscript{65} 
VBNC states of more than 60 species of 
pathogenic and non-pathogenic bacteria have so far 
been described.\textsuperscript{66} However, there has been some 
skepticism on this phenomenon as a current gold 
standard of bacteriology is based on culturability of 
bacteria on, or in, appropriate media.

Several conditions to convert VBNC to cultur-
able state have so far been reported. These are 
temperature upshift,\textsuperscript{60,67} incubation in phosphate 
buffer,\textsuperscript{68} supplementation with \textit{H}_2\textit{O}_2-degrading com-
ounds, such as catalase or sodium pyruvate,\textsuperscript{69} 
addition of heat-stable autoinducer of growth,\textsuperscript{70} 
addition of resuscitation promoting factor,\textsuperscript{71} and 
presence of \textit{Acanthamoeba castellanii}.\textsuperscript{72} However, 
there is no consensus to any of the above conditions 
as the stable condition to convert VBNC to 
culturable state.

Quite recently, we discovered that VBNC \textit{Vibrio cholerae} O1 and O139 were converted to the 
culturable state when co-cultured with eukaryotic 
cells, such as HT-29, Caco-2, T84, HeLa, and 
Intestine 407 and CHO cells (Table 5).\textsuperscript{65} In early 
works by Colwell’s group,\textsuperscript{73} it was demonstrated 
that inoculation of VBNC \textit{V. cholerae} O1 into rabbit 
ileal loops resulted in fluid accumulation from which 
culturable \textit{V. cholerae} O1 could be isolated. Moreover, 
it was shown that VBNC \textit{V. cholerae} O1 converted to the culturable state after ingestion
during a human volunteer study. Our results that showed the cultured eukaryotic cells converted VBNC \textit{V. cholerae} strains to culturable state might have reproduced the phenomenon that Colwell and her colleagues have demonstrated in rabbit and human volunteers.

Table 5. Conversion of VBNC \textit{V. cholerae} O139 VC-280 to the culturable state by co-culture with various eukaryotic cells (from Ref. 65)

|        | HT-29 | Caco-2 | T84 | HeLa | Intestine | CHO |
|--------|-------|--------|-----|------|-----------|-----|
| With eukaryotic cells in MEM-FBS | 1:32  | 1:16   | 1:32 | 1:16 | 1:16      | 1:32|
| With MEM-FBS alone               | —     | —      | —   | —    | —         | —   |

Fig. 7. Morphology of VBNC and culturable \textit{Vibrio cholerae} O139. The cells were labeled by GFP. (A) VBNC state, (B) culturable state. A bar represents 5 µm (from Ref. 65).

Table 5. Conversion of VBNC \textit{V. cholerae} O139 VC-280 to the culturable state by co-culture with various eukaryotic cells (from Ref. 65)

|        | HT-29 | Caco-2 | T84 | HeLa | Intestine | CHO |
|--------|-------|--------|-----|------|-----------|-----|
| With eukaryotic cells in MEM-FBS | 1:32  | 1:16   | 1:32 | 1:16 | 1:16      | 1:32|
| With MEM-FBS alone               | —     | —      | —   | —    | —         | —   |

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Profile

Yoshifumi Takeda graduated from School of Medicine, Osaka University in 1960 and initiated his scientific career in 1961 as a student of Dr. Tsunesaburo Fujino, the discoverer of *Vibrio parahaemolyticus*, at the Research Institute for Microbial Diseases, Osaka University. During last 50 years, he has been working on the pathogenesis of several enteric bacteria, such as *Vibrio parahaemolyticus*, diarrheagenic *Escherichia coli*, and *Vibrio cholerae*. Especially the role of toxins produced by enteric bacteria is the focus of his research interest. Among many contributions he made, several outstanding ones are: demonstration of the cardiotoxicity of the thermostable direct hemolysin produced by *Vibrio parahaemolyticus*, study on the structure–activity relationship of heat-stable enterotoxins produced by enterotoxigenic *E. coli*, demonstration of the RNA N-glycosidase activity of Shiga and Shiga-like toxins produced by *Shigella dysenteriae* and enterohemorrhagic *E. coli* and discovery of *V. cholerae* O139.

He had worked as Professor and Chairman of Department of Bacterial Infections at the Institute of Medical Sciences, the University of Tokyo (1983–1989), Professor and Chairman of Department of Microbiology, Faculty of Medicine of Kyoto University (1987–1995), Director General of the Research Institute of International Medical Center of Japan (1994–1999), and Director General of the National Institute of Infectious Diseases, Japan (1999–2001). Since June 2007, he is working as the Director of Collaborating Research Center of Okayama University for Infectious Diseases in India, which is located at the National Institute of Cholera and Enteric Diseases, Kolkata, India. His current research interest is study on Viable But Nonculturable *V. cholerae* and on a newly emerged *V. cholerae* El Tor variant.