Characteristics of *stx*-Converting Phages of EHEC O157

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Abstract

In this communication, we analyzed primarily an O157 strain isolated during the EHEC outbreak in Tokyo and revealed that all *stx*-converting phages examined were lambdoid and induced with ultraviolet light or norfloxacin and that an optimal dosage required for induction and patterns of time course in VT-specific DNA synthesis and toxin release were essentially similar to those in an O157 Sakai strain as described in the previous report.

But the relationship appears to be rather remote between *stx*-converting phages lysogenized in O157 strains prevalent in Sakai city, west of Japan and those in O157 strains prevalent in Tokyo and Nagano, east of Japan in 1996, since the immunity is different from each other. Thus, *stx*-converting phages derived from the same hosts or the same prevalent area are related to each other but lower degree of relatedness is observed for those derived from different years and areas.

These results may be accounted for by a hypothesis of the presence of a cassette of lysis genes associated with an *stx* gene that is mutually exchangeable by recombination between these phages.

Key words : *stx*-converting phage, EHEC O157, norfloxacin, prophage induction, Vero toxin, VT phage

Introduction

There was an epidemic of EHEC O157 and O26 in many places in Japan during summer 1996. We have reported the results of the studies using a Sakai strain of EHEC in the previous paper (1), since the magnitude of infection was the largest in Sakai city. In connection with high incidence of hemolytic uremic syndrome (HUS), we have discussed a possible relationship between prophage induction with norfloxacin (NFLX) followed by massive toxin production and HUS. In this communication, we investigated *stx*-converting phages from O157 : H 7 strains isolated in the infected area other than Sakai (for example, Nagano and Tokyo) and compared with VT 2 phages from a Sakai strain. The patterns of prophage induction and toxin production after NFLX treatment were very similar among these strains but the immunity of these phages was different.

Materials and Methods

Bacterial and phage strains

EHEC O157 V141 and EHEC O157 V424 were isolated by Tae Takeda and others (National Children's Hospital) during an epidemic in Tokyo and Nagano, respectively, in 1996. Both are single lysogens for VT 2 phage and designated VT 2 -141 and VT 2 -424, respectively. A Sakai strain EHEC O157 RIMD0509894 doubly lysogenic for VT 1 -Sa and VT 2 -Sa that had been characterized in the previous report was also used. An indicator strain was *E. coli* K12 W3110 *str-r* sensitive to all VT 2 phages.

Prophage induction

Prophage was induced by treatment with ultraviolet light (UV) or norfloxacin (NFLX) (2). Overnight cultures of the O157 strains and W3110 lysogens were refreshed for 1 h to an early logarithmic growth phase, resuspended in Tris dilution buffer, and irradiated with

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UV (at an incident dose rate of 1.66 J/m^2 for 30 sec) before growth in LB-broth. Alternatively, 1-h refreshed cultures of EHEC O157 strains and W3110 lysogens were treated with various concentrations of NFLX (an optimum dose of 1 μg/ml) for 30 min, washed by centrifugation, and resuspended in drug-free LB-broth for 2-h phage growth. Infective centers were scored by overlay-plating cells within a few minutes after induction with an indicator W3110 and the number of plaques appeared in the following day was counted ; efficiency of prophage induction being the number of infective centers divided by the number of plated induced cells.

Media and chemicals

E. coli strains were grown in LB (Luria-Bertani) -broth, LB-agar (1.2% agar), and LB-soft agar (0.6% agar) supplemented with vitamin B1. Norfloxacin (NFLX) was provided by Kyorin Pharmaceuticals, Inc. (3).

Toxin assay and toxin gene assay were performed as described in the previous report (1).

Results

Characteristics of *stx*-converting phages

EHEC O157 RIMD0509894 doubly lysogenic for VT1-Sa and VT2-Sa produced these two kinds of phage upon induction. Both phages formed minute or pinpoint plaques on a lawn of an indicator W3110 (λ nonlysogen) and they were indistinguinshable from each other from plaque morphology. By using phage lysates derived from individual plaques, W3110 strain was lysogenized. Many single lysogens were examined by PCR with primers specific for either VT1 or VT2 toxin gene. The results showed that all single lysogens were W3110 (VT2-Sa) but that no stable W3110 (VT1-Sa) was obtained.

EHEC O157 V141 was a single lysogen and produced only VT2-141 phage ; EHEC O157 V424 was also a single lysogen and produced only VT2-424 phage. Plaques formed by phages VT2-141 and VT2-424 were of similar size and much larger than those formed by VT2-Sa. Stable lysogens W3110 (VT2-141) and W3110 (VT2-424) were obtained.

These results indicate that K12 strain is endowed with *att* site and IHF (integration host factor) (4, 5) necessary for lysogenization of VT2 phage but devoid of or defective in those for VT1 phage.

The immunity among VT2 phages was examined by cross-streaking phage lysates on streaks of lysogens. As a result, it is revealed that VT2-Sa has an immunity different from that of VT2-141 or VT2-424 and that VT2-141 has the same immunity as VT2-424.

Although no lysogens of K12 strain for VT1-Sa were obtained, VT1-Sa phage lysates could be prepared starting from single plaques. Cross-streak method using these lysates demonstrated that W3110 (VT2-Sa) was immune against VT1-Sa phage.

Thus, VT1-Sa and VT2-Sa are related to each other comprising a group, and VT2-141 and VT2-424 are related to each other comprising another group ; there exists, however, a distance between these two groups.

Norfloxacin triggers prophage induction

There is a report that under certain conditions various antimicrobial agents including NFLX enhance the production and release of Vero toxin (VT) from EHEC strains (6). It has been shown that the *stx* gene encoding VT was located on the phage genome (7, 8, 9). We have examined whether NFLX caused prophage induction which would result in multiplication of *stx* gene and mass production of VT. Fig. 1 (a) shows the result of the prophage induction with EHEC O157 V141 strain singly lysogenic for phage VT2-141. The number of induced prophages as determined by infective center assay increased as NFLX concentrations increased, reaching a maximum at 1 μg/ml of the drug, and then decreased. The amount of VT2 toxin determined in parallel indicated a similar pattern to that of the prophage induction (Fig. 1 (b)). As expected, VT1 toxin was not detected.

The same type of experiments were performed using EHEC O157 RIMD0509894 strain as reported in the previous paper (1). The prophage induction and VT2 toxin production showed similar patterns to those of the above strains.

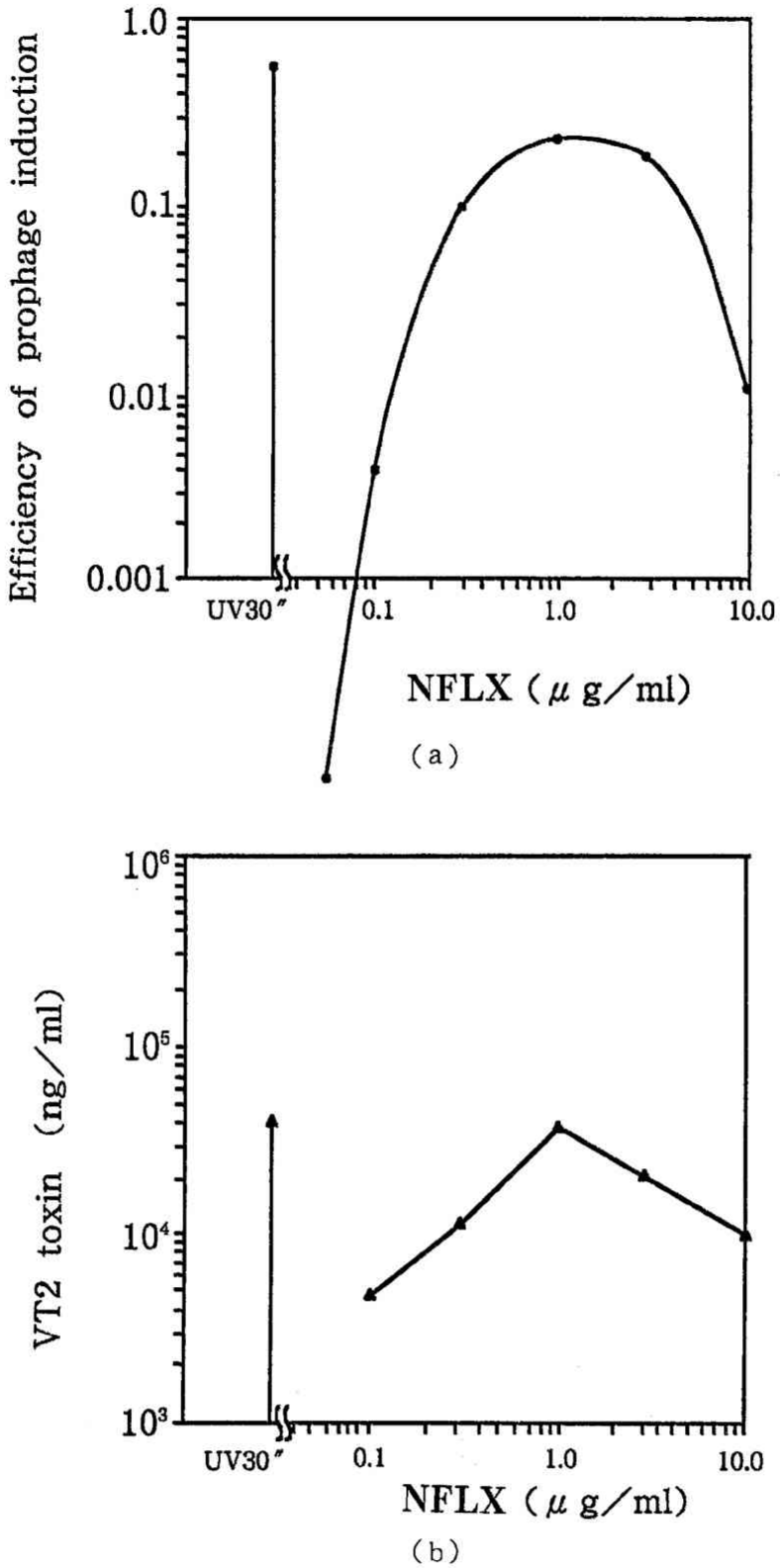

Fig. 1. Prophage induction in EHEC O157 V141 (VT2-141) strain. This strain is a single lysogen ; infective centers (a) and toxin production (b) are caused by phage VT2. The cells were treated with different concentrations of NFLX. The efficiency of prophage induction was maximal at 1 μg/ml of NFLX. The maximum toxin production was also observed at the same dose of NFLX and the amount of toxin produced was equivalent to that obtained by optimal UV irradiation. There is a parallel relationship between induction efficiency and VT production.

Time course analysis after induction

Fig. 2 shows time course experiments using EHEC O157 V141 strain (singly lysogenic for VT 2 -141). After induction with 1 μg/ml of NFLX, infective center (a), synthesis of *stx*-specific DNA (b), and amount of VT protein (c) were followed. All these values increased with time, and reached plateaus. Ninety min later, number of infective centers started to decrease rapidly due probably to inactivation of released phage particles.

The same type of experiments were performed using EHEC O157 RIMD0509894 strain as reported in the previous paper (1). Similar patterns were observed in these different strains.

The rate of multiplication of VT 2 toxin DNA was much faster in VT 2 -141 phage than in VT 2 -Sa phage. This difference will be reflected in their growth rate, amount of produced toxin and size of plaques.

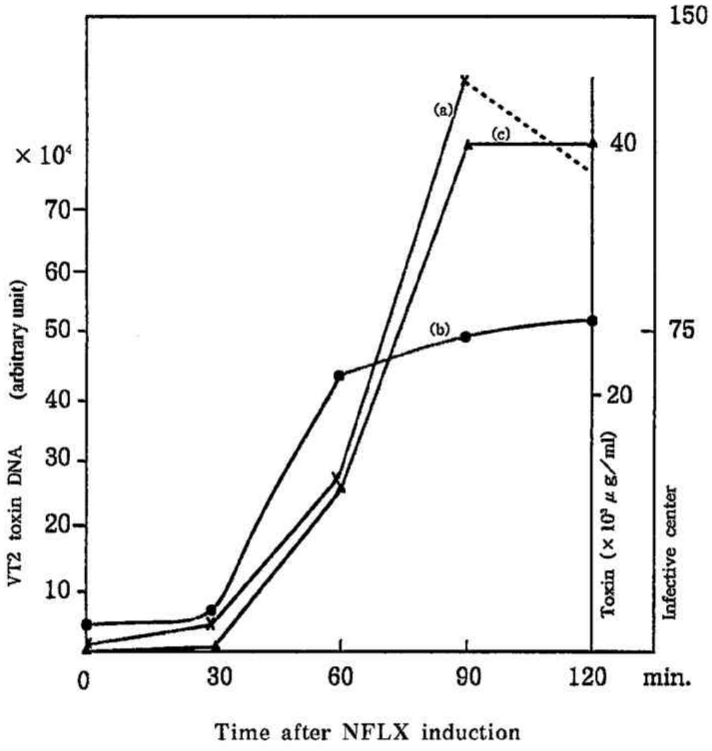

Fig. 2 . Time course patterns after NFLX induction of EHEC O157 V141 (VT 2 -141) strain. (a) Infective center ; (b) Toxin VT 2 ; (c) VT 2 -specific DNA.

Discussion

The finding that VT 1 -Sa and VT 2 -Sa both of which were lysogenized in O157 strains prevalent in Sakai city (west area of Japan), shared the same immunity reminds us of the report by Rietra et al. (10) ; VT 1 and VT 2 phages from a strain O157 E30480 showed the same immunity and so did VT 1 and VT 2 phages from a strain 933. These phages are considered as closely related from the points of phage morphology as revealed by electron microscopy, plaque size, restriction pattern of phage DNA, and other features.

Strain O157 V141 and strain O157 V424 were prevalent, respectively, in Tokyo and Nagano (east area of Japan) and the phages VT 2 -141 and VT 2 -424 they produced had the same immunity, formed large plaques and closely related.

It can be concluded that *stx*-phages derived from strains prevalent in the same hosts or areas are of the same origin and that those derived from different years and areas show a variation, although they are all inducible lambdoid phages. Nevertheless, there are VT 1 phage carrying *stx*- 1 and VT 2 phage carrying *stx*- 2 . This is due to the presence of a cassette of lysis genes associated with an *stx* gene that are mutually exchangeable between lambdoid phages through recombination (11, 12).

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