JOURNAL OF BACTERIOLOGY, Jan., 1966
Copyright © 1966 American Society for Microbiology
Vol. 91, No. 1
Printed in U.S.A.

NOTES

Hemolysin-Destructive Factor of *Vibrio cholerae* (*Vibrio comma*)

AKIRA WAKE AND MAKOTO YAMAMOTO
Department of Bacteriology I, National Institute of Health, Tokyo, Japan

Received for publication 30 July 1965

Feeley and Pittman (Bull. World Health Organ. **28**:347, 1963) speculated that a hemolysin (HL) destructive enzyme produced by El Tor vibrio at a late stage of growth lowers the HL titer. We proved the production of an HL-destructive factor (HDF) by strains of classical cholera and El Tor vibrio and established an in vitro method for quantitative titration of HDF.

HL was prepared by growing a hemolytic El Tor strain in heart infusion broth (Nihon Eiyo) or Syncase (Finkelstein et al., J. Infect. Diseases **114**:203, 1964) at 37 C for 20 hr and by centrifuging the culture in the cold. Filtration of the supernatant liquid through Millipore filters (type PH) decreased the HL titer. Titration of HL was performed by the method of Feeley and Pittman. HDF was prepared by the method used by Finkelstein et al. for preparation of choleragen. An HDF-producing strain was grown in 100 ml of brain heart infusion broth or Syncase in a 500-ml flask at 37 C for 18 hr with shaking at 112 strokes per minute. The supernatant fluid (*p*H 8.0 to 8.6), obtained by centrifugation, was filtered. The Millipore filtrate contained a reduced HDF titer. HL and HDF were kept frozen until use. The method for titration of HDF is given in Table 1.

HDF was detected at the 4th hour of cultivation, although the titer varied depending upon each strain. No HDF was detected when a rugose single colony was used as the inoculum. A high HDF titer was detected in rice water stools from cholera patients. The foregoing results may be an indication that HDF is the choleragenic factor itself. HDF is thermolabile, is resistant to 0.2% formalin, precipitates at 60% saturation of ammonium sulfate, and is active in a *p*H range of 6.90 to 8.60. HL of Ubon-type El Tor and that of a strain of NAG (NCTC 4715) was inhibited by HDF from a strain of classical cholera (Ogawa type).

TABLE 1. *HDF titers* of Vibrio cholerae (V. comma) from various sources† with or without various treatments*

Source of HDF	Source of HL	Treatment	Treated HDF titer	Untreated HDF titer
BHIB 114	Syncase Java 9	Millipore filtration	6	16
Syncase 114	Syncase Java 9		0	4
E 45	Syncase Java 9		0	16
L 45	Syncase Java 9		0	16
E 9	Syncase Java 9		0	32
E 9 smooth	Syncase Java 9		0	32
E 9 rugose	Syncase Java 9		0	0
L 63	Syncase Java 9		0	0
Java 9	Syncase Java 9		0	32
BHIB 114	Syncase Java 9	60 C, 30 min	16	128
BHIB 114	Syncase Java 9	100 C, 30 min	0	
Syncase 114	Syncase Java 9	60 C, 30 min	0	4
Syncase 114	Syncase Java 9	100 C, 30 min	0	
BHIB 114	Syncase Java 9	Formalin, 0.2%	32	64
Syncase 114	Syncase Java 9	5 C, 72 hr	16	16
E 45	Syncase Java 9		64	64
E 9 smooth	Syncase Java 9		64	64
Rice water stool (H.C.)	Syncase Java 9	Freezing	16	—
Rice water stool (J.D.)	Syncase Java 9	Freezing	512	—
Rice water stool (E.H.)	Syncase Java 9	Freezing	128	—
BHIB	NCTC 4715 (NAG)	Millipore filtration	8	—
E 9 smooth	NCTC 4715 (NAG)	Millipore filtration	—	16
L 45	NCTC 4715 (NAG)	Millipore filtration	—	16
BHIB 114	NCTC 3661 (Ubon)	Millipore filtration	16	—
E 9 smooth	NCTC 3661 (Ubon)	Millipore filtration	—	64
L 45	NCTC 3661 (Ubon)	Millipore filtration	—	32

* The method for titration of HDF was: HL was diluted in phosphate-buffered saline (*p*H 7.1) with the largest factor of dilution giving complete hemolysis; HL was diluted with the same factor in a specimen to be titrated for HDF; 1.0 ml of the HL diluted with the specimen was put into the first test tube in a series, and 0.5-ml portions in the other tubes; a series of twofold dilutions of the specimen with HL was made; the mixtures of the specimen and HL (0.5 ml) were incubated at 37 C for 2 hr; 0.5 ml of 1% sheep red cell suspension in the same buffered saline was added to each tube, and the mixtures were further incubated at 37 C for 2 hr; after reading hemolysis, the mixtures were kept overnight in the cold; and final reading was made, and the reciprocal of the highest dilution of HDF showing complete inhibition of hemolysis was taken as the HDF titer.

† The origins of the strains were as follows. BHIB114: classical cholera of Ogawa type, given by SEATO Medical Research Laboratory in Bangkok on 3 March 1962; cultivated in brain heart infusion broth. Syncase 114: the same strain, cultivated in Syncase (Finkelstein et al., 1964). E 34: a nonhemolytic El Tor strain of Ogawa type, isolated in Manila by Wake from a patient on 21 November 1964; has been subcultured twice on meat infusion-agar slants since isolation. L45: the same strain as E45, subcultured five times on the same medium. E 9: a nonhemolytic El Tor strain of Ogawa type isolated in Manila by Wake from a patient on 6 October 1964; has been subcultured twice on meat extract-agar slant since isolation. E 9 smooth: a strain originated from a single smooth colony of strain E 9 on a Trypticase Soy Agar (BBL) plate. E 9 rugose: a strain originated from a single rugose colony of strain E 9 on a Trypticase Soy Agar plate. L 63: a nonhemolytic El Tor strain of Ogawa type isolated in Manila by Wake from a patient on 22 October 1964; subcultured five times since isolation. Java 9: a hemolytic El Tor strain isolated in Semarang, and given to us on 8 November 1961. Besides the strains mentioned above, a classical cholera strain of Inaba type, strain 121, received from SEATO Medical Research Laboratory in Bangkok, produced detectable HDF in brain heart infusion broth and Syncase.