FUNCTION OF NEURAMINIDASE IN VIBRIO TAXONOMY

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The similarity between the morphological, cultural, and other properties of cholera and noncholera vibrios makes their differentiation difficult. Different groups of vibrios and closely related species were compared on the basis of their neuraminidase activity. High neuraminidase activity was found in cholera vibrios and in this respect they differed from noncholera vibrios and bacteria of the genera Pseudomonas and Comamonas.

Among all the microorganisms, Vibrio cholerae evidently possesses the highest neuraminidase activity, and for this reason it is used as a source of the enzyme on a commercial scale. The suggestion has been made that the formation of neuraminidase by Vibrio cholerae takes place parallel with the accumulation of type 2 toxins [7], including the factor that increases capillary permeability [8]. The writers consider that these findings are not accidental and that, consequently, neuraminidase plays an important role in the pathogenesis of cholera. The action of the enzyme is probably directed toward destruction of mucoproteins, which are ascribed [2] an important role, for example, in the protection of the gastro-intestinal tract from various pathogenic agents and the transfer of water or other compounds in the cells. A frequent manifestation of the action of neuraminidase on mucoproteins is its ability to change the surface of erythrocytes so that they are no longer agglutinated by myxoviruses. According to Tikhonenko [5] the essence of this process is that cell receptors of erythrocytes on which myxoviruses are adsorbed contain neuraminic (or sialic) acid, which is both an amino acid and a deoxy-sugar. Neuraminic acid occupies a terminal position in mucoproteins and is linked with the peptide part of the molecule through a glucoside bond. Neuraminidase splits the glucoside bond so that the free neuraminic acid goes into solution and the surface properties, including the charge of the erythrocyte, are sharply modified.

The object of the present investigation was to compare neuraminidase activity of a number of different vibrios and also of bacteria from which the agent of cholera has frequently to be differentiated, i.e., to study a problem which has received no attention in the literature. In addition, an attempt was made to detect correlation between neuraminidase activity and other characteristics of vibrios.

TABLE 1. Neuraminidase Activity of Various Microorganisms

Group	Microorganisms	Specific activity (median and its confidence interval calculated for a probability of 0.99)
1	V. cholerae	0,088 (0,037—0,177)
2	V. El Tor	0,064 (0,038—0,096)
3	V. El Tor	0,039 (0,026—0,062)
4	Noncholera vibrios	0,022 (0,011—0,031)
6	Comamonas	0,006 (0—0,11)
7	P. aeruginosa	0,010 (0,004—0,018)

EXPERIMENTAL METHOD

For most of the investigation eight strains of V. cholerae isolated from human sources (group 1), nine strains of Vibrio El Tor obtained from human sources (group 2), 15 strains of Vibrio El Tor obtained from hydrobionts, water, and sewage (group 3), 12 strains of noncholera vibrios (one strain of Heyberg's type III, three strains of type II, and the rest of type I) including luminescent (group 4), and four strains of NAG-vibrios isolated from patients with cholera or from

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TABLE 2. Change in Neuraminidase Activity during Keeping of V. El Tor in Water

Strain No.	Method of keeping	Specific activity
840	On semiliquid Martin's agar	0,062
(from water)	In water for 2 years	0,018
208	On semiliquid Martin's agar	0,052
(from water)	In water for 2 years	0,029
2044	On semiliquid Martin's agar	0,068
(from man)	In water for 2 years	0,036
7721	On semiliquid Martin's agar	0,052
(from man)	In water for 50 days	0,041

TABLE 3. Dispersion Analysis of Boundary Functions

Group of vibrios	F	F_T
V. cholerae and V. El Tor from man	33,00	6,55
The same from water, sewage, and hydrobionts	16,70	5,42
V. cholerae and noncholera vibrios	122,00	6,99
V. El Tor from man and V. El Tor from water, sewage, and hydrobionts	99,0	5,18
V. El Tor from man and noncholera vibrios	210,00	6,22
V. El Tor from water, sewage, and hydrobionts and noncholera vibrios	84,00	3,24

Note: F) Coefficient calculated from the experimental data; F_T) value of the coefficient for significant level of probability 0.99 [6].

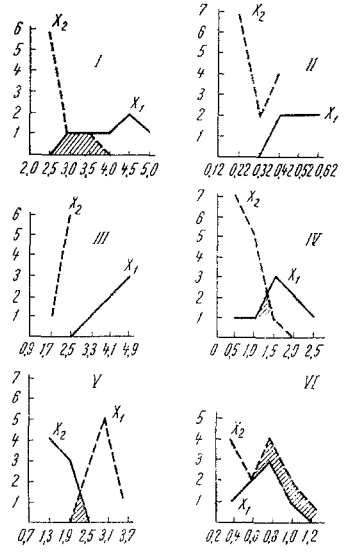

Fig. 1. Results of discriminant analysis of various groups of vibrios. Abscissa, values of boundary functions; ordinate, frequencies; X_1 and X_2) absolute values of boundary functions for compared groups of vibrios; I) groups 1 and 2; II) groups 1 and 3; III) groups 1 and 4; IV) groups 2 and 3; V) groups 2 and 4; VI) groups 3 and 4.

carriers (group 5) were used.* In addition, eight strains of Pseudomonas aeruginosa (group 6) and seven strains of Comamonas (group 7) were used in the investigation.

All strains were kept in semiliquid agar under mineral oil at room temperature. The cultures were seeded from semiliquid agar on Martin's broth, pH 7.6, and, after incubation for 6-8 h at 37°C, on Martin's alkaline agar. Next day, 0.1 ml of the cell suspension (1.5×10^5) was added to 15 ml liquid medium consisting of 3% peptone digest and 0.5% NaCl (pH 8.0) in 100-ml conical flasks. The microorganisms were incubated for 8 h with constant shaking at 37°C. The degree of turbidity of the culture was then determined (FÉK-n57 nephelometer, No. 11 filter); an optical density of 0.1 was taken as the unit of turbidity. After sedimentation of the cells by centrifugation for 30 min at 3000 g, merthiolate was added to the supernatant to a final concentration of $1:10^4$. This liquid as such was used in the investigation ("enzyme"). Before the experiment the enzyme was diluted 1:4.

Strains of Comamonas and Pseudomonas gave no visible growth on medium with 3% peptone and 0.5% NaCl during agitation for 8 h. For this reason, their cultivation was continued for 24 h.

Ovomucoid, prepared by the formula of Orlova and Mekhedov [3], was used as substrate for neuraminidase. Experimental samples consisting of 0.5 ml substrate (3.2 mg protein), 0.5 ml enzyme, and 0.5 ml acetate buffer (0.4 M, pH 5.5) with calcium chloride (final concentration 3×10^{-6} M) were incubated for 15 min in a water bath at 37°C. The reaction was stopped by the addition of 0.5 ml sodium metaperiodate (0.2 M) in phosphoric acid (9 M). Sialic acids were determined by the thiobarbiturate method [9].

*These vibrios are regarded as Vibrio El Tor which has lost its agglutinability by the corresponding O-antisera.

Spectrophotometry was carried out against "zero" samples at 549 nm. Neuraminidase activity was expressed in μM N-acetyl neuraminic acid (coefficient of molar extinction 5.7×10^4) per unit of cell turbidity (see above) per ml undiluted enzyme solution.

EXPERIMENTAL RESULTS

The results of the determination of neuraminidase activity in all species of bacteria investigated are given in Table 1. Only the results relating to NAC-vibrios are excluded, for they were obtained by the study of a small number of strains and were not amenable to statistical analysis with the use of a median [1]. The specific activity of the strains of NAG-vibrios was 0.062, 0.152, 0.156, and 0.175.

The following conclusion can be drawn from these results. By their neuraminidase activity the strains of V. cholerae, including NAG-vibrios, differ significantly from noncholera vibrios. The exceptions are some strains of V. El Tor from group 3, which resemble noncholera vibrios. Meanwhile, vibrios of groups 1-5 are easily distinguished from the microorganisms of groups 6 and 7, in which, because of slow growth on peptone water, even if neuraminidase could be detected this was not so in the first 24 h after seeding.

Turning to the question of the vibrios from group 3, their relatively low neuraminidase level compared with other cholera vibrios is evidently connected with their acquisition of saprophytic properties through their prolonged existence outside their obligate host – man. This was confirmed by the decrease in neuraminidase activity in V. El Tor when kept in sterilized river water (Table 2). Keeping in water also affected other properties of the cholera vibrios and, in particular, their decarboxylase activity [4].

In the next experiments the neuraminidase activity of the vibrios was compared with the activity of other well-studied enzymes, namely alkaline phosphatase and deoxyribonuclease. The method of discriminant analysis [6], by means of which two objects can be compared with respect to several characteristics, was used for the comparison. The results of a study of four groups of vibrios in relation to three characteristics are shown as graphs (Fig. 1). By this means some strains could be differentiated from others, which previously had been impossible on the basis of the same characteristics taken separately. At the same time, this method also reflects to some extent the kinship between the vibrios (the shaded part on Fig. 1). The fact that the difference between all four groups of vibrios is not fortuitous but highly significant is confirmed by the results of dispersion analysis of the boundary functions X_1 and X_2 (Table 3).

To determine the absolute importance of neuraminidase activity when combined with two other characteristics for differentiation between vibrios, correlation analysis was used to compare the strains with each other in relation to activity of two enzymes: neuraminidase and deoxyribonuclease, deoxyribonuclease and alkaline phosphatase, and alkaline phosphatase and neuraminidase. The results showed that the same four groups of vibrios could not be differentiated by this method. Consequently, the results suggest that neuraminidase plays a special role in vibrio taxonomy.

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