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THE ROLE OF SIALIC ACID IN NATURAL MUD IN THE GROWTH AND NEURAMINIDASE SECRETION OF *VIBRIO CHOLERAE non-O1* STRAINS

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Abstract: The growth rate and multiplication of *Vibrio cholerae non-O1* strain in the mud from the Pomorie lake (near the Black sea) were studied. The neuraminidase enzyme secretion and its accumulation at different growth phases and different cultivation temperatures were studied too. The obtained results demonstrate that the mud from the Pomorie lake is a good vibrio growth medium and substrate for the neuraminidase enzyme secretion. The role of the neuraminidase enzyme in the metabolism of mucins and glycoproteins on trophic level was confirmed.

Keywords: *Vibrio cholerae non-O1*, neuraminidase, mud.

1. INTRODUCTION

The representatives of genus *Vibrio* are natural inhabitants of the aquatic environment. Some species are pathogenic for humans. *V. cholerae* strains belonging to serotypes *O1 and O139* cause cholera, a severe epidemic disease, resulting in dehydration and death (Kaper et al., 1995). *V. cholerae* strains, possessing the genetic potential to cause epidemic diseases are generally referred to as *non-O1/non O139 V. cholerae.*

In recent years, sporadic cases or limited outbreaks caused by *non-O1/non O139 V. cholerae* (Bhattacharya et al., 1998; Morris, 1990) have been reported. *V. cholerae* is part of the natural bacterial flora of the aquatic medium, including fresh, brackish and salty waters (Colwell and Hug, 1994). The ecological changes in the sea and estuarine medium provide good conditions for dissemination of *V. cholerae* and further the appearance of new epidemic strains, spread by birds and wastewater (Ruiz et al., 2000). The elucidation of mechanisms of pathogenic microorganisms' adaptation to the changes of environment conditions is a question of practical and theoretical interest. This depends on the genetic and biochemical abilities of bacteria with pathogenic potential to inhabit objects of the environment at various temperatures and limit of the available nutrition substances (Reid et al., 2002). Neuraminidase is an enzyme found in a number of viruses and bacteria, including *V. cholerae* (Burnet and Stone, 1947) and *V. cholerae non-O1* (Muller and Lutticken, 1974).

Bacterial neuraminidases are induced by a number of low- and high-molecular weight substances all of which possess terminal non-reducing N-acetyl-neuraminic groups. A number of natural substrates as soil, mud, water, etc., which contain mucins are suitable substrates for induction of the enzyme, as its secretion at the saprophytic free-living vibrios and aeromonads takes part in the metabolization of mucins and glycoproteins at trophic level.

The aim of the present work was to investigate the role of the Pomorie lake mud as a natural substrate containing mucins in the growth and neuraminidase production of *V. cholerae non-O1* in a wide temperature range and its relation to the growth phases.

2. MATERIAL AND METHODS

2.1. Bacterial strains

Research experiments were carried out with 17 *V. cholerae non-O1* strains of a different origin, pathogenic potential and neuraminidase production from the collection of the National Center of Infectious and Parasitic diseases in Sofia, kindly provided to us by associated professor, Doctor T. Kantardjiev. Tryptic soy broth (TSB) (Difco, Detroit, MI, USA), supplemented with 1% NaCl and pH 8.2, was used as a growth medium for the studied strains.

2.2. Cultivation conditions

The 24-hour culture was resuspended in phosphate buffer to bacterial suspension with density $1x10^6$ cells/ml. Flasks containing 50 ml sterile Pomorie lake mud were inoculated with 1 ml bacterial suspension. Flasks containing 50 ml

nutrient broth supplemented with 0.1% glucomacropeptide and inoculated with 1 ml bacterial suspension were used as control cultures. The bacterial cultures were incubated at 4 ^{0}C, 25 ^{0}C and 37 ^{0}C for 72 hours. The cell quantity was determined by serial dilutions and counting of colonies on tryptic soy agar with pH 8.2 (Difco, Detroit, MI, USA). The results were presented as the arithmetic mean of two experiments, each performed twice.

2.3. Mud from the Pomorie lake.

Mud from the Pomorie firth lake (near the Black sea), sterilized in autoclave at 121 ^{0}C for 15 minutes, was used as a growth medium and inducer for the neuraminidase enzyme secretion.

Table 1. Content of free and bounded sialic acids in mud from Pomorie lake.

Pomorie mud	Bounded sialic acids (μg/ml)	Free sialic acids (ì g/ml)
Sterile mud	0.320 – 0.345	0.175 – 0.210
Non-sterile mud	0.290 – 0.320	0.160 – 0.175

2.4. Neuraminidase activity.

The neuraminidase activity was determined quantitatively according to Aminoff (1961). Glucomacropeptide (Abrashev et al., 1979) was used as a substrate and inducer. We assumed one unit (U) of neuraminidase activity as the amount that releases 1 μg of sialic acid from the glucomacropeptide substrate for 1 minute under standard conditions.

Table 2. Neuraminidase activity of *V. cholerae non-O1* strains isolated from different sources.

Strain	Source	Group according to Heiberg	Neuraminidase activity (U/ml)
V.cholerae non-O1 30	River water	I	20
V.cholerae non-O1 29	River water	II	28
V.cholerae non-O1 14	River water	II	26
V.cholerae non-O1 15	River water	II	24
V.cholerae non-O1 17	River water	II	19
V.cholerae non-O1 16	River water	II	21
V.cholerae non-O1 7	River water	I	17
V.cholerae non-O1 42	River water	I	23
V.cholerae non-O1 19	Sewage	I	25
V.cholerae non-O1 13	Sewage	II	22
V.cholerae non-O1 6	Sewage	II	18
V.cholerae non-O1 27	Sewage	II	27
V.cholerae non-O1 18	Sewage	I	23
V.cholerae non-O1 8	Seawater	I	24
V.cholerae non-O1 9	Seawater	IV	22
V.cholerae non-O1 11	Seawater	III	21
V.cholerae non-O1 1	Seawater	II	25

3. RESULTS AND DISCUSSION

A precondition of these investigations was the suggestion of Műller (1974) that a number of natural substrates as soil, mud and water containing mucins are a suitable substrate for induction of the neuraminidase enzyme. The enzyme secretion in saprophytes, free-living vibrios and aeromonads effects on the metabolization of mucins and glycoproteins at trophic level. We studied the effect of different temperatures and the presence of inducer in the medium on the growth, multiplication and enzyme secretion of *V. cholerae non-O1* in a model system containing firth mud from Pomorie lake. Previous to our studying the role of the firth mud it was tested for presence of sialic acids (Table 1). It appears from the table that the bounded sialic acids content varies from 0.320 μg /ml to 0.345 μg /ml in sterile mud and from 0.290 μg /ml to

0.320 μg /ml in non-sterile mud. Meanwhile, the content of free sialic acids was studied and it was considerably lower – from 0.175 μg/ml to 0.210 μg /ml in sterile mud and from 0.160 μg /ml to 0.175 μg/ml in non-sterile mud. There are no data in the literature available to us about presence of sialic acids in lake sediments. There are no data about the role of the sialic acids in the behaviour of pathogenic microorganisms in natural substrates. The presence of sialic acids in the firth mud is essential, because they are inducer for the biosynthesis of neuraminidase enzyme.

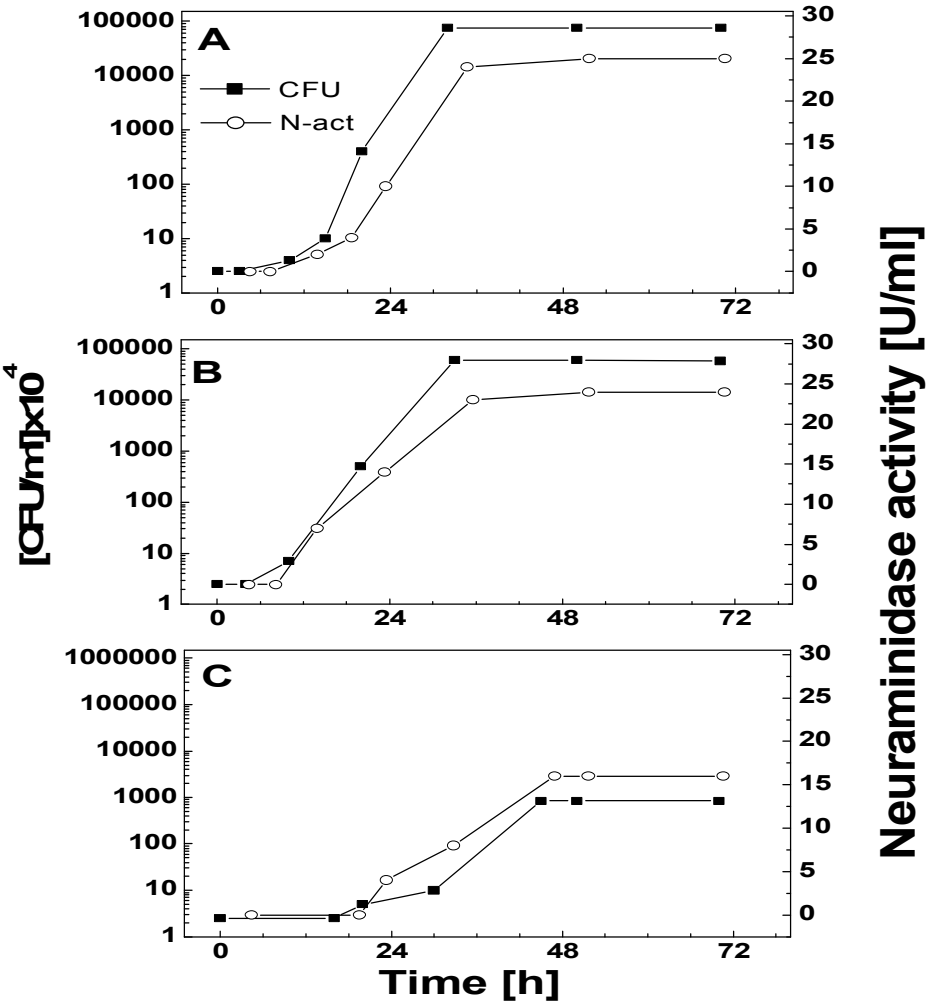

Fig. 1. Growth and neuraminidase activity of *V. cholerae non-O1* 29 strain cultivated in nutrient broth at 37˚C (A), 25˚C (B) and 4˚C (C).

In view of choosing a proper enzyme producer, we carried out a screening of 17 *V. cholerae non-O1* samples isolated from different sources of environment – river, sewage and seawater (Table 2). All the 17 samples produced neuraminidase activity between 17 and 28 U/ml. There was no statistically important difference in the neuraminidase activity levels between the isolates from different sources and the type according to Heiberg ($p>0.05$). *V. cholerae non-O1* 29 strain appeared to be the best producer (28 U/ml) and this determined its following investigation. The results from the experiments on enzyme biosynthesis at each growth phase of this strain are represented on Fig. 1 – in broth and on Fig. 2- in firth mud. It was found that the lag-phase of the broth culture at 37 ^{0}C continues to the 3rd hour and no neuraminidase was produced during this period. The enzyme synthesis starts at the beginning of the log-phase, increases quickly, reaches its maximum (25 U/ml) at the 32nd hour and keeps this level till the 72nd hour of incubation. The lag-phase at 25 ^{0}C and 4 ^{0}C was 4 and 16 hours respectively, as enzyme synthesis in this period was not observed

too. The maximum enzyme secretion was observed in the end of the log-phase and in the beginning of the stationary phase, 24 U/ml and 16 U/ml respectively.

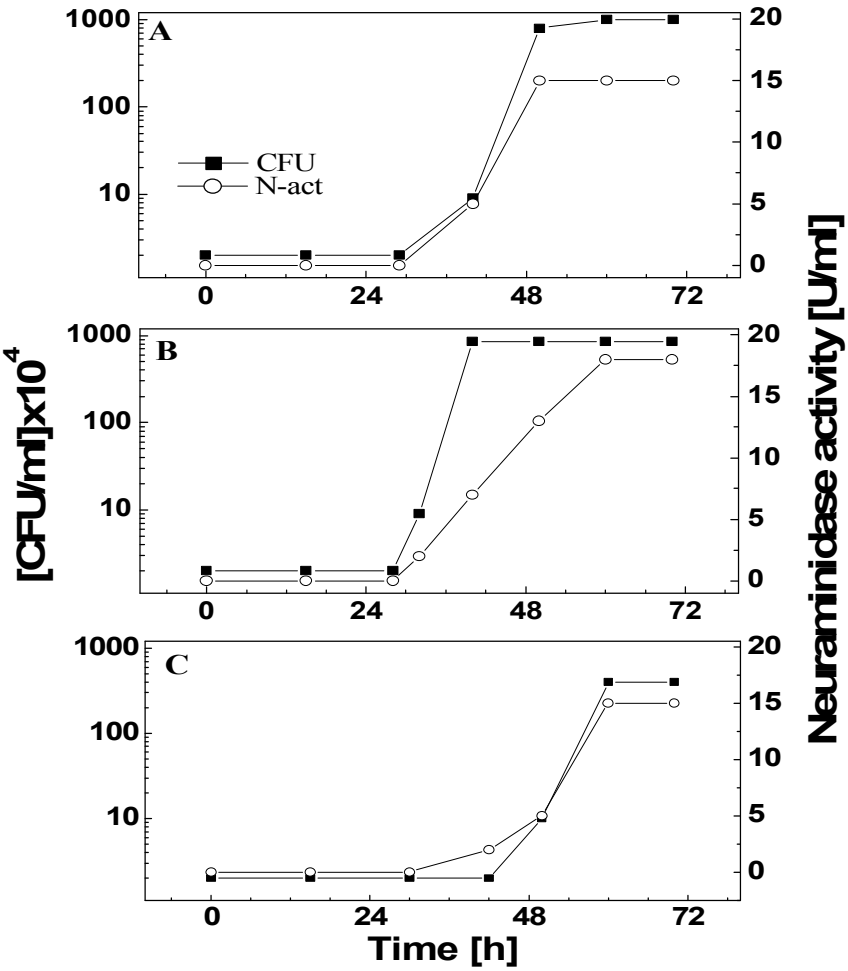

Fig. 2. Growth and neuraminidase activity of *V. cholerae non-O1* 29 strain cultivated in mud from Pomorie lake at 37˚C (A), 25˚C (B) and 4˚C (C).

4. CONCLUSION

The results show that the Pomorie lake mud is an appropriate natural substrate for the vibrios growth, multiplication and neuraminidase secretion. Results obtained elucidate the metabolism of mucins and glycoproteins at trophic level and of preserving some pathogenicity determinants as neuraminidase enzyme as well.

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ÐÎËß ÍÀ ÑÈÀË Î ÂÀÒÀ ÊÈÑËÈÍÀ Â ÅÑÒÅÑÒÂÅÍÀ ÒÈÍß ÏÐÈ ÐÀÑÒÅÆÀ È ÍÓÐÀÌÈÍÈÄÀÇÍÀÒÀ ÑÅÊÐÅÖÈß ÍÀ ÙÀÌ Î ÂÅ *VIBRIO CHOLERAE non-O1*

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Ðåçþìå: Ïðîó÷åíè ñà ðàñòâîðèìîñòòà è ðàçëîæàâàíåòî íà ùàì *Vibrio cholerae non-O1* â êàë îò Ï î ìîðèéñêîòî åçåðî (áëèçî äî ×åðíî ìîðå). Ïðîñëåäåíà å ñúùî ñåêðåöèÿòà íà åíçèìà íóðàìèíèäàçà è íåãàòèâíî íàòðóïàâàíå ïðåç ðàçëè÷íèòå ðàñòâîðèìè ôàçè è ðàçëè÷íè òåìïåðàòóðè íà êóëòèâèðàíå. Ïîêó÷åíèòå ðåçóëòàòè ïîêàçâàò, ÷å êàëòà íà Ï î ìîðèéñêîòî åçåðî å äîáðà ñðåäà çà ðàçâèòèå íà âèáðèîíèòå. Ñèàëîâèòå êèñåëèíè, ñúäúðæàùè ñå â íåÿ, ña èíäóêòîð çà ñâêðåöèòà íà åíçèìà íóðàìèíèäàçà. Ïîâúðõíà å ðîëÿòà íà òîçè åíçèì â ìåòàáîëèçìà íà ìóòèíèòå è ãëèêîïðîòåèíèòå íà òðîô÷íî íèâî.