ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 2, pp. 240–241. © Pleiades Publishing, Inc., 2006.
Original Russian Text © G.A. Vydryakova, T.V. Kirpichenko, 2006, published in Mikrobiologiya, 2006, Vol. 75, No. 2, pp. 287–288.

SHORT COMMUNICATIONS

Proteolytic Activity of Luminous Bacteria

G. A. Vydryakova[a,1] and T. V. Kirpichenko[b]

[a] *Institute of Biophysics, Siberian Division, Russian Academy of Sciences, Akademgorodok, Krasnoyarsk, 660036 Russia*

[b] *Krasnoyarsk State University, Svobodnyi pr. 79, Krasnoyarsk, 660041 Russia*

Received September 15, 2005

DOI: 10.1134/S0026261706020214

In spite of the extensive investigations into the symbiosis between luminous bacteria and marine animals, the mechanism of this phenomenon is far from being well understood. It is believed that microbial synthesis of proteases can be responsible for the colonization of fish tissues with microbial cells. The study of the symbiosis between the squid *Euprymna scolopes* and the luminous *Vibrio fischeri* bacterium showed that the coelomic fluid of the light organ of the squid contains the host-derived amino acid glycine [1]. It was also found that *N*-acetyl-D-galactosamine inhibits the reaction of erythrocyte hemagglutination induced by the symbiotic bacterium *Photobacterium phosphoreum* [2], up to partial or complete hemolysis. It can be assumed that the presence of glycine in the coelomic liquid is either due to the action of the proteases of the symbiotic strains or to the induction of their synthesis in response to the interaction of *N*-acetyl-D-galactosamine–specific bacterial lectin with the receptors of the light organs.

To understand the role of proteolytic enzymes in the formation of symbiosis between luminous bacteria and marine animals, we assayed the proteolytic activity of luminous bacteria of various taxonomic and ecological groups in media both with and without N-acetyl-D-galactosamine.

The experiments were performed using luminous strains and dark spontaneous mutants (including aldehyde-dependent mutants) of symbiotic *P. phosphoreum* cultures; the free-living bacteria *P. phosphoreum, P. leiognathi*, and *Vibrio harveyi*, as well as the *V. fischeri* commensal strains obtained from the collection of cultures at the Institute of Biophysics, Siberian Division, Russian Academy of Sciences (IBSO 836).

The proteolytic activity of the culture liquid was assayed by the Anson's method. One unit of proteolytic activity (U) was defined as the amount of enzyme that catalyzed the formation (in 1 min at37°C) of TCA-soluble products in an amount equivalent to 1 μmol tryptophan, when stained with the Folin reagent.

Proteolytic activity was expressed in U/mg protein.

[1]Corresponding author; e-mail: vg@ibp.ru

Table 1. Proleolytic activity of the symbiotic luminous and nonluminous *P. phosphoreum* strains

Strains	Luminescence	Protease activity, U/mg protein
Symbiotic *P. phosphoreum* strains		
1844	+	0.010
1909	+	0.011
1940	+	0.011
1949	+	0.010
Mean		0.011 ± 0.005
Spontaneous dark mutants of symbiotic *P. phosphoreum* strains		
1780 (aldehyde-dependent)	–	0.012
1911	–	0.012
1911 (aldehyde-dependent)	–	0.011
1940	–	0.012
Mean		0.012 ± 0.0004
Symbiotic *P. phosphoreum* strains in the presence of *N*-acetyl-D-galactosamine		
1780 (aldehyde-dependent)	–	0.010
1844	+	0.010
1909	+	0.010
1911	–	0.011
1911	+	0.005
1940	–	0.011
1940	+	0.005
1949	+	0.021
Mean		0.011 ± 0.004

Table 2. Proteolytic activity of free-living luminous and commensal bacterial strains

Strains	Source	Protease activity, U/mg protein
P. phosphoreum		
2096	Sea water	0.010
1697	Sea water	0.005
1708	Sea water	0.014
1693 (in the presence of *N*-acetyl-D-galactosamine)	Sea water	0.010
1693	Sea water	0.012
Mean		0.010 ± 0.005
P. leiognathi		
208	Sea water	0.043
54	Sea water	0.043
302	Sea water	0.040
212	Sea water	0.032
530	Sea water	0.050
Mean		0.042 ± 0.004
V. fischeri		
2089(1)	Stomach	0.060
2089(2)	Stomach	0.070
1920	Intestines	0.032
1231	Intestines	0.047
Mean		0.052 ± 0.012
V. harveyi		
94	Sea water	0.070
72	Sea water	0.075
103	Sea water	0.075
Mean		0.073 ± 0.002

Protein was quantified by the method of Lowry et al.

The induction of protease synthesis by symbiotic luminous *P. phosphoreum* strains was studied by adding *N*-acetyl-D-galactosamine at a concentration of 0.1% to the cultivation medium.

The experiments showed the presence of proteolytic activity in all the cultures under study (Table 1), which was almost the same in luminous *P. phosphoreum* strains (0.011 ± 0.005 U/mg protein) and nonluminous mutants (including aldehyde-dependent ones) of the symbiotic *P. phosphoreum* strains (0.012 ± 0.0004 U/mg protein). The addition of *N*-acetyl-D-galactosamine to the cultivation medium did not induce the synthesis of proteolytic enzymes in *P. phosphoreum* (Table 1). The proteolytic activity of the marine *P. phosphoreum* cultures was close to that of the symbiotic strains of this bacterium, comprising 0.010 ± 0.005 U/mg protein (Table 2). The activity of extracellular proteases of the free-living bacterium *P. leiognathi* was four times that of the marine *P. phosphoreum* strains and comprised 0.042 ± 0.004 U/mg protein. The proteolytic activities of the commensal *V. fischeri* strains and free-living *V. harveyi* were still greater, making up 0.052 ± 0.012 and 0.073 ± 0.002 U/mg protein, respectively (Table 2).

Thus, the proteolytic activity of symbiotic and free-living *P. phosphoreum* strains is close, it is not induced by *N*-acetyl-D-galactosamine, and is considerably lower than that of the other luminous bacteria studied. This finding suggests that proteases might not be responsible for the colonization of marine animals with the luminous bacterium *P. phosphoreum*.

REFERENCES

1. Graf, J.L. and Ruby, E.G., Host-Derived Amino Acids Support the Proliferation of Symbiotic Bacteria, *Arch. Microbiol.*, 1998, vol. 95, pp. 1818–1822.
2. Vydryakova, G.A., The Carbohydrate Specificity of Luminous Bacteria, *Prikl. Biokhim. Mikrobiol.* (in press).