APPLYING TRANSCRIPTOME PROFILING TO EXPLORE THE QS-SYSTEMS IN THE FISH PATHOGEN *ALIIVIBRIO SALMONICIDA*

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Bacteria coordinate activities as a population, which likely provides a selective advantage in the natural environment by allowing them to alter their morphology and physiology quickly to adapt to environmental changes. In order to adapt, bacteria need a wide variety of mechanisms for sensing and responding to these changes. Recent work has clarified many aspects of how bacteria communicate and synchronize cell behaviour through an elegant process known as quorum sensing (QS). QS mediated by signal molecules, referred to as Autoinducers (AI), enable bacteria to control and synchronize behaviour such as motility, biofilm formation, virulence factor production and bioluminescence under different environmental conditions. Although some details of QS are known for a few model organisms, the understanding of the broader role of QS in gene regulation and the diversity of adaptive responses and how these responses are linked to virulence remains fragmented.

To address the lack of knowledge of the diversity of adaptive stress responses, such as intra- and inter-species communication, population-level cooperation, and the principles underlying signal transduction and information processing during infection *Aliivibrio salmonicida*, the causative agens of cold-water vibriosis has been used as a model system. The observed phenotypic variability due to gene knockouts in the sensing and responding systems (QS-systems), using transcriptome profiling (microarray), will be presented.

ACHIEVEMENTS AND PROSPECTS IN LONG-DISTANCE TRANSPORTATION OF LIVE RED KING CRAB *PARALITHODES CAMTSCHATICUS*

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Red King Crab (*Paralithodes camtschaticus*) is one of the most valuable and expensive seafood delicacies. It comes to the market in different forms: cooked-frozen legs, raw-frozen legs, boiled meat, etc... The most valuable product, providing the best preservation of food quality, is live red king crab. But there are some problems associated with operational live crab transportation from place of catch to the final consumer. Crabs habitats are far away from major economic centers and transportation hubs. That's the Sea of Okhotsk in Russia, where the catch of crab in the declining population has virtually closed, the coast of Alaska in the United States, the Barents Sea in Russia and northern Norway. Experiments show that crabs can be kept for a long time out of water and are transportable by air. But when transportation takes more then 24 hours, there is significant mortality. Works on developing new and improving existing methods of life crabs transportation are actual and are aimed at increase in duration of transport and reducing mortality.

Experiments were conducted on the land based water tank complex Norway King Crab (Byugoynes, Norway) and storage complex with closed recirculation water system (CRWS) – «La Maree» LTD. (Moscow, Russia). In addition, were analyzed the outcome of life crabs sending from the northern part of Norway to Belgium, France, Italy, Korea, Japan and China. Crabs for experiments were caught in the Varanger Fjord (Norway).

Polystyrene boxes with wet or dry material inside were used for crab's transportation. Low temperature maintained using frozen gel-ice.

Fig. 1. Polystyrene transport box with crabs

The monitoring of physiological condition was carried out using the method of noninvasive pulsometry. To read the heartbeat parameters and transmit information to the computer an optic fiber sensor was fastened on crab's carapace. As a result crab's heart rate and stress-index were recorded.

To establish the maximum crabs are still alive out of water, as well the impact of various parameters on the physiological condition of the crab, the experimental group was formed. Crabs from this group were subjected to simulate transport without water. Crabs from this group were put in same transport boxes with frozen gel-ice and different materials inside.

The influence effect of humidity level inside the container on the transport duration was studied on a group of 14 crabs. Half of them were put inside the transport boxes with wet foam. Other crabs were kept out of water for 5–10 minutes to remove moisture from the gill chamber and after that were set inside the boxes with dry material.

Crabs mortality data and general physical condition of the surviving animals after the transport from northern part of Norway to the cities of Europe and Asia was collected. For estimation of crabs activity after transportation visual census method was used.

Before and after transport the washings from crab's gills were carried and the hemolymph samples were taken for determination of the quantity of substances. Ammonia content in the washings was quantified by Sedghi- Solorzano Method.

In the proccess of experiments it was found that crabs can stay alive in polystyrene containers without water for up to 81 hours at a temperature up to 7 degrees. The heart rate of four different crabs presented in Figure 2.

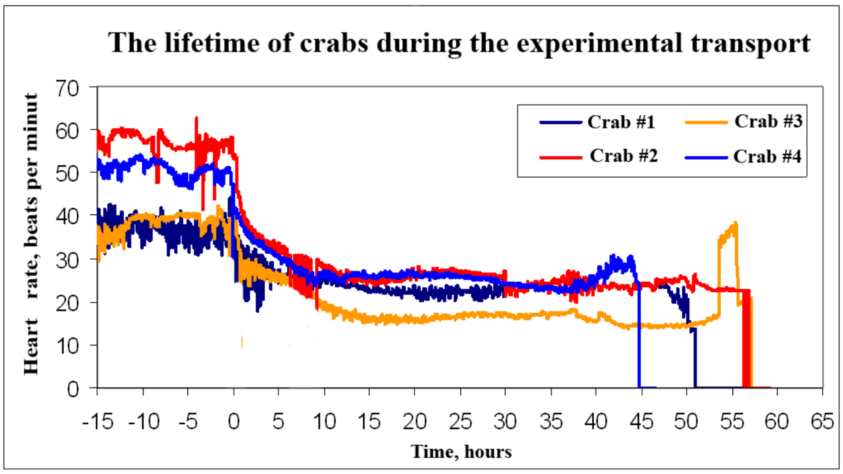

Fig. 2. The lifetime of crabs during the experimental transport determined through monitoring of cardiac activity

Using dry material inside containers and pre-transportation exposure of crabs out of water show significantly not worse results than the traditionally used method where the crabs are covered by wet foam ore paper. General results presented in the Table1.

Table1. The results of crab's life expectancy in boxes with wet and dry material

	Wet material	Dry material
Number of crabs	7	7
Minimum lifetime, hours	27	41
Maximum lifetime, hours	59	61
Average lifetime, hours	43	53
Standard deviation, ± hours	10	8,3

Consequently average lifetime was 43 hours for crabs in wet boxes and 53 hours for dry transport.

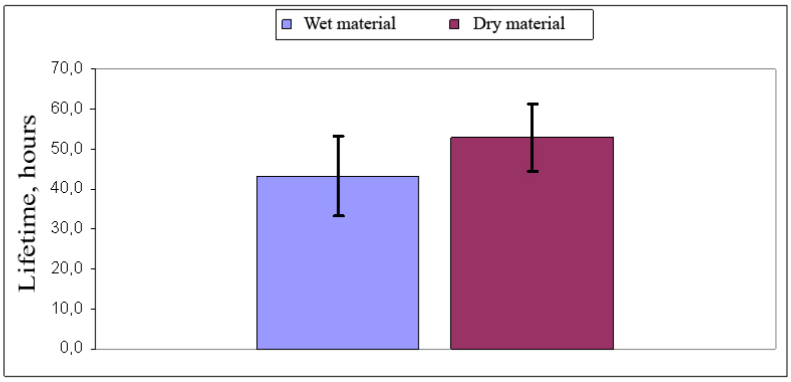

Fig. 3. The average lifetime of crabs during the dry and wet transport

It was established that the minimum mortality of crabs observed when the duration of transport was up to 36 hours.

A correlation between the average crabs mortality during the transportation, pre-transportation storage period and the season was found. When the transportation time was up to 36 hours and crabs were kept in tanks with sea water for one week, mortality was 2.5%. When crabs overexposure lasted less than 2 days – mortality was 10%. In autumn and winter, when air and water temperature is low, mortality does not exceed 3%. In warm season when temperature is high mortality of crabs increases sharply and can reach 20% during transport up to 24 hours and 50% during longer transport.

The high content of NH_4^+ ions in the washings from crab's gills after transportation was found. The content of ammonia on the gills after 36-hour staying out of water was 8–14 mg/kg body weight.

Transportation of crabs in containers without water is the most efficient and cost-effective way to deliver live crabs to consumers. This method does not require complicated technical devices. For reducing mortality and increasing transport duration have to use some innovations. Thus we have proved that high humidity inside the container is not a factor in the success of transportation. An obvious necessity of overexposure crabs in the tanks or cages without food for removing metabolic products. Influence of season on the success of transport is very high. An important reason of high mortality in summertime is not only high temperature but also weakened crabs condition during first 3–5 months after molting in January – April. Starting from September mortality after transport up to 24 hours is significantly reduced compared with the summer period. After the molting, calcium in crabs shell is not enough, and it becomes more fragile and in addition, falling meat content in legs, which reduces the viability of individuals in extreme conditions. Requires further study the possibility of reducing mortality by using materials absorbent ammonium inside the containers. Deceleration of metabolic processes in crabs by reducing the water temperature before transportation can be effective.

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