

## **MICROARRAY AS A TOOL TO DISCOVER ENZYMES INVOLVED IN DETOXIFICATION OF OXYGEN-DERIVED OR IRON LIMITATION STRESS AND THAT MAY HAVE POTENTIAL IN BIOTECHNOLOGY**

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In general, enzymes have potential in biotechnology and can be used in for instance medicine and life-science industry. Our group utilise the fish pathogenic bacterium *Vibrio (Aliivibrio) salmonicida* as a model organism for various studies including gene regulation, bacterial communication networks and virulence mechanism. *V. salmonicida* causes “cold-water vibriosis” (or “Hitra disease”) in fish, including marine-reared Atlantic salmon. Here, we have studied genes and proteins involved in stress management in this bacterium.

Generally, stress to microorganisms can be defined as any deviation from optimal growth conditions that result in a reduced growth rate. In their natural habitat, bacteria can meet various types of stresses such as nutrient availability, radiation, reactive oxygen species or iron limitation. Pathogenic bacteria, for instance, may be subjected to oxidative stress through the oxidative burst from phagocytic cells.

Faced with stress or stressors, bacteria will induce regulatory networks that control the expression of selected gene responses. These regulatory networks are called “stress responses” as the level of the response is highest during a stress condition.

By studying differentially regulated genes and proteins involved in different stress conditions, we get insight into the molecular mechanisms of *V. salmonicida*. In this work, we have studied the response of this bacterium to oxidative-derived free radical ( $O_2^-$ ,  $H_2O_2$  and  $OH^\cdot$ ) stress and stress caused by iron limitation. Hydrogen peroxide, paraquat or iron chelator was added to the growth media and microarray was used as a tool to examine the response of the bacteria to these stress conditions.

## **THE USE OF BROWN ALGAE OF THE LAMINARIALES FOR MANUFACTURING OF DIETARY JAMS ENRICHED WITH SELENIUM AND CHROMIUM**

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Balanced nutrition is one of the factors determining the health of the population of Russia. The topical issues now are the development and manufacturing application of the healthy dietary products. Laminaria is one of the best raw material for such products. It is a natural selective absorbent of macro- and micronutrients accumulated in tissues, contains a large amount of polysaccharides and organic iodine. Biologically active substances of laminaria can reduce blood pressure, increase resistance to infectious diseases, as well as have a positive effect in treatment of cardiovascular diseases, anemia, osteoporosis (Sovershaeva S. N. et al, 2002).

Useful properties of products based on kelp can be enhanced by the addition of micronutrients required for healthy people, and for people suffering from alimentary-dependent diseases.

Cardio-vascular diseases have the leading role among the pathologies associated with poor diet. One of the effective approaches to this socially important issue is the development and manufacturing of new dietetic products intended for use in nutrition of persons suffering from such diseases as coronary heart disease and hypertension.

Epidemiological studies have shown that consumption of selenium not only reduced risk of developing of cardiovascular disease, but also contributed to an increase of life expectancy.

An achievement of the national biotechnology was to develop and commercialize the method of cultivation of baker's yeast *Saccharomyces cerevisiae* with a high content of organic forms of selenium (Zolotov P. A. et al, 1998). Broad use of selenium yeast in the diet of the population, however, was

prevented by the presence of poorly digestible cell membrane. The membrane significantly reduced the absorption of the contents of yeast cells and was potentially allergenic. In contrast the water-soluble fraction of yeast autolysate enriched with selenium, was produced by hydro-processing, then the autolysis at 50 °C and removal of damaged cell membranes by centrifugation (Mazo V. K. et al, 2002). Under the title "Selenium-Vitasil" this additional source of food selenium is used in a number of dietary supplements.

Cardiovascular diseases are often accompanied by diabetes mellitus – a disease associated with dismetabolism. The high frequency of this disease in our country determines the special interest in the medical community to the problems of its prevention and treatment. Besides diabetes, along with abdominal obesity, hypertension and other metabolic disorders is an indicator of patients with metabolic syndrome (Chaplin S., 2005; Ushakova T. I., 2007).

A big role in the regulation of carbohydrate and lipid metabolism plays a trace element chromium, which supports normal glucose tolerance and forms a complex compound with insulin, more effective than the free insulin. It is known that chromium in foods is present as inorganic salts and complex compounds with organic ligands, which is the active form of chromium and has a marked effect on the absorption and blood level of glucose. This compound is considered as the glucose tolerance factor. Assimilation of inorganic salts of chromium is extremely low – 0.5 – 0.7% from food quantity. Absorption of the glucose tolerance factor in the intestine is much more intensive, and may reach 25% of dietary chromium.

Chromium is also involved in the regulation of cholesterol metabolism and during the treatment in some cases caused reduction of cholesterol in the blood. Ability to synthesize the glucose tolerance factor in humans is limited, and this determines the need of the intake of the compound with food (Tutelian V.A. et al, 2002).

We suggested the technology of the dietary products – Laminaria jams enriched with selenium and chromium for the nutrition of persons with alimentary-dependent diseases.

The objects of investigation were commercial samples of kelp harvested in various regions of Russia and China, and Laminaria jams with the addition of selenium or chromium. The contents of dry matter, alginic acid, iodine and ash was determined by GOST 26185–84, protein – according to GOST 7636–85 by avtoazot analyzer «FOSS Tecator» 2300 (Sweden) by the Kjeldahl method. Chromium content was determined by mass spectrometry with inductively coupled plasma. The content of selenium – by mikrofluorimetric method by measuring the fluorescence of hexane extract. The composition and amount of carbohydrates was determined by high performance liquid chromatography. Determination of insoluble and soluble fractions of dietary fibers was performed by the method of Gordon and Ohkuma, 2004, through the enzymatic hydrolysis of protein and starch materials in the products and subsequent filtering. Authors thank very much the colleagues from the Institute of Nutrition, RAMS for the help in determination of the content of selenium, dietary fibers and carbohydrates.

Analysis of raw materials has shown that its chemical composition was greatly influenced by the primary processing and harvesting area (Table1).

**Table 1. The chemical composition of the commercial samples of kelp**

Sample	Origin	Contents (% of dry matter)			
		alginic acid	protein	ash	iodine
Salted kelp (blades)	Sakhalin, Russia	33.33±3.33	7.30± 0.73	31.47±1.57	0.200±0.020
Salted kelp (shredded)	Sakhalin, Russia	26.90±2.69	8.19±0.82	38.23±1.75	0.170±0.017
Dried kelp premium grade	White Sea, Russia	29.74±3.02	8.39±0.94	28.18±1.91	0.179±0.009
Dried kelp first grade	White Sea, Russia	31.86±3.32	9.42±1.16	19.02±0.93	0.062±0.007
Washed shredded kelp	China	49.53±4.95	13.46±1.35	19.52±0.98	0.001±0.0001

The samples of shredded kelp from China were poor of mineral substances and iodine because of the primary treatment – additional washing and drying at high temperatures. Stable and high content of iodine in the White Sea kelp allowed recommending it for making jams, enriched with chromium or selenium.

Various berries and fruits (cranberry, dried apricots, apples, lemon) were added to Laminaria Jams for taste improvement. Selenium was added in organic form (Selenium-Vitasil). As a sweetener for the anti-diabetic jam synthetic sweetener sucralose, derived from sugar was selected. Sucralose has no effect on carbohydrate metabolism, has a pleasant sweet taste, easily soluble in water and is stable for cooking. Chromium was added to the product in a complex with a milk protein hydrolysate.

Laminaria Jams were manufactured in a hydrodynamic rotary cooker which provided simultaneous grinding, mixing and pasteurization at a constant temperature of 90–95 °C.

Analysis of chemical composition and nutritional value of different samples of jams showed that they were of low caloricity (50 – 81 kcal) ; 20 g of jam satisfied up to 30% of the daily requirements of an adult in iodine, selenium or chromium. They were also rich in dietary fibers, which played an important role in the process of digestion: improved intestinal motility, had a prebiotic effect, reduced the rate of mono- and disaccharides absorption, and thus protected the body from high blood glucose and increased insulin synthesis.

Selenium was tested in the department of cardiovascular pathology of Clinic of medical nutrition for the study of the effectiveness of its use in the diet of patients with coronary heart disease (CHD), hypertension and obesity. The study showed that the product had a beneficial effect on the dynamic of clinical and instrumental parameters that characterized the functional state of the cardiovascular and nervous system. Also jam intake contributed significantly to the availability of selenium to patients with initially low level of the element in serum.

Chromium-enriched Laminaria Jam has passed the clinical trials among the patients with insulin-dependent diabetes mellitus type in the therapy department of the Ryazan State Medical University, where the possibility of dietary and medical treatment of metabolic syndrome on an outpatient basis was explored. Fifteen patients aged from 33 to 63 years suffering from arterial hypertension, dismetabolism of carbohydrates and lipids, diseases of the gastrointestinal tract took part in the study.

According to preliminary data, the use of dietary jam enriched with chromium was associated with improved overall health for all patients, normalization of the defecation, weight loss and reduction in waist circumference in some cases. Throughout the study neither of the patients observed adverse reactions. The improvement in lipid and glucose profile indicators was shown. The patients with increased glucose tolerance after 3 months of the product intake showed normal results. A rapid decrease in total cholesterol in the blood of all patients was observed.

Laminaria Jam enriched with selenium can be recommended for use as a dietary product in the nutrition of people with low selenium level and with the pathology of the cardiovascular system (coronary heart disease, hypertension, hyperlipoproteinaemia).

Inclusion of Laminaria jam enriched with chromium in a complex dietary therapy can reduce the manifestations of the metabolic syndrome, namely, improve lipid and carbohydrate metabolism, decrease body weight and waist circumference and subjectively improve the patients life.

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