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SYNTHESIS OF BACTERIORHODOPSIN BY *Halobacterium sodomense* K91r

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The light-dependent protein bacteriorhodopsin deserves special attention among physiologically active compounds from halophilic Archeans. It is associated with the development of a new type of information-storage system, the creation of an artificial eye, and the use as biologically active additives with clearly pronounced antioxidant and radioprotector properties [1].

We isolated previously two strains of extreme halophilic bacteria from Barsakelmes salt marsh (South Sub-Aral) that were capable of synthesizing carotinoid pigments [2]. The goal of the present study was to identify and determine quantitatively bacteriorhodopsin in cells of the previously isolated strain *Halobacterium sodomense* K91r and to study the protein accumulation as a function of culture age.

The capability of cells of an extreme halophile to synthesize bacteriorhodopsin was studied for the first time in Uzbekistan. Cell-free extract of *H. sodomense* K91r was obtained by the standard method using CFE buffer [3]. The composition of the resulting protein extract was analyzed by electrophoresis under denaturing conditions according to Laemmli [4]. The electrophoregram of the studied extract showed 16 bands corresponding to proteins of molecular weights in the range 110–7 kDa. Among them, a protein band of molecular weight of the order of 26 kDa was observed. This corresponded to bacteriorhodopsin standard (Fluka). The identification of this protein in the extract from the halophilic strain was promising for further work to isolate and purify bacteriorhodopsin.

The quantitative content of the synthesized bacteriorhodopsin was determined as a function of the cultivation time of the strain and the composition of the growth medium for No. 44 and Braun liquid media using spectrophotometry based on the light-sensitivity of the protein [5]. For this, cells were collected from growth medium (2 mL) by centrifugation and disintegrated by distilled water containing DNAase 1 (0.01 mg). The lysate was stirred until homogeneous and treated with NaOH (4 M) and NH_4OH (4 M) in a 9:0.5:0.5 (lysate:NaOH:NH_4OH) ratio in the dark. Absorption at 568 nm (A_{568}) was first measured in the dark (A^0_{568}). The mixture was exposed to light for 24 h. The absorption was measured again (A^{24}_{568}). Considering that the molecular weight of bacteriorhodopsin is 26 kDa and the molar extinction coefficient is 63,000 $M^{-1}\cdot cm^{-1}$, the bacteriorhodopsin concentration was determined from the equation

$$BR\ (g/L) = 26{,}000 \cdot (A^0_{568} - A^{24}_{568})/63{,}000.$$

Bacteriorhodopsin began to accumulate heavily starting on the 5–6th day after the start of cultivation. This was due to an increase of the NaCl concentration in the medium and; therefore, a decrease of the aeration of the growth medium and a switch to the conversion of light energy into chemical mediated by bacteriorhodopsin. Despite the fact that *H. sodomense* K91r biomass grew more vigorously in Braun medium, this did not affect the accumulation of bacteriorhodopsin. A study of bacteriorhodopsin accumulation during growth of *H. sodomense* K91r found that the yield of pigment was greater during cultivation in No. 44 medium than in Braun medium. The values were 11.3 and 10 mg/L, respectively, on the 11th and 10th day of cultivation. The bacteriorhodopsin concentration decreased sharply on the 14th day of cultivation. Bacteriorhodopsin accumulation by the local strain of *H. sodomense* K91r did not depend directly on the amount of biomass and the medium composition but moreso on several other factors such as the illumination and age of the cultivation medium. Thus, our data enabled the cultivation conditions of the microorganism to be optimized in order to increase the synthesis of the target protein.

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According to the literature, strains of *Halobacterium* can accumulate 12–20 mg/L of bacteriorhodopsin without optimizing the conditions [6]. In this respect, the strain isolated by us, which accumulated 10–11 mg of bacteriorhodopsin in 1 L of medium, could be recommended for further investigation on the optimization of the cultivation conditions.

We selected culture stored in semi-liquid agar under mineral oil at +4°C as the most available and convenient method for storing the halophilic strain in the laboratory.

After storing the strain for one year, it was checked for the capability to grow *H. sodomense* K91r and to accumulate bacteriorhodopsin in Braun liquid medium containing 25% salt at 45°C. During the storage time the strain did not lose its intrinsic pigmentation and also retained its morphological-culture and biochemical properties. Electrophoresis [4] showed that the capability of the culture to accumulate bacteriorhodopsin was restored after storage for one year. In this respect we consider the method used to store the local halophilic strain *H. sodomense* K91r to be suitable for use under laboratory conditions.

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