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Response of pigmented *Serratia marcescens* to the illumination

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ABSTRACT

Variations in the illumination conditions (light/darkness) affected both the biosynthesis of prodigiosin and energy metabolism of the pigmented strain ATCC 9986 *Serratia marcescens* growing aerobical in the batch culture were shown. In the process incubation the transition of the pigmented culture from illumination within (24 h, 48 h) in the dark conditions increased the prodigiosin synthesis by 2.0, 2.5 times, respectively. At the same time, the illumination did not influence the prodigiosin biosynthesis in the stationary growth phase. In the initial period of prodigiosin synthesis the rate of oxygen consumption was higher than later when the pigment synthesis gradually decreased. The respiration activity of colorless strain 24-5 is not independent from the lighting conditions. The regulation of energetic pathways in the light and in darkness has been revealed. Prodigiosin is associated with the hydrophobic protein and it is represented pigment protein complex by diameter of particles less 100 kDa. Fluorescence spectrum of prodigiosin and it the absorption spectra of derivatives of high orders D^{IV} and D^{VIII} were described.

1. Introduction

Chemical structure of pigments is of great importance for the identification of phototrophic bacteria. Fundamental researches concerning chemistry, ecology, and *de novo* synthesis of carotenoids by new forms of marine bacteria have been reviewed [1]. Several different carotenoid groups can be identified in phototrophs. Representatives of the genus *Pseudomonas* produce various nonphotosynthetic pigments, such as pyocyanin and pioverdin.

Serratia marcescens is a gram-negative, facultative anaerobic bacterium of the genus *Serratia* and family *Enterobacteriaceae*. Strains isolated from natural sources synthesize membrane-linked pigment, prodigiosin linear trypyrrole–pyrryldipyrrylmethene [2]. The ecological aspect is great interest since these pigmented strains are widespread in the environment, whereas the colorless variants are isolating mainly from the clinical isolates [3,4]. Prodigiosin acts as the potent proapoptotic and antimetastatic factors at higher concentrations, and is has also antimicrobial, immunosuppressant, and anticancer properties [5,6]. Recently, it was reported on the action of prodigiosin cytotoxicity against different human neuroblastoma cell lines [7].

Prodigiosin is a typical secondary metabolite, and its biosynthesis reaches a maximum in the stationary phase of bacterial growth and also depends on many factors [8]. However, the physiological role of prodigiosin in metabolism of the bacterial producers remains unclear and stimulates authors to the subsequent studies. Prodigiosin is itself interesting as trypyrrole, in contrast with chlorophylls and bilins which are cyclic and linear tetrapyrroles, respectively. Moreover, the influence of visible light on the pigmented heterotrophic bacteria is less understood. It was shown to act on the growth processes, metabolism, and reproduction of heterotrophic bacteria [9]. From this point of view, the pigmented strains of *S. marcescens* are suitable objects for study the effect of illumination on the cell growth, and elucidating the physiological role of pigments in a prokaryotic cell.

In this work, we studied the influence of the light–dark conditions on cell metabolism of the growing culture, the character of prodigiosin biosynthesis, aerobic respiration in the batch culture of pigmented and colorless *S. marcescens*.

2. Materials and methods

2.1. Strains and growth conditions

The study was carried out with pigmented strain *S. marcescens* ATCC 9986 and its colorless dissociant *S. marcescens* 24-5 obtained from the Culture Collection of Kazan Federal University, Russia. The colorless strain 24-5 *S. marcescens* was isolated from strain *S. marcescens* ATCC 9986 [10]. Strains were maintained and cultivated in the liquid medium [8] and in broth containing meat peptone (5 g/L) and glycerol (10 g/L) [11]. In some experiments, meat peptone

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was replaced by vegetable peptone (Fluka) for the pigment extraction from biomass.

The culture was grown for 24 h on a shaker (type 357, Poland, 200–220 rpm) and then used as an inoculum (0.2%, v/v). Both strains were cultivated in 0.25-l Erlenmeyer flasks containing 50 mL of medium (pH 7.2) at 28 °C, on a shaker either under continuous lighting with the fluorescent lamp (white, Hitachi) at a photosynthetic light flux of 22.8 μmol photons m^{-2} s^{-1} or in darkness no less than 3–5 days. The same light flux did not produce any changes in the growth parameters of both strains such as specific growth rate and generation time. Cell growth was followed by the measurement of the optical density at 670 nm (OD) so that spectra prodigiosin and optical density of growing culture have been divided and were not imposed against each other. In the growing culture prodigiosin concentration was measured on the scanning spectrophotometer a Lambda – 25 (Perkin Elmer, USA).

The oxygen consumption by cell suspension was measured by the Warburg's manometric method [12], which permitted to assay several parallel samples. The results presented are mean values of three independent experiments from three to five of separate flasks.

2.2. Prodigiosin essay

To extract prodigiosin from the cells, 1 ml from culture to 9 ml of acidified ethanol (10 ml of ethanol and 1 ml of 1 N HCl) was added. Time of extraction made 20-min, the cell debris was removed by centrifugation at 9200*g*, 10 min, 5 °C, the absorbance of supernatant was determined spectrophotometrically at 535 nm on a Specord (Carl Zeiss, Germany). Pigment concentration was calculated using extinction coefficient for prodigiosin (51.5×10^3 l/g/cm) [13].

2.3. Respiration assay

The rate of the oxygen consumption was measured by the Warburg's manometric method permitting the recurrences and the experimental variants at the same time. The samples of growing culture were withdrawn every 24 h and cells were harvested by centrifugation at 6000*g*, 20 min, washed, and resuspended in 50 mM Tris-HCl buffer (pH 7.5). The optical density of the cell suspension was 1.0–1.2 OD at 670 nm. The Warburg's vessels were incubated at 28 °C in the repetition no less three vessels in darkness and/or at fluorescent lamp (white) of 22.8 μmol photons m^{-2} s^{-1}.

2.4. Identification of prodigiosin

The pigmented cells were suspended in 10 mM Triton X-100, cellular lysate was centrifuged (9200*g*, 20 min, 5 °C). Supernatant was saturated with 0.2 N, 0.4 N, 0.6 N, and 0.8 N solutions of ammonium sulfate. The prodigiosin protein complex was in the fraction saturation with 0.2 N by ammonium sulfate. The fraction was dialyzed for overnight. The remaining supernatant (50 μl) was used for the separation of polypeptides in a linear gradient; it was loaded on PAGE (6–16%) and 2.5% of SDS according to described method [14]. Protein aliquots with equal radioactivity were placed on each lane. Protein content of trichloride acetic acid (TCA) precipitates was estimated [15].

2.5. Definition the size of the pigment protein particles

Pigmented biomass suspended consistently two times in 10 mM Triton X-100 and the lysate was centrifuged at 9200*g*, 20 min, 5 °C. To extract of the pigment protein fraction the supernatant was saturated by ammonium sulfate 0.2 N, as it has been shown earlier. The isolated prodigiosin protein fraction dialyzed overnight, and separated by centrifugation at 9200*g*, 20 min, 5 °C. Then the incorporated colored of pigment protein fractions were resuspended in the minimal volume bidistillate. By method of dynamic light scattering (DLS) the hydrodynamic diameter of pigment protein particles was measured at 25 °C using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). Samples transferred in low-volume spectroscopic plastic cells. The viscosity and the refractive index of solvent were those of water. The indexes of particles (complex) and Triton X-100 were set at 1.45 and 1.605, correspondingly. The angle of the laser bean was 173°. To calculate the radii from the auto- correlative data the CONTIN method was used. The hydrodynamic diameter was measured in triplicate. Each measurement corresponded to five auto-correlative functions recorded during 30 s. The experiment was realized in duplicate for each sample.

2.6. Spectral methods

Prodigiosin from biomass was extracted with acetone; the sediment was separated by centrifugation 9200*g* 20 min, 5 °C. The supernatant was treated with the solvent system acetone–chloroform–H_2O (1:10:80); pigment was dissolved in chloroform phase and purified by thin-layer chromatography in the solvent system chloroform–ethylacetate–acetic acid (8:1:1) with the subsequent elution with acidic ethanol. The fluorescence intensity of prodigiosin was recorded on an MPF-44B analyzer (Perkin-Elmer, United States). The excitation wavelength is at 535 nm. Acidic ethanol was used as control. Differential absorption spectrum of prodigiosin was determined on a Specord spectrophotometer (Carl Zeiss, Germany).

Absorption of prodigiosin derivatives D^{IV} and D^{VIII} was examined by the method of derivative spectra. The registration of the absorption spectra of prodigiosin and their derivatives of high order was carried out using spectrophotometer DW-2000 FA-220 (SLM-Aminco, Germany) and Specord (Carl Zeiss, Germany) [16]. The spectra were scanned and then processed with the help of the Graph Digitizer 1.0 program. Graphs were constructed with the help of the Microcal Origin 5.0 software. The latter program enables one to calculate error bars and to carry out differentiation concurrently. Samples to definition the absorption prodigiosin derivatives of high order were extracted as cited above.

3. Results and discussions

3.1. Physiological factors

Potential capability of a majority of *S. marcescens* strains for pigment production varied considerably in dependence on environmental and physiological conditions. It is well known that prodigiosin is produced from intermediates and is accumulated as a secondary metabolite.

Fig. 1 demonstrates the curve of growth and the pigment biosynthesis by strain *S. marcescens* ATCC 9986 grown on glycerol and meat peptone. In batch aerobic culture, in dark conditions, prodigiosin was revealed beginning from 18–20 h of incubation and reached the maximum concentration during the exponential growth phase 60–70 μg/l. The results from three experiments (mean + standard deviation [SD]) demonstrate the correlation of growth and prodigiosin concentration during fermentation. Growth continued with aeration at 28 °C. As a rule three major population metabolic phases based upon the kinetics of cell *S. marcescens* were defined. In the exponential phase of growth from 20 to 24 h until 58 to 60 h of incubation the cellular population was maximum. We registered prodigiosin content in the active growth phase of culture at 42 h (Fig. 2). The latter expression suggests that