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Pigment-dependent light influence on the energetics of *Serratia marcescens* ☆

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Abstract

This paper describes a direct determination of the light energy accumulation by pigmented *Serratia marcescens* using a photomicrocalorimetric method. At 5400 lux the rate of energy storage by the pigmented strain 9986 was 1.5 J h^{-1} (g dry wt)$^{-1}$. The stored energy accumulation by pigmented strain 33 and by its non-pigmented equivalent, and also by strain 24-5 (control), was in the range 0.4–0.77 J h^{-1} (g dry wt)$^{-1}$. Growing cultures contain monomer and dimer forms of the photosensitive pigment, prodigiosin. The visible light directly affected the pigment itself and provoked its phototransformation.

Keywords: Bacteria; Illumination; Photomicrocalorimetry; Phototransformation; Pigment; *Serratia*

1. Introduction

It is well known that almost all phototrophic prokaryotes store luminous energy in chlorophyll-containing complexes and that only the genus *Halobacterium* does so by using bacteriorhodopsin [1]. However, the influence of visible light on other bacteria is less well understood. Although it has been shown that light affects the

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growth processes, metabolism, and reproduction of heterotrophic bacteria [2,3], the literature contains no information on the pigmented microorganisms, as distinct from phototrophic ones, which store light energy with the participation of their pigments. Therefore, a study of the pigments considered to be secondary metabolites may clarify their physiological role.

Serratia marcescens is able to synthesize a red pigment, prodigiosin [4], which is a natural pyrryldipyrrylmethene [5] linked to the inner membrane [6]. This bacterium may be used as a means to discover the physiological role of bacterial pigments. Moreover, *S. marcescens* itself is interesting because prodigiosin is a linear tripyrrole, in contrast with the compounds of classical photosynthesis such as chlorophylls and bilins which are cyclic and linear tetrapyrroles, respectively.

This work is a preliminary study of the energetics of *S. marcescens* and, specifically, of the function of prodigiosin in metabolism, including the action of visible light on it.

2. Experimental

Two pigmented strains of *Serratia marcescens*, ATCC 9986 and 33, were employed in this study. The controls were the strains 24-5, obtained experimentally as non-pigmented, and 33 with pigmentation, but grown in non-permissive conditions. The flasks containing 50 ml of culture medium were incubated in a gyratory shaker at 28°C and 200 rpm. Cell growth was monitored as optical density at 670 nm. Prodigiosin was extracted with acidic ethanol and spectrophotometrically assayed at 535 nm according to Williams et al. [7].

The amount of light energy accumulated by the bacterial cells was determined directly using a differential adiabatic photomicrocalorimeter with a light apparatus [8].

In these experiments, cultures were grown in the dark. The cellular suspension in 50 mM Tris-hydrochloride (pH 7.5) was introduced into the calorimetric vessel (volume, 0.65 ml, constituting $0.7–1.1 \times 10^{-4}$ g of dry weight). The rate of stored energy accumulation was measured from the start of prodigiosin synthesis by *S. marcescens*. The illumination of the cullular suspension in the calorimetric vessel was either 2000 or 5400 lx. The experiments were repeated five times and triplicate measurements were made for each time point from three separate flasks.

3. Results and discussion

At 2000 lx, pigmented cells of *S. marcescens* did not show any accumulation of light energy. On increasing the illumination by visible light to 5400 lx, pigmented cells of strain 9986 increased their energetic level in comparison with the non-pigmented control (strain 24-5) up to 1.5 J h^{-1} (g dry wt)$^{-1}$. These data are statistically reliable (not less than 95% probable). Under similar conditions the rates of stored energy accumulation by pigmented and non-pigmented strain 33 and also by strain 24-5 (control) were the same, 0.4–0.7 J h^{-1} (g dry wt)$^{-1}$ (Fig. 1). The

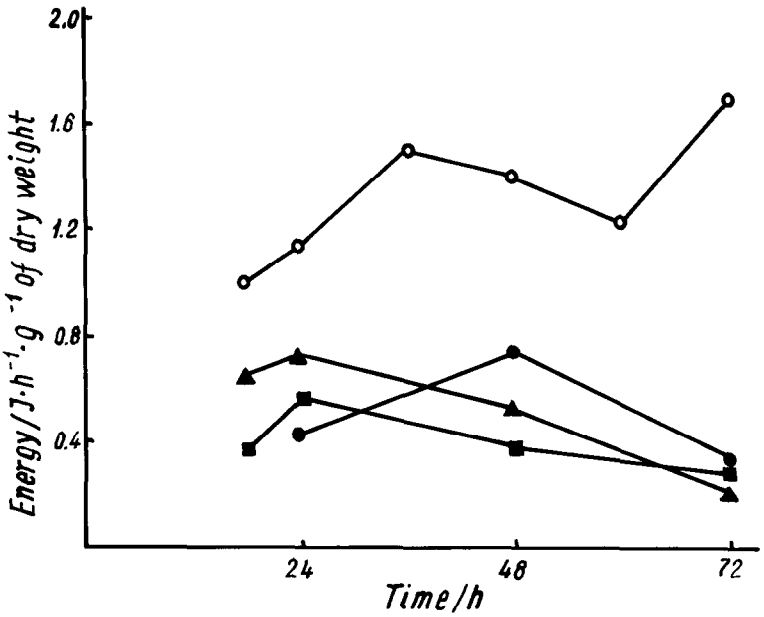

Fig. 1. The storage of light energy by *S. marcescens* strains: ○, 9986; ■, 24-5; ▲, 33; ●, 33 (non-pigmented).

unicellular alga *Chlorella* was used as a typical photosynthetic organism. Its level of energy storage was 175 J h^{-1} (g dry wt)$^{-1}$. This confirms the view that the storage of light energy takes place in pigmented cells. The results also indicate that the action of visible light on the pigmented cells of *S. marcescens* is a complicated physiological process and that the prodigiosin incorporated in the cullular membrane is probably not the only component involved. Apparently, strain 33 is unable to undergo subsequent stages of phototransformation under the present conditions.

These preliminary experiments showed that illumination at 2000 lx of growing cells of strain 9986 for 1–7 days did not cause any changes in two growth parameters, into the specific growth rate and the generation time. The same illumination (2000 lx) was used in subsequent experiments. Under continuous light, during the exponential growth phase, pigment synthesis was slightly higher than that in the dark. However, during the later stationary phase, the rate of pigment accumulation increased under dark conditions. Subsequently the content of prodigiosin in the culture grown in darkness was essentially higher than that of cells grown in light. The maximum prodigiosin content in the dark and light cultures was observed at 3–4 days and at 2–3 days, respectively. The dark culture retained its strong red colour during the whole time of the experiments (up to 9 days).

The action of the differential light spectrum on the cell pigmentation showed that illumination of the growing culture in the range 500–600 nm at 200–250 lx, corresponding to the absorption by the prodigiosin dimer form (535 nm), decreased the pigment content by 50%. The same effect for the monomer form of the pigment

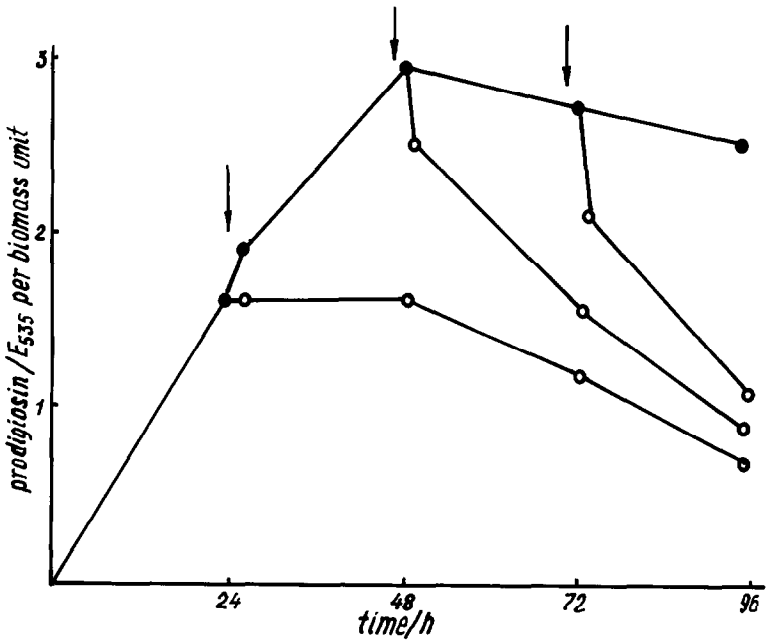

Fig. 2. The action of illumination on the prodigiosin biosynthesis: ●, dark; ○, light. Arrows indicate the time of switching on the light.

(460–470 nm) took place at an illumination of 55 lx. Moreover, in the range of 370–500 nm and at 200–250 lx, the prodigiosin content in the growing culture was reduced by 80%. Thus, mono- and dimer forms of prodigiosin were present in the growing cells and the light influence occurred through these forms. It can be suggested that the monomer is the more photosensitive form of the pigment.

Fig. 2 shows the influence of the illumination conditions (light/darkness) on the pigmentation of *S. marcescens*. Illumination with visible light for 4 h resulted in a slight decrease in prodigiosin content. The dynamics of the accumulation of the pigment was the same as in dark conditions. It should be noted that switching on the light for cells grown in the dark for 24, 48 and 72 h resulted in a decrease in pigmentation. In contrast, cultures grown in light for 24 and 48 h recovered the high level of pigmentation after switching off the light (Fig. 3). Later in the period of prolonged stationary phase (at 72 h), switching off the light did not affect the prodigiosin content, which signified the cessation of pigment synthesis (Fig. 3). These data allow the conclusion that visible light directly affects the pigment itself, stimulating its phototransformation.

It is interesting to discuss some aspects of the storage of visible light energy and the correlation of pigmentation under the action of visible light on non-photosynthesizing cells such as *S. marcescens*. The experimental data confirmed the storage of light energy by pigmented cells of *S. marcescens* which is a well-known heterotrophic bacterium. The phototransformation process seems to be very com-

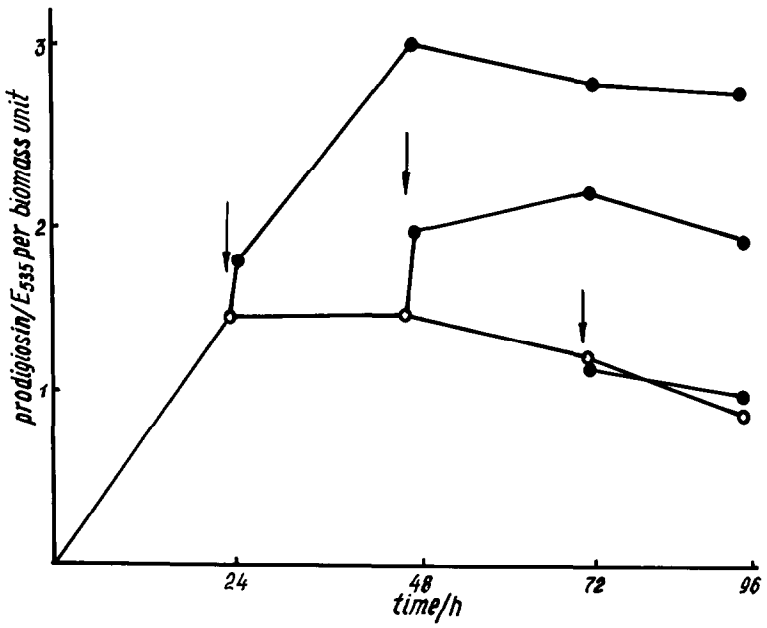

Fig. 3. The action of illumination on the prodigiosin biosynthesis: ○, light; ●, dark. Arrows indicate the time of switching on the light.

plicated, involving other components besides prodigiosin. In our opinion, the results obtained for the energy storage by pigmented strain 33 support this fact.

The quantity of pigment in the cells was reduced under conditions of continuous illumination with visible light, indicating the photosensitivity of prodigiosin. It was also shown that the pigment photosensitivity is more developed for the spectrum absorption of prodigiosin monomer form. The experiments on the light/dark effects on pigmented cells suggest that visible light acts directly on the pigment itself synthesized by the culture. These data extend our knowledge of the physiological role of prodigiosin in *S. marcescens*.

References

[1] W. Stoeckenius, R.H. Lozier and R.A. Bogomolni, Biochim. Biophys. Acta, 505 (1979) 215.
[2] E.P. Pheophilova, M.V. Volokhova, B.A. Velichko, A.M. Karpov, M.V. Polotebnova, G.V. Tcherkasova and G.F. Shilkova, Biotechnologia, 4 (1988) 719.
[3] O. Tiflova and T. Karu, Biomed. Eng., 18 (1991) 387.
[4] P.A.D. Grimont and F. Grimont, in N.R. Krieg (Ed.), Bergey's Manual of Systematic Bacteriology, Vol. 1, Williams and Wilkins, Baltimore, 1984, p. 477.
[5] A.H. Jackson, G.W. Kenner, H. Budzikiewicz, C. Djerassi and J.M. Wilson, Tetrahedron, 23 (1967) 603.
[6] M. Vinas, I.G. Loren and I. Giunea, Microbios Lett., 24 (1983) 19.
[7] R.P. Williams, C.L. Gott and I.A. Green, J. Bacteriol., 81 (1961) 376.
[8] V. Ye. Petrov and N.L. Loseva, Physiol. Rastenii, 17 (1970) 1110.