Color of Actinomycetes

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(Received Oct. 17, 1991)

Introduction

It is well known that the colors of bacteria are regarded as rather variable features even under the strictly standardized culture conditions, though they have been dealt as important characters by many investigators. Why are they relatively variable and unstable? There must be a key to solve this question. One possibility is that light may be one of the important factors influencing the coloration of bacterial cultures. The present paper deals with the effect of light on the pigmentation in actinomycetes; and with pigments of *Streptomyces luteoreticuli* as an example of constitutive type of pigmentation.

Photochromogenicity

In the course of our investigation on the metabolites of *Nocardia brasiliensis* IFM 82, we observed the fact that colonies of this strain developed rich orange pigment when grown under diffuse sunlight, but remained colorless to beige in the dark. When the strain was exposed to light at 0°C and then was kept at low temperature (0-5°C), little or no pigmentation was observed. But subsequent incubation at higher temperature (27-28°C) in the dark caused pigmentation.

Among nonphotosynthetic bacteria, it has been known that a number of mycobacteria such as *M. kansasii*^{1,2)}, *M. marinum*³⁻⁶⁾, a *M. sp.*^{7,8)} and others^{9,10)}, three species of *Myxococcus*^{11,12)}, *Flavobacterium dehydrogenans*¹³⁾, and *Micrococcus roseus*¹⁴⁾ exhibit a specific response to light which is referred to photochromogenicity²⁾ or photoinduced carotenogenesis. Thus, early investigations of photochromogenicity in actinomycetes and related microorganisms have dealt exclusively with mycobacteria and no attention has been paid to other bacteria. Therefore, it was of our interest to

investigate the effect of light on the pigmentation of various strains of actinomycetes and related microorganisms.

As results of our investigation, it was revealed that numbers of strains belonging to genera *Streptomyces*^{16,17)}, *Nocardia*¹⁵⁾, *Micromonospora*^{15,18)}, *Microbispora*^{15,18)}, *Actinoplanes*^{15,18)}, *Corynebacterium*¹⁵⁾, *Brevibacterium*¹⁵⁾, *Arthrobacter*¹⁵⁾ and *Mycobacterium*¹⁹⁾ exhibited photochromogenicity as indicated in Table 1. Table 2 shows the coloration of various strains of actinomycetes and related organisms, which were arbitrarily selected from the tested bacteria, when grown under illumination and in the dark.

These results suggested that photochromogenicity was not a specific phenomenon in a particular species of microorganisms, but a common and widely distributed phenomenon in various species of nonphotosynthetic microorganisms.

Table 1. Photochromogens in Actinomycetes and Related Microorganisms

Genus	No. of species tested	No. of photochromogenic species
<i>Arthrobacter</i>	2	2
<i>Brevibacterium</i>	7	2
<i>Corynebacterium</i>	8	2
<i>Nocardia</i>	19	8
<i>Mycobacterium</i>		9*
<i>Actinoplanes</i>	3	3
<i>Micromonospora</i>	8	4
<i>Actinomadura</i> group	2	2
<i>Microbispora</i>	4	2
<i>Streptomyces</i>	348	70
<i>Streptoverticillium</i>	10	1

*prior to present investigation, it has been already known that 7 out of 9 species of mycobacteria are photochromogenic.

Table 2. Coloration of photochromogenic strains belonging to actinomycetes and related microorganisms when grown under illumination and in the dark

Organism*	Color of colonies grown	
	under illumination	in the dark
<i>Arthrobacter simplex</i> IAM 1660	yellow	colorless
<i>Brevibacterium sulfureum</i> AU-31	yellow	colorless
<i>Corynebacterium aquaticum</i> IFO 12154	brilliant yellow	colorless
<i>Nocardia asteroides</i> IFM 1	orange	white
<i>Nocardia brasiliensis</i> IFM 82	orange	beige
<i>Mycobacterium smegmatis</i>	orange	pale orange
<i>Actinoplanes philippinensis</i>	orange	pale orange
<i>Micromonospora echinospora</i> KCC A-0073	orange	light brown
<i>Actinomadura madurae</i> IFM 73	orange	beige
<i>Microbispora chromogenes</i> KCC A-0022	orange	whitish
<i>Streptomyces canus</i> ISP 5017	yellow	whitish
<i>Streptomyces fradiae</i> ISP 5063	orange	pale yellow
<i>Streptomyces chibaensis</i> ISP 5220	orange	lemon yellow
<i>Streptomyces massasporeus</i> ISP 5035	yellow	buff(violet)

*)Strains are arbitrarily selected from the test strains.

Fig. 1 Dependence of Carotenoid Synthesis of *B. sulfureum* on the Duration of Light Exposure.

After 12 hr incubation in the dark, the cultures were exposed to light (10000 lux) for a certain time in an ice bath, then incubated in the dark for 24 hr at 27°C. The cells were harvested and extracted with acetone-methanol (1:3). The extract was concentrated *in vacuo* and the residue was dissolved in methanol. OD₄₄₀ of the methanol solution was measured.

Fig. 2 Time Dependence of Carotenoid Synthesis of *B. sulfureum* after Photoinduction.

After 12 hr incubation in the dark, the cultures were exposed to light (5000 lux) for 60 min in an ice bath, then incubated in the dark for a designated period. The amount of carotenoids produced was measured as described in the Fig. 1.

Mechanism of Photochromogenesis

The photoinduced pigments produced in the light-exposed cells of photochromogenic strains were carotenoids as far as tested. To gain a better insight into the phenomenon, the mechanism of photoinduced pigmentation with *Brevibacterium sulfureum* AU-31²⁰ was studied. As shown in Fig. 1, the amount of carotenoids produced in *B. sulfureum* AU-31 was proportional to the light dose below saturation, as observed in the known photochromogenic bacteria^{4,7,8,11-13}. Figure 2 shows the carotenoid production as a function of time of dark incubation following initial illumination. After 15 min. of lag period, carotenoid production began at the maximum rate and tapered off after 4 hrs. This fact suggested that photoinduction was followed by a series of dark metabolic reactions which led to the synthesis of carotenoids *de novo*. If chloramphenicol was added immediately after photoinduction, carotenoid production was completely inhibited, as shown in Fig. 3. When the chloramphenicol addition was delayed, inhibition of carotenoid formation decreased. The carotenoid formation showed sensitivity to chloramphenicol up to 60 min. The results

Fig. 3 Inhibition of Carotenoid Synthesis by Chloramphenicol during Dark Incubation of the Photoinduced *B. sulfureum*.

After incubation for 12 hr in the dark, the cultures were exposed to light (10000 lux) for 60 min in an ice bath. Then, the cultures were incubated at 27°C in the dark for 24 hr. Chloramphenicol (5µg/ml) was added at various time during the first 4 hr of the dark incubation.

can be explained as follows. Illumination of the dark-grown colonies resulted in derepression of genetic sites that led to the formation of enzymes involved in carotenoid production. When the strain AU-31 was initially illuminated and then incubated in the dark, a repression of further synthesis of carotenoid-forming enzymes set in after 60 min. Thus it seemed likely that the same mechanism as previously known photochromogenic microorganisms was operating in *B. sulfureum* AU-31. It is noteworthy that substantially identical results with *B. sulfureum* AU-31 were obtained with *Streptomyces canus* ISP 5017¹⁶, *Micromonospora echinospora* KCC A-0073¹⁸, *Microbispora chromogenes* KCC A-0023¹⁸, *Actinoplanes philippinensis* KCC A-0043¹⁸ and *Mycobacterium smegmatis*¹⁹.

Thermal Expression of Photochromogenesis

Nocardia asteroides IFM 9 was classified into scotochromogen according to Runyon's definition², because both the light-exposed and the dark-grown colonies of the strain developed carrot red color when grown at 27°C. However, when *N. asteroides* IFM 9 was grown at 20°C after subculturing in the dark once at 20°C, the light-exposed colonies developed carrot red color whereas the dark-grown colonies were virtually pigmentless, exhibiting its photochromogenic character. The reason that the strain grown at 27°C behaved like scotochromogen might be due to its thermolabile regulation system, possibly a thermolabile repressor. Similarly, some photochromogenic strains, *N. rubropertincta* IFM 33 and *N. pretoriana* IFM 36, showed different coloration between the colonies grown at 34°C and those grown at 27°C as shown in Table 3. It was also assumed that the pigmentation of the dark-grown colonies of these strains was due to thermolabile regulation system. On the other hand, both the light-exposed and dark-grown colonies of *N. brasiliensis* IFM 14 and IFM 15 exhibited photochromogenic pigmentation at 27°C incubation whereas they were virtually pigmentless when grown at 34°C. It was therefore assumed that the appearance of the pigmentless colonies grown under illumination was due to thermolabile transcription system, translation system or enzymes.

Photochemical Coloration and Decoloration

Streptomyces purpurogeniscleroticus ISP 5271 formed an intra- and extra-cellular purple pigment when grown under illumination, whereas no pigment was formed when the strain was grown in the dark (Table 4). This phenomenon resembles photochromogenicity. However, when the dark-grown pigmentless colonies were exposed to light at low temperature, permitting no growth, the purple pigment was formed. Thus, this experiment revealed that the pigmentation of the strain was not due to photochromogenesis but a photochemical event without accompanying *de novo* synthesis of pigment-forming enzymes. Similar phenomenon was reported with a *Chainia* sp.²¹⁾. The pigment, SS-228R, was photochemically converted from SS-228Y under illumination, as elucidated by Kitahara *et al*²¹⁾. On the other hand, it has been well known that in many cases pigments are susceptible to light so that a certain photochemical reaction sometimes causes decoloration of pigmented colonies. *Streptomyces violaceus* ISP 5082 and *S. caeruleus* ISP 5103 are the cases. They produced pigment when grown in the dark, but little or no pigment was formed when grown under illumination (Table 4).

Constitutive Type of Pigmentation

Streptomyces luteoreticuli (*Streptoverticillium luteoreticuli*) develops brilliant yellow colonies. To characterize the pigment, its mycelial cake was acetone-extracted, concentrated and reextracted with ethyl acetate. The ethyl acetate extract was subjected to silica gel TLC using the solvent system of benzene : methylethylketone (5 : 1). As shown in Fig. 4, fifteen pigment spots were observed on the chromatogram. These colored components were isolated and their structures were determined as illustrated in Fig. 5²²⁾. It was consequently suggested that the pigments were synthesized *via* several different biosynthetic pathways. All of these pigments turned out to be produced in both the light-exposed and dark-grown colonies of this strain, indicating a constitutive pigmentation in the mycelia. Numbers of bacteria belonging to actinomycetes and related microorganisms showed constitutive type of pigmentation similar to that of *S. luteoreticuli*. Scotochromogenicity in mycobacteria according to Runyon's definition²⁾ may be regarded as this type of pigmentation, with exception of the pigmentation of bacteria showing gradual change or deepening of the color of colonies in the light¹⁹⁾.

Table 3. Color of various strains of *Nocardia* showing different expression of photochromogenesis caused by changing the incubation temperature

Organism	Grown at	under light	in the dark
<i>N. asteroides</i> IFM 9	20°C	carrot red	whitish
	27°C	carrot red	carrot red
	34°C	carrot red	carrot red
<i>N. brasiliensis</i> IFM 14	20°C	orange	beige
	27°C	orange	beige
	34°C	buff	buff
<i>N. brasiliensis</i> IFM 15	20°C	orange	beige
	27°C	orange	beige
	34°C	beige	beige
<i>N. rubropertincta</i> IFM 33	20°C	orange	whitish
	27°C	orange	whitish
	34°C	orange	orange
<i>N. pretoriana</i> IFM 36	20°C	orange	pale orange
	27°C	orange	pale orange
	34°C	orange	orange

Table 4. Color of bacteria showing constitutive type of pigmentation, photochemical coloration or photochemical decoloration

Organism*	Color of colonies grown	
	under illumination	in the dark
<i>Brevibacterium linens</i> AM-25	carrot red	carrot red
<i>Corynebacterium fascians</i> IFO 12155	orange	orange
<i>Nocardia asteroides</i> IFM 45 3Y	orange	orange
<i>Streptomyces luteoreticuli</i>	yellow	yellow
<i>Streptomyces violaceus</i> ISP 5082	whitish	purple
<i>Streptomyces caeruleus</i> ISP 5103	pale yellow	yellow
<i>Streptomyces purpurogeniscleroticus</i> ISP 5271	reddish purple	whitish

* Strains are arbitrarily selected from the test strains.

Fig. 4 Schematic Representation of Thin Layer Chromatogram of the Acetone Extract from Mycelial Cake of *Streptomyces luteoreticuli*.

General Discussion

From the viewpoint of biosynthesis of secondary metabolites, the pigmentation of microorganisms may be classified as two types; one is euchromogenesis and the other is pseudochromogenesis. Euchromogenesis means that the pigmentation is due to *de novo* synthesis of pigment-forming enzymes. This is divided into two types; constitutive and inducible types. Inducible type of euchromogenesis means that light, heat or chemicals triggers pigment biosynthesis. Scotochromogenicity is classified into a constitutive type of pigmentation, as mentioned above, and photochromogenicity is classified into an inducible type. On the other hand, pseudochromogenesis is also divided into two types; type I and type II. Type I pseudochromogenesis means that an exogenous substance is converted to a pigment by

enzymes presynthesized in the cells or secreted in the medium. Type II means that metabolites preexisted in the cells are converted to colored or colorless compounds by means of physical or chemical condition. Photochemical coloration and photochemical decoloration are classified into this type.

Microorganisms probably exhibit two types of pigmentation or more at the same time in the same cells, because the bacterial color consists of various colored-components synthesized *via* different biosynthetic pathways²². A biosynthetic pathway leads to a type of pigment and another one may lead to a certain different type of pigment. Consequently, it is not strange that certain microorganisms such as *Streptomyces chibaensis* ISP 5220 and *S. massapensis* ISP 5035 (Table 2) exhibit multiple photoresponses in pigmentation at the same time and in the same cells¹⁷. Thus, the coloration of microor-

Fig. 5 The Colored Components of *Streptomyces luteoreticuli*

ganisms is apparently complicated and conditional. However, it is evident that biosynthetic pathways leading to individual pigment are genetically controlled even if their genetic expression is affected by cultural conditions, e.g. light, temperature, chemicals and others. Therefore, it may be considered that in spite of fluctuation in color, the kinds of pigmentation exhibited by certain microorganisms reflect their genetic character and may be a differential property of real taxonomic value.

ACKNOWLEDGMENTS

The author feels very honored to receive the Merit Award of the Society for Actinomycetes Japan (SAJ). The achievement for this award was "Contribution to the improvement of the basis for scientific and social exchange in the Society". This achievement was done with combined effort and assistance of my colleague directors as well as members of the society; fortunately I had been at the position of the director and then the chairman.

In around 1984, having Dr. Y. Okami as a key person who contributed to our Society as the chairman as well as an executive member of the board of directors, we planned to invite ISBA'88 to Japan and also to reform SAJ, which remained to be only a group for researchers, to a formal scientific society registered to the Science Council of Japan. I was assigned the work to prepare the draft of regulation of SAJ. What I aimed at in preparing regulation was "to reorganize the Society, which was very active but only a group of intimate friends interested in the same microorganisms, into the one inspired by scientific and social necessity". This continues to be the basis of the present regulation of SAJ. Succeeding in the establishment of regulation, SAJ was registered to the Science Council of Japan, and Dr. Okami was inaugurated the first chairman and I was appointed the second chairman. In the meantime, studies on actinomycetes developed and deepened rapidly during 1980s, especially after 1985. It is well known that ISBA'88 was held in Tokyo taking the opportunity of this trend, and actinomycetes became familiar subject of study for many scientists. In Japan, there had been another group of actinomycetes researchers, namely, the Society for Applied Genetics of Actinomycetes, Japan. At the opportunity of ISBA'88, the opinion that this society and SAJ should be combined was voiced and became the majority. Fortunately with understanding and cooperation of the members of both societies, the union was realized on July 13, 1990. The basis for scientific exchange in actinomycetes study was thus established. I believe that hereafter the level of actinomycetes study would be further heightened in our country, and SAJ would continue to develop as the national center with both domestic and international roles.

I consider that it was as a representative of all members of the two societies who made effort for improvement of scientific exchange in the field of actinomycetes that I was given the Merit Award.

The author is grateful to Dr. Fumio Kato, Faculty of Pharmaceutical Sciences, Toho University, for his cooperation in the study of color of actinomycetes, and to many students who learned in my laboratory for their help.

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