SHORT COMMUNICATION

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CYTOCHROMES IN SPORES OF ACTINOMYCETES

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Certain deficiencies in respiration systems of bacteria (1–3), and, perhaps, some fungal (4, 5) spores were among the first recognized features which promoted the current interest in comparative biochemistry of dormant and vegetative states in microorganisms. Recent studies with spores of actinomycetes suggested that one might find among them cells widely differing in their structure, composition, and metabolic activity. The present paper reports a survey of cytochrome content in different kinds of spores of actinomycetes of the following species.

- (1) Aerobic mesophilic species which are thought to produce aerial conidia typical for the genus *Actinomyces* (*Streptomyces*). Spores not thermoresistant. Contain no dipicolinic acid (DPA). A number of enzymes were shown to be operative in intact spores of this type (6). Strains *A. fluorescens* 527 and *A. albus* 968 were both obtained from the collection of the Institute of Microbiology USSR Academy of Sciences.
- (2) Aerobic thermophilic species which are thought to produce conidia typical for certain thermophilic *Micropolyspora*. Spores weakly thermoresistant. Contain trace amounts or no DPA. The strain *M. rectivirgula* 1325 was obtained from the collection of Moscow State University.
- (3) Aerobic thermophilic species which are thought to produce spores, typical for the genera *Thermoactinomyces* and *Actinobifida*. Spores markedly thermoresistant. Contain appreciable amounts of DPA and have rather specialized internal structure reminiscent of bacterial endospores (7–11). The strains *Th. (Micromonospora) vulgaris* 136 and *A. dichotomica* 188 were obtained from the culture collection of Moscow State University.

Actinomycetes were cultured at respective optimal temperatures on cellophane strips placed on the surface of potato agar and thick suspensions of washed aerial spores were prepared as described earlier (12). Submerged mycelium (late exponential phase) was obtained from shaken cultures em-

Table 1. Cytochromes in mycelium of

Organisms and strains	Cytochrom		
	Submerged mycelium (shaken culture)		
	Cytochrome type		
	<i>c</i>	<i>b</i>	<i>a</i>
<i>Actinomyces fluorescens</i> 527	16.5	21.3	7.3
<i>Actinomyces albus</i> 968			
<i>Micropolyspora rectivirgula</i> 1325	25.5	60	10
<i>Actinobifida dichotomica</i> 188	41.5	35.5	18
<i>Thermoactinomyces vulgaris</i> 136	41.5	30.6	13

ploying a medium of the following composition: glycerol 20 g; peptone 50 g; fish extract 6.2 ml; NaCl 5.0 g; tap water 1 liter; pH 7.0. Cytochrome content was determined by using a low-temperature differential spectrophotometry (13). Suspensions of intact washed cells were prepared in 0.3 M sucrose, sodium hydrosulfite was used as reducing, potassium ferricyanide as the oxidising agent. For quantitative calculations the wave lengths used were 540 and 572 m μ for cytochromes of *b* and *c* type, and 590 and 624 m μ for cytochrome *a*. The amount of cytochromes was expressed in arbitrary units (1D per mg of dry biomass). If there were more than one peak of absorption corresponding to a particular cytochrome type, the calculations were based on the main peak.

In Table 1, data on cytochrome content in mycelia of different kinds and age, as well as in spores, are summarized.

As will be seen from Table 1, there was a considerable difference in cytochrome content in the young mycelium of actinomycetes grown in shaken or in surface culture. As a rule, the cytochrome content in the latter case diminishes 1.5–3 fold (depending on the culture and cytochrome type). One is reminded in this way of data showing increased functional activity of flavin-mediated oxidations in the surface mycelium of certain moulds (14). Cytochrome content in the mycelium of actinomycetes decreases with age. In spores one finds 13–20% of cytochrome content of submerged mycelium or 30–40% of that in young surface mycelium. The mycelium of thermophilic species is always richer in cytochromes.

The general cytochrome pattern (Table 1 and Fig. 1) does not change significantly if one compares mycelium and spores of actinomycetes producing nonresistant spores (that is, *A. fluorescens*, *A. albus*, and *M. rectivirgula*). One might assume that only quantitative changes in cytochrome system accompany spore formation in these organisms. There is no reason to exclude the functional role of cytochromes during maintenance and early germination events

different age and spores of Actinomycetes.

content ($\Delta D \cdot 10^{-4}/\text{mg}$ dry weight)								
Young mycelium (surface growth)			Aerial mycelium+spores (surface growth)			Spores		
Cytochrome type			Cytochrome type			Cytochrome type		
<i>c</i>	<i>b</i>	<i>a</i>	<i>c</i>	<i>b</i>	<i>a</i>	<i>c</i>	<i>b</i>	<i>a</i>
9.7	15.3	6.5	6.3	12.6	4.95	3.4	4.6	1.6
9.8	18.3	5.3	7.3	7.7	3.0	6.0	5.2	1.8
15	21	6.8	4.9	13.7	3.2	4.1	9.0	2.0
17	15.4	5.6	15	12.8	3.6	5.3	5.7	0.8
22	19	3.9	21.5	18.5	2.2	8.2	6.7	0.0

Fig. 1. Cytochrome pattern in aerial mycelium and spores of *A. fluorescens* 527.
Low temperature difference spectra.
1. Young (24 hr) aerial mycelium.
2. Old (120 hr) aerial mycelium.
3. Spores.

Fig. 2. Cytochrome pattern in aerial mycelium and spores of *Th. vulgaris* 136.
Low temperature difference spectra.
1. Young (12 hr) aerial mycelium.
2. Old (40 hr) aerial mycelium.
3. Spores.

in these spores. However, an additional possibility is also suggested since aerial conidia of mesophilic *Actinomyces* species were reported to contain significantly increased amount of catalase (15).

The situation is quite different with thermoresistant spore producers (Table 1 and Fig. 2). In the mature aerial mycelium of *T. vulgaris*, there is decrease in the cytochrome *a* content, while there was not enough distinction in the absorption peaks corresponding to cytochromes *b* and *c*. In mature spores of *T. vulgaris*, cytochrome *a* could not be detected. (The results with *A. dichotomica* were rather variable in this respect. Mean values from four independent determinations are presented in the Table.) One broad and flat absorption maximum is seen to correspond to cytochromes of *b* and *c* type. The possibility is not excluded that this absorption is due to some kind of reserve heme compound(s), as suggested for *Bacillus cereus* spores (3). Thus, qualitative changes in respiration system accompany transition from vegetative mycelium to heat-resistant spores in thermophilic actinomycetes.

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