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Biosynthesis of Ergothioneine and Hercynine by Fungi and *Actinomycetales*

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Unlike other bacteria, aerobic members of the order *Actinomycetales* show a close biochemical relationship to the fungi by their capacity to synthesize hercynine and ergothioneine. The myxomycete *Physarum polycephalum*, possessing the same synthetic ability, also shows this relationship. Contrariwise, the unusual position of yeasts as fungi is indicated by the inability of all yeastlike *Ascomycetes* and all except a few false yeasts to synthesize these two betaines.

Although the biosynthesis of ergothioneine and its precursor hercynine has been demonstrated in mycobacteria and a few fungi (5, 10), the role of these betaines in metabolism has yet to be discovered. However, from a taxonomic viewpoint, this synthetic capacity, shared by both these groups of organisms and not by other bacteria (10), pointed to the existence of a significant physiological relationship between the fungi and the mycobacteria. The relationship of certain other families of the order *Actinomycetales* in regard to this ability is examined in the present report.

The lack of production of ergothioneine by a few yeasts examined earlier (9) invited further investigation by suggesting a correlation between yeastlike forms and inability to produce ergothioneine. This concept is reinforced by data obtained during this investigation.

MATERIALS AND METHODS

Most of the fungi and a few *Actinomycetales* examined were obtained from the American Type Culture Collection (ATCC) and their numbers appear in the tables; strains without such numbers were cultures maintained in this laboratory. The two strains of *Nematospora*, Y-1808 and Y-2077, were supplied by L. J. Wickerham from the Northern Regional Research Laboratory, U.S. Department of Agriculture. *Physarum polycephalum* was obtained from J. W. Daniel, and the bulk culture for analysis was grown by R. M. Klein at the New York Botanical Garden.

The organisms were grown on a variety of chemically defined media (free of ergothioneine and hercynine) as indicated in Tables 1 and 2. Each organism was harvested by filtration or centrifugation, washed with water, dried in a vacuum oven at 80 C, and stored in a desiccator until processed for analysis. The dried organisms were extracted with hot water; the extract was concentrated to dryness and then suspended in 75% ethanol and chromatographed on an alumina column (5). In most instances the fractions containing ergothioneine and hercynine were concentrated and again chromatographed, with 80% ethanol used as solvent to obtain a good separation.

Assays for ergothioneine and hercynine were performed as previously described (5) except that, in most cases, 1% formic acid was omitted from the column solvent. Content of the betaines was expressed as milligrams per 100 g of dried organisms.

RESULTS

Organisms selected from among the fungi and the bacterial order *Actinomycetales* were examined for their capacity to produce ergothioneine and hercynine on chemically defined media free of these betaines (Table 1). Members of the fungal classes *Zygomycetes*, *Ascomycetes*, *Deuteromycetes*, and *Basidiomycetes*, as well as one *Myxomycete*, were observed. In addition, a few strains of *Streptomyces*, one of *Nocardia*, and one of *Actinoplanes*, aerobic members of the order *Actinomycetales*, were examined. For the *Mycobacteriaceae*, data summarized from earlier work (5) was included.

All the organisms examined in Table 1 produced ergothioneine and all but *Aspergillus niger* produced hercynine. However, failure to detect hercynine in this instance is not considered significant because the organism does synthesize ergothioneine and presumably must do so through the hercynine pathway (2, 11). The fungi made small to moderate amounts of ergothioneine (1.7 to 46.6 mg/100 g). Earlier determinations done on *Neurospora crassa* and *Geotrichum rugosum* had established maximum yields of 85 to 110 mg per cent (9). These data, considered along with the current results, show that the range of ergothi-

TABLE 1. *Production of ergothioneine and hercynine by fungi and Actinomycetales cultured on synthetic media*

Classification	Organisms	ATCC no.	Growth conditions[a]		Dry wt analyzed	Yield of betaines	
			Medium[b]	Time		Ergothioneine	Hercynine
				days	*g*	*mg/100 g*[c]	*mg/100 g*[c]
Mycota							
Eumycotina							
Zygomycetes	*Rhizopus stolonifer* (−)	12939	W	9	1.3	20.3	15
	R. stolonifer (+)	12938	W	9	1.6	8.6	1
Ascomycetes	*Aspergillus nidulans*	10074	W+	17	0.9	1.7	8
	A. niger		W	12	0.6	12.5	0
	Neurospora crassa		R	8	2.8	46.6	5
	Penicillium roqueforti		W	16	1.0	4.3	76
	P. notatum	9178	W	10	2.4	2.5	4
Deuteromycetes	*Geotrichum rugosum*	757	W	5	0.5	41.8	8
	Rhodotorula glutinis	2527	W	5	0.5	3.1	12
Basidiomycetes	*Sporobolomyces salmonicolor*[d]	623	W	10	2.1	3.9	3
Myxomycotina							
Myxomycetes	*Physarum polycephalum*		D	10	0.9	46.0	3
Protophyta							
Schizomycetes							
Actinomycetales							
Mycobacteriaceae	Many species[e]		S	7–77	0.1–3.0	1–118	3–68
Actinomycetaceae	*Nocardia asteroides*		W+	21	1.5	51.8	67
Streptomycetaceae	*Streptomyces albus*	3004	RN	7	1.3	1.7	6
	S. fradiae	10745	RN	9	0.6	30.8	7
	S. griseus	10137	RN	7	1.4	50.0	104
Actinoplanaceae	*Actinoplanes philippinensis*	12427	RN	14	0.8	64.0	13

[a] All organisms except the mycobacteria were grown at 25 C; *S. albus*, *S. griseus*, and *A. philippinensis* were grown as shake cultures.

[b] Composition of media: D, Daniel et al., medium AV-40 including hematin (4); R, Ryan (13); RN, Romano and Nickerson medium (12), including 0.5% asparagine (omitted for *S. fradiae*); S, Sauton (14); W, Wickerham (17); W+, 1% mannitol and 0.4% asparagine added to W.

[c] Dry weight of cells.

[d] Alexopoulos (1), citing several investigators, classifies this as a *Basidiomycete* rather than as one of the *Cryptococcaceae*.

[e] Data summarized from Genghof and Van Damme (5) includes 101 human and nonhuman strains.

oneine synthesis among fungi is comparable to that observed for the *Mycobacteriaceae*. Values for hercynine, the sulfurless precursor of ergothioneine, were generally lower than those of ergothioneine, in keeping with past experience. In a few cases, the hercynine levels were higher, as may happen with time, when the amount of sulfur in the medium becomes depleted (6).

Table 1 shows the existence of a point of biochemical similarity between the fungi and certain members of the bacterial order *Actinomycetales* in that the two groups synthesize both ergothioneine and hercynine, indicating possession of a synthetic pathway common to both groups. No other bacteria have ever been shown to synthesize ergothioneine. A selection of bacteria from 10 different genera tested earlier (9, 10) proved negative in this respect. In unpublished work (D. S. Geughof), three additional organisms, *Escherichia coli* W (3 g, dry weight), *Clostridium histolyticum* (7 g, dry weight), and *Propionibacterium shermanii* (0.5 g, dry weight), showed no synthesis of ergothioneine or hercynine. Thus far, the following genera have shown no evidence of ergothioneine synthesis: *Bacillus*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Lactobacillus*, *Propionibacterium*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Vibrio*.

P. polycephalum, a slime mold and free-living plasmodium of the subdivision *Myxomycotina*, reveals a relationship to the *Eumycotina*, at the biochemical level, in its capacity to synthesize these betaines (Table 1).

Among the fungi examined was a group that

did not synthesize the betaines (Table 2); all except one of these, *Sordaria fimicola*, were yeasts. Earlier observations (9) that several yeasts seemed incapable of producing ergothioneine suggested there might be a correlation between the yeastlike state and inability to form ergothioneine. A group of six strains in the class *Ascomycetes* (true yeasts) and six strains of the *Deuteromycetes* (false yeasts) were grown, extracted, and examined for content of ergothioneine and hercynine (Table 2). In contrast to the filamentous fungi (Table 1), none of these yeasts synthesized either of the betaines when grown in a chemically defined medium. Three false yeasts, *G. rugosum* [classified as *Trichosporon cutaneum* by Lodder and Kreger-Van Rij (8)], *Rhodotorula glutinis*, and *Sporobolomyces salmonicolor*, were exceptions in that they did produce both betaines (Table 1). But the characteristic pattern among the yeasts seems to be a lack of capacity to synthesize ergothioneine and hercynine.

DISCUSSION

The fact that several genera of the order *Actinomycetales*, including *Nocardia asteroides*, three strains of *Streptomyces*, and a strain of *Actinoplanes philippinensis*, were found to synthesize ergothioneine is of interest in that it suggested a hitherto unappreciated source of soil ergothioneine which is available for incorporation into plants. Melville et al. (10) first proposed that the fungi in soil be considered the chief, if not only, source of the ergothioneine found in oats and other cereal grains. Later work by S. Eich in Melville's laboratory (9) showed the capacity of oat seedlings to incorporate radioactive ergothioneine via the root system and suggested this as the probable mechanism for ergothioneine incorporation into plants. More recently, Tan and Audley (16) reported the presence of very large amounts of ergothioneine (8.2 to 9.5 mg/g) in *Hevea brasiliensis* latex and, in a later communication (3), indicated that this was probably obtained from soil by the growing plant. Indeed, the occurrence of hercynine (16) as well in this plant product is probably also due to absorption of the compound produced by microorganisms in the soil.

Recently, Scott and Henderson (15) suggested that ergothioneine is an artifact of isolation derived from an imidazolidine-2-thione present in the original biological material and formed during isolation (acid conditions). It seems unlikely that this is the case in any of our work because of the very mild conditions of extraction employed. In the experiments reported here, hot-water extraction (90 to 100 C) of dried organisms was used, but more recently this has been modified to consist of extraction with 75% ethanol at room temperature of unheated, moist mycobacteria (6). D. B. Melville (*personal communication*) has obtained no evidence so far that ergothioneine is derived from another compound, changed in structure during isolation.

TABLE 2. *Fungi showing no evidence of ergothioneine or hercynine production*

Classification	Organism[a]	ATCC no.	Growth period[b]	Dry wt
			days	*g*
Ascomycetes	*Nematospora* sp.[c]		4	0.2,0.3
	Pichia membranefaciens	2254	7	0.3
	P. neerlandica	10653	7	0.3
	Saccharomyces carlsbergensis	9080	2	0.4
	S. cerevisiae		1	0.9
	Sordaria fimicola		31	0.4
	Torulospora rosei	10664	4	0.6
Deuteromycetes	*Candida albicans*		7	0.8
	Oospora sp.		17	0.6
	Pityrosporum ovale	12078	4	0.6
	Torulopsis utilis	8206	1	0.5
	Trichosporon fermentans	10675	4	0.4
	T. pullulans	10677	8	0.2

[a] Several of these organisms were grown and analyzed for the betaines by this investigator in the laboratory of D. B. Melville at Cornell University Medical College. *T. utilis* was analyzed by E. Inamine.

[b] All grown on Wickerham medium (17) at 25 to 30 C.

[c] Two strains analyzed.

The inability of *Sordaria fimicola* (Table 2), an unusual fungus, to synthesize ergothioneine and hercynine could conceivably be associated with its inability to make asexual spores. Heath and Wildy (7) observed that only the spores and not the mycelium of *Claviceps purpurea* synthesize ergothioneine. A thorough investigation of synthesis by mycelium as opposed to synthesis by spores has not been made, though Melville (9) states that he has also found the betaine present in the conidia of *A. niger* and *N. crassa*.

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LITERATURE CITED

1. Alexopoulos, C. J. 1962. Introductory mycology, 2nd ed. John Wiley and Sons, New York.
2. Askari, A., and D. B. Melville. 1962. The reaction sequence in ergothioneine biosynthesis: hercynine as an intermediate. J. Biol. Chem. **237**:1615–1618.
3. Audley, B. G., and C. H. Tan. 1968. The uptake of ergothioneine from the soil into the latex of *Hevea brasiliensis*. Phytochemistry **7**:1999–2000.
4. Daniel, J. W., K. L. Babcock, A. H. Sievert, and H. P. Rusch. 1963. Organic requirements and synthetic media for growth of the myxomycete *Physarum polycephalum*. J. Bacteriol. **86**:324–331.
5. Genghof, D. S., and O. Van Damme. 1964. Biosynthesis of ergothioneine and hercynine by mycobacteria. J. Bacteriol. **87**:852–862.
6. Genghof, D. S., and O. Van Damme. 1968. Biosynthesis of ergothioneine from endogenous hercynine in *Mycobacterium smegmatis*. J. Bacteriol. **95**:340–344.
7. Heath, H., and J. Wildy. 1956. The biosynthesis of ergothioneine and histidine by *Claviceps purpurea*. 1. The incorporation of 2-^{14}C acetate. Biochem. J. **64**:612–620.
8. Lodder, J., and N. J. W. Kreger-Van Rij. 1967. The yeasts. North Holland Publishing Co., Amsterdam.
9. Melville, D. B. 1959. Ergothioneine. Vitamins Hormones **17**:155–204.
10. Melville, D. B., D. S. Genghof, E. Inamine, and V. Kovalenko. 1956. Ergothioneine in microorganisms. J. Biol. Chem. **223**:9–17.
11. Reinhold, V. N., Y. Ishikawa, and D. B. Melville. 1970. Conversion of histidine to hercynine by *Neurospora crassa*. J. Bacteriol. **101**:881–884.
12. Romano, A. H , and W. J. Nickerson. 1956. The biochemistry of the Actinomycetales. Studies on the cell wall of *Streptomyces fradiae*. J. Bacteriol. **72**:478–482.
13. Ryan, F. J. 1950. Selected methods of Neurospora genetics p. 51–75. *In* R. F. Rushmer (ed.), Methods in medical research, vol. 3. Year Book Medical Publishers, Inc., Chicago.
14. Sauton, B. 1912. Sur la nutrition minerale du bacille tuberculeux. Compt. Rend. **155**:860–861.
15. Scott, J. E., and G. Henderson. 1968. A new route to the imidazole-2-thiones from 2-thiohydantoins. Biochem. J. **109**:209–215.
16. Tan, C. H., and B. G. Audley. 1968. Ergothioneine and hercynine in *Hevea brasiliensis* latex. Phytochemistry **7**:109–118.
17. Wickerham, L. J., and K. A. Burton. 1948. Carbon assimilation tests for the classification of yeasts. J. Bacteriol. **56**: 363–371.