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Fatty acids and production of tylosin-like compounds in *Streptomyces fradiae*

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The composition of fatty acids and the spectrum of macrolide antibiotics produced in 7 mutant strains of *Streptomyces fradiae*, a tylosin producer, were investigated. The strains under investigation differed in the production level and representation of individual tylosin-like compounds. The composition of fatty acids in the mycelium did not depend on the total production. However, the strains producing relomycin in addition to tylosin produced a significantly higher fraction of fatty acids with a higher melting point, and, on the contrary, the strains producing only tylosin or tylosin and desmycosin synthesized a significantly lower proportion of these acids. The results obtained indicate that in addition to the activity and substrate specifity of secondary metabolism enzymes, the composition of the tylosin-like compounds produced can be influenced by the cell membrane and its function.

The occurrence and biosynthesis of fatty acids in streptomycetes have already been studied from different points of view, however, often as a part of the study of the biosynthesis of secondary metabolites. A considerable attention was particularly devoted to the composition of fatty acids in streptomycetes producing secondary metabolites of oligoketide type as metabolic pathways leading to both types of compounds utilize identical precursors (Běhal *et al.* 1969 a, b).

It is known that the composition of fatty acids in microorganisms is highly influenced by cultivation conditions under which the cell population is grown. However, physiological changes caused by differences in fatty acid composition are less understood (Arima *et al.* 1973, Okazaki *et al.* 1973, 1974). Lipids produced by streptomycetes contain branched iso and anteiso fatty acids, whose amount and mutual ratio are important with respect to membrane functions and are regulated both at the genetical and physiological level (Batrakov *et al.* 1978).

Gräfe *et al.* (1982 a, b) isolated and characterized mutants of *Streptomyces hygroscopicus* and *Streptomyces griseus* that cannot form aerial mycelium and have a different ratio of iso and anteiso acids, viz. i-16:0 and ai-15:0, respectively. The authors assume that this fact is caused by a genetically determined change of the biosynthesis of corresponding branched amino acids, i.e. valine and isoleucine, and that a mechanism regulating the composition of fatty acids exists in streptomycetes and that the function of such a regulatory mechanism is necessary for differentiation.

We studied the composition of fatty acids in *Streptomyces fradiae*, a tylosin producer. Tylosin is a 16-membered macrolide antibiotic, often produced together with other structurally related compounds, macrocin, lactenocin, relomycin and desmycosin (Seno *et al.* 1977). As the spectrum of these compounds is influenced not only by regulation of synthesis and activity of enzymes of secondary metabolism but can also be regulated at the level of transport of macrolides from the cell, we studied the relationship between the composition of fatty acids and spectrum of macrolides produced.

Materials and methods

Strains and their cultivation: All strains used were from the collection of the Research Institute of Biofactors and Veterinary Drugs in Kouřim, Czechoslovakia. Strains 13/1, 13/20 and 13/4 were induced by mutagenic treatment (UV light) of one parent strain, strains 30/37, 30/42, 60/48 and 180/48 were obtained in the same way from another parent strain. The strains were cultivated in 500 ml flasks containing 40 ml of medium on a reciprocal shaker (1.7 Hz) for 7 days. The first generation was cultivated for 2 days, the second generation was inoculated with 5% of this culture. A chemically defined medium contained (g/l): sucrose 20, sodium L-glutamate 2, $CaCO_3$ 3, NaCl 5, $MgSO_4 \cdot 7\,H_2O$ 1, $K_2HPO_4 \cdot 3\,H_2O$ 0.3, and 1 ml of a solution of trace metals. This solution contained: $FeSO_4 \cdot 7\,H_2O$, $ZnCl_2 \cdot 4\,H_2O$, $ZnSO_4 \cdot 7\,H_2O$, $CuSO_4 \cdot 5\,H_2O$, $CoCl_2 \cdot 2\,H_2O$, all of them at a concentration of 1 g/l. Before heat sterilization pH was adjusted to 7.0 with 1 N NaOH.

A complex medium was prepared according to BALTZ and SENO (1981) and contained (g/l): beet molasses 2.0, corn meal 15, fish meal 9, corn gluten 9, NaCl 1, $(NH_4)_2HPO_4$ 0.4, $CaCO_3$ 2, crude soybean oil 30. Before sterilization pH was adjusted to 7.8.

Analytical methods: Total concentration of tylosin-like compounds was determined by means of the diffusion plate method with *Sarcina lutea* or after chloroform extraction according to absorbance at 290 nm and comparison with known standards. Relative amounts of individual tylosin analogues were determined by quantitative thin layer chromatography according to SENO *et al.* (1977). The compounds were applied using the application device LINOMAT 3 and the chromatograms were monitored in a densitometer CAMAG (MUTTENS, Switzerland).

Analysis of fatty acids: Cells were centrifuged, washed twice with water and hydrolyzed with boiling 1 N NaOH. The mixture was acidified with HCl to pH 1 and extracted twice with diethylether. Combined extracts were evaporated, derivatized (BF_3-methanol, boiling for 3 min), separated and identified in the GC-MS HEWLETT-PACKARD 5995 B (CA, USA) apparatus under the following conditions. Capillary column 25 m × 0.25 mm ID × 0.2 μm film with SE-54 phase. Injector temperature 250 °C, programmed temperature from 140 °C to 250 °C at 4 K/min. Carrier gas (helium) flow 1.1 ml/min, split 1:50, ionization voltage 70 eV.

Results

The occurrence and relative representation of fatty acids in the mycelium of individual *Streptomyces fradiae* strains are presented in Table 1. The identified fatty acids were within the C_{14}—C_{18} range, in agreement with literature data (BATRAKOV and BERGELSON 1978). The content of branched fatty acids was high, from 66% of total fatty acids in the strain 30/37 up to 76% in the strain 30/42. Homologues of tuberculostearic acid described in *Streptomyces* by BALLIO and BARCELLONA (1968) were not detected. Acids ai-15:0, i-16:0, 16:0, 16:1, ai-17:0 were most frequent in all strains tested. Table 2a shows the sum of homologues of iso, anteiso, saturated and unsaturated fatty acids. The highest differences among individual strains were detected in iso-fatty acids with even carbon number (precursor isobutyryl-CoA), where the difference in the relative representation was as high as 100%. In the strain 180/48 no substantial qualitative or quantitative difference in the spectrum of fatty acids was detected.

Table 2b illustrates the production of tylosin and tylosin-like compounds in the complex and chemically defined media. In the complex medium the strains 13/1, 13/20 and 13/4 produced tylosin and relomycin and the strains 13/1 and 13/4 produced macrocin in addition. In the complex medium the strains 30/37, 30/42 and 60/48 produced tylosin and the strains 30/42 and 60/48 also produced macrocin and desmycosin.

Discussion

In the present communication the profile of fatty acids in *Streptomyces fradiae*, a tylosin producer, has been first described. No significant differences with respect to other streptomycetes could be detected (BATRAKOV *et al.* 1977, EFIMOVA and TZYGHANOV 1969, KONOVA *et al.* 1974, KOVALČUK *et al.* 1980, KOZYRITSKAIYA *et al.* 1979, ŘEZANKA *et al.* 1984).

Table 1
Content of fatty acids (%) in *Streptomyces fradiae* mutants

Fatty acid[a]	Strain						
	13/1	13/20	13/4	30/37	30/42	60/48	180/48
14:0	1.4	1.5	2.2	2.4	0.6	2.8	0.2
i-14:0	3.5	3.4	3.1	4.6	2.8	2.0	1.6
15:0	1.0	0.9	1.0	1.2	1.4	1.2	0.9
i-15:0	6.7	7.1	7.1	4.5	5.6	4.8	4.3
ai-15:0	17.3	13.5	10.4	18.8	28.0	21.0	19.5
16:0	8.9	10.8	12.3	8.4	8.6	11.4	8.9
i-16:0	17.9	22.5	26.4	8.7	21.5	17.4	18.1
16:1	4.7	2.8	2.1	9.2	9.5	7.2	13.8
i-16:1	3.3	2.8	2.9	3.4	tr	2.0	2.0
17:0	3.4	2.6	3.6	2.9	0.6	0.8	0.9
i-17:0	2.3	3.4	5.8	4.1	2.7	2.1	2.4
ai-17:0	13.5	9.9	8.3	13.7	14.3	14.2	14.1
17:1	1.1	1.2	1.0	1.3	tr	0.9	1.2
17:1	0.6	0.8	0.8	0.7	tr	0.8	1.1
i-17:1	2.5	2.1	2.3	2.6	0.5	2.2	2.4
ai-17:1	4.2	6.1	4.6	5.4	tr	5.0	5.1
18:0	0.5	1.1	1.2	0.9	1.0	1.6	0.5
18:1	3.3	3.8	2.9	3.4	0.9	0.7	1.3
18:1	0.9	0.8	0.7	0.6	tr	0.6	0.9
18:2	3.0	2.7	1.3	3.2	1.3	1.3	0.8
20:0	tr	0.1	tr	—	0.7	tr	—

[a] First number, number of carbon atoms in the chain; second number, number of double bonds; i, isoacid; ai, anteisoacid; tr, traces

Table 2a
Fatty acid composition of mutant strains of *Streptomyces fradiae*. Classification according to structure

Fatty acid (%)	Strain						
	13/1	13/20	13/4	30/37	30/42	60/48	180/48
Even iso-branched (ie)	24.7	28.7	32.4	16.7	24.3	21.4	21.7
Odd iso-bracnhed (io)	11.5	12.6	15.2	11.2	8.8	9.1	9.1
Anteiso-branched (ai)	35.0	29.5	23.3	37.9	42.6	40.2	38.7
Saturated (s)	15.2	17.0	20.3	15.8	12.2	17.8	11.4
Unsaturated (u)	13.6	12.2	8.8	18.4	11.7	11.5	19.1
Ratio $\frac{ie + io + s}{ai + u}$	1.06	1.40	2.12	0.78	0.83	0.93	0.73

Table 2b
Production of tylosin and tylosin-like compounds in the complex and chemically defined media

	Strain						
	13/1	13/20	13/4	30/37	30/42	60/48	180/48
Total production (μg/ml)[a]	6200/76	4200/55	6000/67	7000/85	10400/134	6600/92	—
Relative amount:							
tylosin	81/100	75/100	46/96	100/100	58/100	63/100	—
desmycosin	—	—	—	—	8/0	14/0	—
macrocin	11/0	—	32/0	—	34/0	23/0	—
relomycin	8/0	25/0	22/4	—	—	—	—

[a] Production in the complex medium/production in the chemically defined medium

As tylactone, the tylosin aglycone, is common for all tylosin-like compounds, a direct correlation between the quantitative representation of individual fatty acids and mutual ratio of structural analogues, described for instance in the biosynthesis of monensins A and B in regulatory mutants of *Streptomyces cinnamonensis* (POSPÍŠIL *et al.* 1985), cannot exist here. However, mutant strains with a changed profile of fatty acids, in which the uptake of certain nutrients by the cell or excretion of some metabolic products by the cell are facilitated can be isolated (GRÄFE *et al.* 1982b). Mutants of *Streptomyces fradiae*, a neomycin producer, with a changed spectrum of fatty acids, required oleic acid for the antibiotic production and accumulated increased amounts of amino acids under conditions of the antibiotic biosynthesis (ARIMA *et al.* 1973, OKAZAKI *et al.* 1973, 1974). These results indicate that the biosynthesis of products of secondary metabolism in microorganisms is closely associated with the construction and function of the cell membrane. Therefore, we concentrated on possible relationships between the profile of fatty acids representing to a certain extend properties of the membrane with respect to its permeability and spectrum of the antibiotic produced. Tylosin, macrocin, relomycin and desmycosin differ in their chemical structure (i.e. even polarity) and it may be thus assumed that their transport out of the cell proceeds with different velocity and depends on properties of the cell membrane. The composition of fatty acids with different melting point is an important characteristic with respect to the cell membrane function. Unbranched fatty acids have melting points comparable with those of corresponding iso acids, whereas anteiso acids and unsaturated acids have lower melting points (e. g. n-17:0, 61.3 °C; i-17:0, 60.5 °C; n-17:1, 13.5 °C; ai-17:0, 38.0 °C). The function and behaviour of individual fatty acid in the cell membrane are also determined by their structure. From this point of view, the ratio presented in Table 2a should thus characterize the membrane with respect to its permeability. It is of interest that this ratio is the highest in the strains 13/1, 13/20 and 13/4 producing relomycin in addition to tylosin. High amounts of relomycin are produced just by strains having highest values of the above mentioned ratio. The strains 30/42 and 60/48 produce less polar desmycosin in addition to tylosin and macrocin and have a lower value of this ratio. With respect to the profile of fatty acids, the strain 1180/48, in which the biosynthetic pathway is blocked prior to the production of tylactone and which consequently does not produce any macrolide antibiotic, resembles the strains 30/37, 30/42 and 60/48. On the basis of the results referred to here it may be assumed that membrane permeability determined by the representation of individual fatty acids can, to a certain extent, modify the spectrum of tylosin-like compounds, which is otherwise determined genetically and mediated by the synthesis, activity and substrate specificity of enzymes of secondary metabolism and to a lesser extent also by cultivation conditions (SENO *et al.* 1977). The cell membrane can thus favour the excretion of a metabolite by the cell. These problems have not yet been studied in this connection. In order to understand better the effect of the structure of fatty acids on the spectrum of tylosin-like compounds produced it will be necessary to modify cultivation conditions in such a way which would lead to a purposeful change of the fatty acid profile and thus to changed properties of the membrane and ratio of individual tylosin-like compounds.

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Book Review

H. WERNER, Anaerobier-Infektionen — Pathogenese, Klinik, Therapie, Diagnostik (2. überarbeitete u. erweiterte Auflage). 200 S., 51 Abb., 140 Tab. Stuttgart-New York 1985. Georg Thieme Verlag. DM 26,00. ISBN: 3-13-598702-7.

Lange Zeit standen die durch Clostridien verursachten Krankheiten Tetanus und Gasbrand im Mittelpunkt der Anaerobierinfektionen. In den letzten Jahrzehnten fand gleichermaßen bei Klinikern und Mikrobiologen die Gruppe der anaeroben sporenlosen Bakterienspezies großes Interesse. Sie verursacht im wesentlichen septisch-eitrige Infektionen. Diese neue Entwicklung ist nicht zuletzt auf große Fortschritte zurückzuführen, die auf dem Gebiet der anaeroben Kulturverfahren und durch neue Methoden zur selektiven Anzucht und Differenzierung dieser sehr heterogenen Keimgruppe gemacht wurden. Der Autor des vorliegenden Buches hat selbst maßgebliche Pionierarbeit auf diesem schwierigen und mühevollen Spezialgebiet der medizinischen Mikrobiologie geleistet. Durch seine Untersuchungen sind wesentliche neue Erkenntnisse u. a. zur Pathogenese, Mischinfektionen, Standortflora, Antibioticaresistenz und zur klinischen Bedeutung einzelner Anaerobierspezies gewonnen worden. Das Buch gibt daher nicht nur einen kompetenten Überblick über die durch Anaerobier bedingten Infektionen, sondern es werden auch die vielen eigenen Erfahrungen und Untersuchungsergebnisse mit in den Vordergrund der Betrachtungen gestellt. Die vorliegende zweite Auflage folgt bereits 4 Jahre nach der ersten. Wesentliche neue Erkenntnisse auf diesem Gebiet wurden eingearbeitet, auch ist jetzt die Laboratoriumsdiagnostik als besonderer Abschnitt vorhanden. Zuerst werden wichtige Eigenschaften der Anaerobier abgehandelt, wobei auf neue Forschungsergebnisse der Taxonomie, Genetik, Immunologie und Pathogenese eingegangen wird. Ihrer Bedeutung entsprechend werden dann die durch Anaerobier verursachten vielfältigen Infektionen ausführlich dargelegt. Im Vordergrund stehen Krankheitsprozesse mit mono- oder polymikrobieller Beteiligung von Anaerobiern. Dabei wird jeweils auf die Pathogenese, Ätiologie, klinisches Bild, Diagnose und Therapie eingegangen. Im Abschnitt Grundlagen der Anerobierchemotherapie wird entsprechend ihrer besonderen Problematik der gegenwärtige Kenntnisstand umfassend behandelt. Die weltweit durchgeführten prospektiven Studien, besonders auch die der Arbeitsgruppe des Autors, werden im Ergebnis dargelegt und durch viele instruktive Tabellen und Abbildungen veranschaulicht. Für den Kliniker dürften vor allem auch die Therapiekonzepte für die sehr problematischen Mischinfektionen von Interesse sein. Die Laboratoriumsdiagnostik wird im wesentlichen unter praktischen Aspekten abgehandelt. Auch hier spiegeln sich die großen Erfahrungen, die der Autor selbst sammeln konnte, z. B. Chemotherapeutica-Empfindlichkeitstestung, wider. Das ausführliche Literaturverzeichnis (771 Zitate) ermöglicht durch eine Untergliederung in Abschnitte eine rasche Information über wichtige Arbeiten zur entsprechenden Thematik.

Dieses Buch vermittelt einen umfassenden und eindrucksvollen Überblick über die Anaerobier und die durch sie verursachten Infektionen. Im deutschen Schrifttum gibt es nichts Vergleichbares, so daß dieses gut ausgestattete und preiswerte Werk sehr brgrüßt wird. Für Kliniker und Mikrobiologen wird es gleichermaßen von großem praktischen Nutzen sein.

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