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Identification of odorous compounds from Streptomyces avermitilis.

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SUMMARY

The production of avermectin-type antibiotics and the odours of a strain of *Streptomyces avermitilis* was investigated. With the increasing production of the total of avermectins (0-600 ug/ml), the synthesis of geosmin is also enhanced by more than one order of magnitude and, in contrast, the production of homologues of oxolone (dihydrofuranone) decreases in a similar range. In addition to these compounds, more than twenty other volatile chemical products have been identified by means GC-MS, *i.e.* pyrazine derivatives, acetoin and its homologues, aromatic esters, furan derivatives, *etc.*).

Microorganisms produce many volatile substances with strong odours. In the 19th century, the odours of the soil were subjected, for first time, to scientific scrutiny [Berthelot and André, 1891]. Recently, substances have been isolated from cultures of actinomycetes which can be held responsible for these odours. The substance responsible for the typical earthy odour of the soil could be extracted from soil by steam and was probably neutral.

As microorganisms began to be grown in pure culture, mainly actinomycetes, produced an earthy odour. Ether-soluble extracts were obtained [Gerber and Lechevalier 1965; Gerber 1968] from actinomycetes which had an odour at high concentration, but which, upon dilution, became earthy-smelling. *Streptomyces griseoluteus,* an odouriferous strain was described [Romano and Safferman 1963]. The metabolites of actinomycetes were studied [Dougherty *et al.* 1966] by nuclear magnetic resonance and mass spectrometry. They obtained some partial structures (fife or six membered lactones), which were revised in the further paper [Gerber 1973].

In this paper were proved these structures as five membered lactones with two alkyl substituents. Based on previous research [Vaněk *et al.* 1990] of the oligoketides antibiotics production from *Streptomyces*, we selected an industrially important strain of *Streptomyces avermitilis* as a suitable model. This strain produces a mixture of avermectines that are biologically active compounds with strong anthelmintic effect. Our study shows that during cultivation and in the dependence on the production of antibiotics great difference in the secretion of smelling compounds was obtained.

MATERIALS AND METHODS

Strain and cultivation

The mutant strain *S. avermitilis* C-18 obtained by genetic improvement [Cimburková *et al.* 1988] of ATCC 31267 was cultivated in a 7 l laboratory fermenter (MBR Bioreactor AG, Sultzer, Swiss) at 28 C. The twin Rushton turbine mixer in a vessel equipped with four baffles was used at 500 rpm and aeration 0.6 VVM.

Antibiotic quantification

Avermectins in the broth were determined by HPLC [Čurdová *et al.* 1989]. Measurements of avermectins were carried out on HPLC-chromatograph SP 8000 B (Spectra Physics, Fremont, CA, USA) with reverse phase column RP18; mean diameter of particles 5μm, ID 0.4 cm, length 25 cm (Tessek, Prague, Czech Republic), mobile phase methanol:H_2O (85:15) and detection at 252 nm.

Structures of volatiles

All samples of supernatant were distilled until 20% of their volume had been collected as distillate. The distillate was extracted with 10% (v/v) of methylene chloride. After drying, the solutions were direct inject in to GC-MS.

GC-MS (a double focusing tandem Jeol DX 303, Tokyo, Japan) was coupled with 60 m long capillary column SPB-5 (ID 0.32 mm); helium as carrier gas and the oven temperature was held at 50 °C for 2 min and then increased by a gradient of 10 °C/min to the final temperature of 250 °C. Ionization energy was 70 eV

RESULTS AND DISCUSSION

Table 1 shows the relative proportions of the individual volatile compounds from a supernatant of *S. avermitilis* identified by GC-MS. Three different cultures grown in a fermenter were compared (non-production, low-production and high-production conditions), the respective productions of antibiotics in the two latter strains being 90 and 600 µg/ml. The individual compounds (*cf.* Table 1) were analyzed by GC-MS on a capillary column immediately after being steam distilled. The peaks obtained were identified on the basis of the characteristic splitting in the mass spectrum and/or the spectrum library. The individual volatile, malodorous compounds were quantified by using the total ion current. The data indicate high proportions of pyrazine-derived heterocyclic compounds.

Their formation can be explained by a reaction between the acetoin-type compounds and amino acids and by a following oxidation-dehydration aromatization. Therefore, their presence cannot be considered surprising.

Among the low-molecular, aliphatic-chain compounds it is the oxygen-containing derivatives of butane and hexane that dominate. These derivatives are formed by degradation of saccharides with pyruvic acid being an intermediate. The following reaction can be ensured by the enzyme diacetyl synthase or by decarboxylation of acetolactate formed by the reaction of pyruvic acid with acetyl lipoate. The formed derivatives that include mainly 4- and 5-methyl-substituted hexanols and hexanolones suggest that the first reaction can be dominating.

Geosmin is a well-known and widely-studied compound [Rosen *et al.* 1968] whose presence in water is recognized by the characteristic earthy smell even at a dilution of 1 to 10^9. The smelling water is difficult to drink. Also, the fish living in a water contaminated with geosmin tastes of mud. Many papers have been published on this topic [Bowner *et al.* 1992; Aoyama 1990; Ploeg *et al.* 1992]

Having analysed our experiments we found that there is a correlation between production of avermectins and odours represented by geosmin. No such finding has been reported for *Streptomyces*. With an increasing concentration of the antibiotic produced the content of geosmin was rising by more than one order of magnitude and, on the contrary, the relative proportions of the oxolone homologues decreased as much as tenfold. A total of five oxolone homologues were identified in the previous works [Gerber 1973]. In this study, we were able to identify other members of the homologous series by using their mass spectra: 3-methyl, 3-ethyl, 3-propyl, and 3-heptyl-5-methyl-3-oxol-2-one. We suppose that some of the unidentified peaks represent other unknown oxolone homologues. In keeping with this observation seems to be a well-known fact that the producing conditions are characterized by the earthy smell, most probably caused by geosmin. In contrast, the production of oxolones having a musty smell is correlated with a low production of the antibiotic.

A similar trend as with the oxolone homologues was observed in case of other furan derivatives, *i.e.* 2,3-dimethyl tetrahydrofurane and methyl 2-furan carboxylate and, also, the hexa-membered, heterocyclic compound 5, 6-dimethyl-tetrahydro-2-pyrone. 5-(2-methylenecyclohexyl)-2-pentanone and

Table 1. Composition (% of total) of odour compounds from *Streptomyces avermitilis*

Compound	Cultivation		
	1	2	3
3-hydroxy-2-butanone	1.14	1.21	0.80
pyrazine	8.72	13.82	7.98
3-hydroxy-3-methyl-2-butanone	2.35	5.25	5.00
hexenal	2.01	3.39	1.47
methylpyrazine	5.09	6.24	6.04
n-hexanol	3.37	0.27	1.36
4-hexen-3-one	0.71	0.98	2.23
4-hydroxy-3-hexanone	0.80	1.50	1.11
4-methyl-2-hexanol	1.16	1.22	1.94
5-methyl-3-hexanone	0.36	0.61	0.54
2,5-dimethylpyrazine	0.87	0.94	2.03
vinylpyrazine	6.95	6.69	8.03
2,3-dimethyltetrahydrofurane	7.40	2.81	0.47
methyl 2-furancarboxylate	2.69	0.66	0.32
3,5-dimethyl-3-oxol-2-one	2.41	1.53	0.46
nonenone	0.79	2.01	1.81
methyl benzoate	1.16	3.15	6.06
3-ethyl-5-methyl-3-oxol-2-on	6.77	3.08	0.53
2-fenylethanol	2.18	3.29	4.27
4-pyridincarboxylic acid	1.16	1.22	0.93
3-propyl-5-methyl-3-oxol-2-one	3.64	2.76	1.10
unknown	1.00	2.09	2.02
unknown	3.12	3.77	1.60
3-butyl-5-methyl-3-oxol-2-one	2.47	1.03	0.21
5,6-dimethyl-tetrahydro-2-pyrone	1.54	1.95	2.44
indole	0.79	0.73	0.96
3-isohexyl-5-methyl-3-oxol-2-one	6.29	1.21	0.27
3-hexyl-5-methyl-3-oxol-2-one	9.03	3.50	0.88
unknown	0.64	1.42	1.74
geosmin	1.90	6.32	22.92
5-(2-methylenecyclohexyl)-2-pentanone	0.64	1.04	0.53
3-isoheptyl-5-methyl-3-oxol-2-one	1.72	1.39	0.17
3-heptyl-5-methyl-3-oxol-2-one	5.04	4.64	1.94
3,4,4a,5,6,7,8,9-octahydro-4a-methyl-2H-benzocycloheptен-2-one	1.24	4.02	5.10
methyl 2-aminobenzoate	1.90	3.40	4.30
terpenic alcohol (probably $C_{16}H_{26}O$)	0.97	0.85	0.43

[1] non-production cultivation (0 µg/ml)
[2] production cultivation (90 µg/ml)
[3] production cultivation (600 µg/ml)

3,4,4a,5,6,7,8,9-octahydro-4a-methyl-2H-benzocyclohepten-2-onewerealso found in the mixture. These compounds were only partially identified or were not identified at all. The synthesis and function of those compounds are not known.

The synergism of the syntheses of the antibiotic and of geosmin and the antagonism between the antibiotic production and the synthesis of oxolone has been not yet reported.

REFERENCES

1 Berthelot, M., and André,G.(1891). *Compt. Rend.* 112, 598-599.
2 Gerber,N.N., and Lechevalier, H.A.(1965). *Appl. Microbiol.* 13, 935-938.
3 Gerber,N.N.(1968). *Tetrahedron Lett.* 25, 2971-2974.
4 Romano,A.H., and Safferman, A.S.(1963). *J. Am. Water Works Assoc.* 55, 169-176.
5 Dougherty,J.D., Campbell,R.D., and Morris,R.L.(1966). *Science* 152, 1372-1373.
6 Gerber,N.N.(1973). *Tetrahedron Lett.* 10, 771-774.
7 Vaněk,Z., Hošťálek,Z., and Spížek,J.(1990). Overproduction of microbial products - Facts and ideas. In *Biotechnology advances,* M.Moo-Young, J.D.Bu´Lock, C.L.Cooney, B.R.Glick, eds. Vol.8, p.1-27, Pergamon Press, Oxford.
8 Cimburková, E., Zima,J., Novák,J., and Vaněk,Z.(1988). *J. Basic Microbiol.* 28, 491-499.
9 Čurdová,E., Jechová,V., Zima,J., and Vaněk,Z.(1989). *J. Basic Microbiol.* 29, 341-346.
10 Rosen,A.A., Safferman,R.S., Mashni,C.I., and Romano,A.H.(1968). *Appl. Microbiol.* 16, 178-179.
11 Bowner,K.H., Padovan,A., Oliver,R.L., Korth,W. and Ganf,G.G.(1992). *Wat. Sci. Tech.* 25, 259-267.
12 Aoyama,K.J.(1990). *Appl. Bacteriol.* 68, 405-410.
13 van der Ploeg,M., Tucker,C.S. and Boyd,C.E.(1992). *Wat. Sci. Tech.* 25, 283-290.