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Identification of *Streptomyces* Odor Spectrum

J. JÁCHYMOVÁ[a], J. VOTRUBA[b], I. VÍDEN[a], T. ŘEZANKA[b]*

[a]*Institute of Chemical Technology, 166 28 Prague, Czechia*
[b]*Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 00 Prague, Czechia*

fax +420 2 4106 2347
e-mail rezanka@biomed.cas.cz

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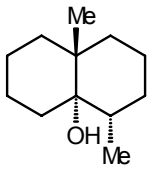
ABSTRACT. The chemical composition of odors produced by nine strains of *Streptomyces* was determined. Strains *Streptomyces aureofaciens*, *S. avermitilis*, *S. cinamomensis*, *S. coelicolor*, *S. griseus*, *S. lividans*, *S. rimosus*, *S. spectabilis*, *S. virginiae* (as representatives of producers of biologically active compounds) were cultivated at the same time statically in dishes and in shaken flasks at similar cultivation conditions. According to the GC–MS analysis of odor compounds, more than twenty noteworthy volatile chemical individuals were identified. As the main component of odor spectrum geosmin and homologues of oxolones (dihydrofuranones) were found; the other compounds (pyrazine derivatives, acetoin and its homologues, aromatic esters, furan derivatives, *etc*.) were in minority.

Usually, the production of important biologically active compounds by actinomycetes (Shapiro 1989; Běhal 2000) is followed by formation of volatile substances with strong odor. In the 19th century, the odors of the soil were subjected, for the first time, to scientific scrutiny (Berthelot and André 1891). The substances responsible for the typical earthy odor of the soil could be extracted from soil by steam distillation. Among the number of soil microorganisms, actinomycetes growing in a pure surface or submerged culture are characterized by production of a strong and earthy odor. Gerber and Lechevalier (1965) and Gerber (1968) obtained diethyl ether-soluble extracts from actinomycetes that contained a high concentrated odor, which, after dilution, became earthy-smelling. Rosen *et al*. (1968) described odor production by the odoriferous strain *Streptomyces griseoluteus*. Dougherty *et al*. (1966) studied the volatile metabolites of actinomycetes by NMR and MS spectrometry. They obtained some partial chemical structures of the odor component (five- or six-member lactones). Later, the chemical structure was revised by Gerber (1973).

Geosmin (**1**) was analyzed by enantioselective multidimensional gas chromatography and a single enantiomer has been identified in pure cultures of *Streptomyces* sp. strain isolated from rotten grapes. Darriet *et al*. (2001) has found that the geosmin (chiral compound) is in the (–) form much more odoriferous than in the (+) one. The effects of geosmin, the main source of soil odor produced by microorganisms, on plant seed germination were examined in 15 species of *Brassicaceae* (Ogura *et al*. 2000). The genetic determinants for production of the most common off-flavor compounds, such as geosmin, was demonstrated by DNA-curing analysis (Saadoun *et al*. 1998).



1 geosmin

As a part of an extensive research of physiology of different *Streptomyces* strains (Pospíšil 1991; Řezanka and Votruba 1998*a*,*b*; Běhal 2001) (producers of important biologically active compounds) we studied the effect of the type of cultivation on the total odor composition in selected streptomycetes.

MATERIALS AND METHODS

Organisms. Nine *Streptomyces* strains from laboratory collection, viz. *S. aureofaciens* (Běhal *et al*. 1979), *S. avermitilis* C-18/6 (Novák *et al*. 1993), *S. cinnamomensis* C100-5 (Pospíšil 1991), *S. coelicolor* 165, *S. griseus* IPB 101, *S. lividans*, *S. rimosus*, *S. spectabilis* NRRL 2494, *S. virginiae* ATCC 13161 were examined for production of odors.

Cultivation. The inoculum was cultivated for 1 d in a complex medium containing (g/L): glucose 5, soybean meal 15, yeast extract 5. The fermentation flasks and Petri dishes were inoculated with 2 % of the

*To whom correspondence should be addressed.

inoculum (1-d old) and cultivated for 10 d. For submerse cultivation a synthetic production medium was used (g/L): glucose 30, $(NH_4)_2SO_4$ 2, $CaCO_3$ 5, NaCl 2, K_2HPO_4 0.5, $FeSO_4 \cdot 7H_2O$ 0.05, $MnSO_4 \cdot 7H_2O$ 0.05, $ZnSO_4 \cdot 7H_2O$ 0.05, $MgSO_4 \cdot 7H_2O$ 0.1. The cultivation was performed in 500-mL round-bottom flasks on a rotary shaker (2.8 Hz) at 28 °C (Novák *et al*. 1993). The same synthetic medium with addition of 1 g/L agar was used for a 10-d surface cultivation in Petri dishes.

Analytical methods. The volatile compounds in agar or liquid medium were determined after steam distillation and extraction by capillary GC–MS (*Jeol* DX 303). 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 (Řezanka *et al*. 1994). Measured data were processed by the SPSS 10 software (Student version; *SPSS Inc.*, USA). Each value (Tables I and II) represents the mean of four independent chromatographic analysis.

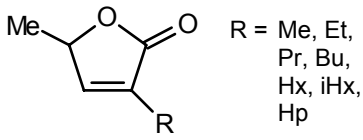
RESULTS AND DISCUSSION

Pyrazine-derived compounds represented a relatively high proportion of the individual volatile compounds of the *Streptomyces* odor spectrum (Table I). Their formation can be explained by a reaction between the acetoin-type compounds and amino acids and by subsequent oxidation–dehydration aromatization. Among the low-molar-mass aliphatic compounds, oxygen derivatives of butane and hexane dominated. Geosmin (**1**) 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 concentrations of 1.3 nmol/L; the smelling water is difficult to drink (*e.g.*, fish living in geosmin-contaminated water taste also of mud). Papers published on this topic (Bowmer *et al.* 1992; Aoyama *et al*. 1991; Ploeg *et al*. 1992) emphasized that geosmin is a main odor component produced by streptomycetes.

Comparing Table I and data given by Běhal (2000), one can find a relation between production of secondary metabolites, especially those synthesized in oligoketide pathways, and composition of the odor spectrum. We showed in *S. avermitilis* (Řezanka and Votruba 1998*b*) that there is a correlation between secondary metabolite production and geosmin formation; no such findings have been reported in most other streptomycetes. In contrast, higher production of geosmin was found in *S. cinnamonensis* C100-5 (Table I), a monensin-producing strain (Pospíšil 1991). The proportion of geosmin was higher in surface static cultures than that in submerse shaken ones (it can be explained by higher ventilation and subsequent stripping of volatile compounds from shaken flasks).

Monensins, tetracyclines and avermectins (representatives of oligoketide-type compounds synthesized *via* condensation of malonyl-CoA and/or other acyl-CoA) were in *S. cinnamonensis*, *S. aureofaciens* and *S. avermitilis* accompanied by high amounts of geosmin (synthesized from 9 units of malonyl-CoA) (Table I). Thus, in this case, geosmin can be considered to be a 'marker' of overall biosynthetic activity. In contrast, in *S. griseus*, which produces streptomycin, where glucose represents the main biosynthetic precursor, is the relative content of geosmin in odor spectrum low.

The other important group of identified odor components was represented by oxolone homologues (3-alkyl-5-methyl-2-oxolones; **2**). These compounds were identified and characterized by Gerber (1973). According to MS, we identified the other homologous series, *viz*. 3-methyl-, 3-ethyl-, 3-propyl -, 3-butyl-, 3-hexyl-, 3-isohexyl- and 3-heptyl-5-methyloxol-2-one (Table I); some of the unidentified peaks can be attributed to new and/or yet unknown oxolone derivatives. As the production of geosmin is characterized by earthy smell, the presence of oxolones can be easily identified as a musty or dank smell. Similarly to geosmin, there is a correlation between the formation of some oxolones and secondary metabolite production by streptomycetes. For example, in monensin biosynthesis by *S. cinnamonensis* 5 acetate, 7 propionate and 1 butyrate subunits take part. As the major portion of oxolones in the related odor spectrum is composed of butyl and hexyl oxolones, their synthesis can be assumed to proceed from abundant 'acetate units'. In contrast, the tetracycline overproduction by *S. aureofaciens* is characterized by prior consumption of 'acetate units' (Běhal 2000) and the abundance of unused propionate units can take part in enhancement of propyloxolone formation. Generally, the odor spectrum can reflect the physiological state of high-production streptomycete strains.



2 3-alkyl-5-methyloxol-2-ones

Table I. Odor spectrum[a] of selected *Streptomyces* strains (relative %)

Compound	*S. aureofaciens*		*S. avermitilis*		*S. cinnamomensis*		*S. coelicolor*		*S. griseus*		*S. lividans*		*S. rimosus*		*S. spectabilis*		*S. virginiae*	
Bis-3,4-hydroxymethylfurfural	2.00	0.31	2.09	0.43	1.11	0.17	0.34	0.33	1.52	2.73	0.14	4.08	0.53	2.88	1.49	1.93	0.42	4.81
3-Butyl-5-methyloxol-2-one	2.02	1.72	2.13	2.57	5.77	3.01	5.41	4.25	2.71	1.90	4.13	1.67	3.35	3.10	3.11	1.44	1.77	3.11
3,5-Dimethyloxol-2-one	0.69	0.33	0.66	0.22	0.85	0.52	0.33	0.38	0.44	1.23	0.41	0.97	0.32	0.87	0.70	0.77	0.20	1.54
2,5-Dimethylpyrazine	1.23	1.33	0.51	1.34	2.29	2.05	7.38	5.52	6.17	1.66	4.96	1.64	11.64	5.44	9.43	3.01	6.18	2.15
2,3-Dimethyltetrahydrofuran	5.66	4.30	1.66	4.99	0.99	2.05	2.15	1.78	6.64	1.86	2.54	2.73	7.52	1.67	4.11	3.11	3.79	2.43
5,6-Dimethyltetrahydroxy-2-pyrone	1.19	0.49	0.96	0.91	1.83	0.33	0.26	0.38	1.12	1.36	1.15	1.99	0.81	0.83	2.21	1.80	1.18	3.18
3-Ethyl-5-methyloxol-2-one	2.09	1.19	3.40	0.71	3.74	2.35	0.61	0.86	1.05	3.20	0.84	2.58	0.52	2.79	1.53	2.76	1.49	2.73
Furfuryl isobutyrate	2.13	2.64	1.59	2.14	1.60	1.56	1.57	1.92	1.60	2.65	1.50	1.05	2.59	0.71	2.50	1.73	1.59	1.95
N-Furfurylpyrrole-2-aldehyde	0.48	0.45	0.58	0.51	1.08	0.36	0.36	0.35	0.30	0.87	0.31	1.44	0.68	0.71	1.02	0.41	0.46	0.89
Geosmin	21.86	35.37	26.98	40.66	33.50	37.39	30.45	34.31	31.34	15.31	19.30	25.92	15.84	26.90	17.67	18.78	25.57	19.12
3-Heptyl-5-methyloxol-2-one	0.09	0.04	0.13	0.13	0.13	0.13	0.07	0.05	0.06	0.11	0.06	0.14	0.07	0.16	0.14	0.14	0.03	0.08
Hexanol	0.44	0.23	0.66	0.46	0.37	0.26	0.24	0.37	0.30	0.67	0.40	0.82	0.44	1.09	0.64	0.53	0.13	0.48
Hexenal	1.26	0.47	2.34	1.31	2.34	1.25	0.29	0.31	1.16	2.69	0.82	2.02	1.35	2.35	1.44	1.83	1.58	2.35
4-Hexen-3-one	0.66	0.29	0.79	0.76	0.29	0.14	0.27	0.23	0.21	0.87	0.23	0.70	0.30	0.59	1.01	0.79	0.29	1.11
3-Hexyl-5-methyloxol-2-one	1.92	1.99	2.86	2.56	3.04	4.89	1.56	1.89	1.98	2.56	1.35	1.77	1.49	1.29	1.71	2.10	1.23	0.96
3-Hydroxybutanone	1.88	2.61	1.31	2.41	2.07	3.83	2.83	2.28	2.42	1.92	2.20	1.27	2.69	1.94	2.98	1.54	2.67	2.58
4-Hydroxy-3-hexanone	0.83	0.23	0.40	0.47	0.68	0.39	0.96	0.57	0.60	0.69	0.59	1.18	0.76	1.06	0.19	0.86	0.35	0.46
3-Hydroxymethylbutanone	4.08	1.79	1.90	2.12	2.56	1.84	2.14	2.52	2.61	2.44	2.17	5.34	1.79	2.33	1.75	4.50	1.21	3.52
Indol	0.12	0.63	0.18	0.51	0.33	1.56	0.69	0.88	0.99	0.54	1.27	1.20	1.20	0.41	1.81	0.40	1.01	0.28
3-Isohexyl-5-methyloxol-2-one	0.19	0.14	0.14	0.11	0.14	0.18	0.07	0.07	0.12	0.16	0.09	0.25	0.12	0.16	0.24	0.23	0.14	0.31
Isopentyl octanoate	0.35	0.80	0.33	0.79	0.69	1.07	0.29	0.65	0.48	0.81	0.73	0.48	1.34	0.49	1.63	1.54	0.98	1.52
Methyl 2-aminobenzoate	1.91	1.29	1.33	1.31	0.82	0.55	0.42	0.44	0.53	1.23	1.70	0.35	1.71	2.54	1.71	2.21	0.73	1.64
Methyl benzoate	0.82	0.43	1.57	0.44	1.27	1.44	0.56	0.64	0.80	1.16	1.26	1.31	1.12	2.59	1.49	1.20	1.43	2.17
Methyl 2-furancarboxylate	0.19	0.30	0.21	0.15	0.37	0.26	0.31	0.28	0.30	0.68	0.15	0.38	0.21	0.18	0.66	0.64	0.42	0.48
8-Methylgeosmin	2.77	1.56	1.96	1.57	3.74	2.51	1.58	1.76	1.76	2.34	1.19	1.99	1.19	2.25	1.44	2.31	1.29	1.74
4-Methyl-2-hexanone	1.18	0.54	0.97	0.60	0.43	0.26	0.19	0.18	0.44	0.23	0.49	0.36	0.29	0.68	0.44	1.29	0.43	1.27
5-Methyl-3-hexanone	0.73	0.31	0.68	0.46	0.64	0.60	0.41	0.46	0.30	1.23	0.67	0.87	0.40	1.21	0.37	0.59	0.49	0.35
5-Methyl-3-propyloxol-2-one	4.08	5.38	1.98	2.13	2.38	3.73	1.95	2.43	1.76	2.29	1.56	1.36	2.22	1.50	1.55	0.87	1.02	0.45
Methylpyrazine	10.56	4.31	7.57	3.46	6.09	3.78	3.15	4.16	8.05	9.49	11.97	9.27	7.49	11.97	9.00	10.23	4.54	9.56
Nonenone	0.45	0.53	0.17	0.22	0.69	0.87	0.43	0.57	0.44	1.06	0.59	0.79	0.44	0.35	0.24	0.27	0.40	0.35
2-Phenylethanol	5.46	2.22	1.74	5.09	2.29	2.46	1.64	1.93	2.03	2.43	1.60	1.96	1.96	1.48	3.22	1.76	2.19	4.32
2-Phenylethyl isobutyrate	0.35	0.29	0.55	0.34	0.56	0.37	0.24	0.18	0.16	0.44	0.27	0.23	0.38	0.67	0.38	0.41	0.42	0.63
Pyrazine	13.26	5.66	19.68	7.43	6.09	3.41	6.15	6.97	7.98	26.13	5.44	18.61	5.88	14.56	6.82	17.34	3.57	15.50
4-Pyridinecarboxylic acid	2.00	2.64	4.45	1.90	6.03	5.37	0.76	0.91	0.94	2.44	1.32	2.02	4.26	1.29	2.68	9.15	1.20	2.46
Vinylpyrazine	5.07	17.19	5.53	8.79	3.19	9.05	23.94	19.19	7.54	2.62	16.63	1.26	17.10	0.96	12.69	1.53	29.60	3.52

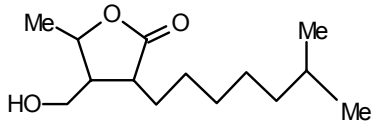
[a]*First columns* – surface static cultivation in Petri dishes; *second columns* – submerse cultivation in shaken flasks.

Table II. Content of oxol-2-one derivatives (μg/L; *first lines* – surface cultures, *second lines* – submerse cultures) in *Streptomyces* strains

Strain	3,5-Dimethyl-oxol-2-one	3-Ethyl-5-methyl-oxol-2-one	5-Methyl-3-propyl-oxol-2-one	3-Butyl-5-methyl-oxol-2-one	3-Hexyl-5-methyl-oxol-2-one	3-Isohexyl-5-methyl-oxol-2-one	3-Heptyl-5-methyl-oxol-2-one	Total
S. aureofaciens	19.9	60.2	118	58.2	55.3	5.5	2.6	319
	9.5	34.3	155	49.6	57.4	4.1	1.2	311
S. avermitilis	19.0	98.0	57.1	61.4	82.4	4.1	3.7	326
	6.3	20.5	61.4	74.1	73.8	3.2	3.7	243
S. cinnamomensis	24.5	107.8	68.6	166	87.6	4.1	3.7	463
	15.0	67.7	107	86.7	141	5.3	3.7	427
S.coelicolor	9.5	17.6	56.2	156	45.0	2.0	2.0	288
	11.0	24.8	70.0	123	54.5	2.0	1.4	286
S. griseus	12.7	30.3	50.7	78.1	57.1	3.5	1.7	234
	35.4	92.2	66.0	54.8	73.8	4.7	3.2	330
S. lividans	11.8	24.2	45.0	119	38.9	2.6	1.7	243
	28.0	74.4	39.2	48.1	51.0	7.2	4.0	252
S. rimosus	9.2	15.0	64.0	96.5	42.9	3.5	2.0	233
	25.1	80.4	43.2	89.3	37.2	4.6	4.6	285
S. spectabilis	20.2	44.1	44.7	89.6	49.3	6.9	4.0	259
	22.2	79.5	25.1	41.5	60.5	6.6	4.0	240
S. virginiae	5.8	42.9	29.4	51.0	35.4	4.0	0.9	170
	44.4	78.7	13.0	89.6	27.7	8.9	2.3	265

The furan derivatives, *i.e.* 2,3-dimethyltetrahydrofuran and methyl 2-furancarboxylate, bis-3,4-hydroxymethylfurfural, *N*-furfurylpyrrole-2-aldehyde, furfuryl isobutyrate and 5,6-dimethyltetrahydroxy-2-pyrone, were identified as another significant component of the odor spectrum; however, not all odor compounds could be identified. The mechanism of synthesis of these compounds and their possible role in the metabolic regulation of secondary metabolite production is not yet known (Shapiro 1989; Běhal 2000, 2001).

It is noteworthy to mention the similarity between the structure of oxolone homologues and regulatory factor A (**3**; 4-hydroxymethyl-3-isocaproyl-γ-butyrolactone; Pliner *et al*. 1975; Yamada and Nihira 1999); considering its role in antibiotic production we can assume a potential role of oxolone derivatives in the regulation of antibiotic production. A similar effect was observed in *S. avermitilis* where the other odorous 'marker' (geosmin) was described to induce the overproduction of avermectins (Řezanka and Votruba 1998*b*).



3 factor A

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