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Pyrazines Responsible for the Potatolike Odor Produced by Some *Serratia* and *Cedecea* Strains

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The pyrazines responsible for the potatolike odor produced by some *Serratia* and *Cedecea* cultures were identified by gas-liquid chromatography (with flame ionization and thermoionic ionization detectors) and mass spectrometry. Alkylpyrazines were produced by the six strains studied irrespective of their odors. The major alkyl-methoxypyrazine produced by *Cedecea davisae* was 3-*sec*-butyl-2-methoxypyrazine, and that produced by odoriferous *Serratia* strains (*S. rubidaea*, *S. odorifera*, and *S. ficaria*) was 3-isopropyl-2-methoxy-5-methylpyrazine. Other pyrazines produced by some strains were 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxy-5-methylpyrazine, and 3-isobutyl-2-methoxy-6-methylpyrazine. Some of these pyrazines had not previously been found as natural products or to be produced by bacteria.

Pyrazines are heterocyclic, nitrogen-containing compounds with unique organoleptic properties. Alkylpyrazines cause nutty or roasted flavors of several heated foods. Alkyl-methoxypyrazines cause musty, earthy, bell-pepper or potatolike odors of many vegetables and other plants (for reviews, see references 5, 17). The food industry uses pyrazines to improve the flavor of some products (17).

Several microorganisms have been found to produce pyrazines. Tetramethylpyrazine is the alkylpyrazine most often produced (e.g., by *Bacillus subtilis* [15] or *Corynebacterium glutamicum* [2]), although 14 other alkylpyrazines are produced by *Aspergillus oryzae* (16). 3-Isopropyl-2-methoxypyrazine is the alkyl-methoxypyrazine most often produced. Different *Pseudomonas* strains (referred to as *P. taetrolens* or "*P. perolens*") have been found to be responsible for the production of this compound, which causes a musty, potatolike, off-flavor in stored fish (18), milk (19), eggs (21), and cheese (4). *Aspergillus parasiticus* (1) and *Septoria nodorum* (3) also produce 3-isopropyl-2-methoxypyrazine. 3-*sec*-butyl-2-methoxypyrazine was tentatively identified in "*P. perolens*" cultures (18), and very recently, 3,5-dimethyl-2-methoxypyrazine was found to be produced by an unclassified microorganism with an obnoxious musty odor (20).

Among members of the family *Enterobacteriaceae*, most strains of *Serratia odorifera* (9) and *S. ficaria* (10) and a few strains of *S. rubidaea* (synonym: *S. marinorubra*) (7) and *Cedecea davisae* (8) are known to produce potatolike odors when grown on agar media.

The purpose of the present work was to identify the compounds responsible for the potatolike odor produced by some members of the family *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. Four strains (*S. odorifera* 1073, *S. ficaria* 4024, *S. rubidaea* 393, and *C. davisae* 016) formed a sample of potatolike-odor producers. Two strains (*S. marcescens* 504 and *C. davisae* 005) formed a control sample with no or unrelated odors.

All cultures were carried out at both 23 and 30°C either in tryptic soy (TS) broth (Difco Laboratories, Detroit, Mich.) or on TS agar (Difco). For the extraction of volatile compounds, 1-liter Erlenmeyer flasks containing 250 ml of TS broth were inoculated with 2.5 ml of an overnight TS broth culture, plugged with cotton, and incubated on a rotary shaker (200 rpm) for 72 h. Preliminary experiments had indicated that odors were maximally produced after 64 to 72 h. Each culture was checked for purity before inoculation and before extraction. The whole process of strain purification, cultivation, and analysis of pyrazines was repeated three times for each strain.

Odor evaluation. Cultures on TS agar (in petri dishes) and in TS broth (in Erlenmeyer flasks) were sniffed for odor evaluation after 36 and 72 h of incubation.

Extraction of volatile compounds. Cultures were centrifuged at 16,300 × *g* for 15 min. The supernatant was saturated with sodium chloride (300 g/liter), adjusted to pH 9.0 with sodium carbonate, and extracted with 3 × 15 ml of diethyl ether (R. P. Normapur grade; Prolabo, Paris, France). This solvent was chosen after preliminary extraction trials showed diethyl ether to give more than 95% recovery of known pyrazines. The diethyl ether extract was collected, dried on sodium sulfate, and concentrated to approximately 100 μl with a Dufton-type reflux column.

Gas-liquid chromatography. A Girdel 300 gas chromatograph (Delsi, Suresnes, France) was equipped with a flame ionization detector and a thermoionic ionization detector (synonym: alkaline flame ionization detector), which allows the specific detection of nitrogen and phosphorous compounds. Chromatographic separations were performed with two columns. Column A was a glass capillary column (39 m by 0.4 mm [inner diameter]) with a nonpolar silicone phase SE54 (1% vinyl-, 5% phenyl-, 94% methylpolysiloxane) immobilized by the method of Grob et al. (13) and Grob and Grob (11, 12). Column B was a fused silica capillary column (30 m by 0.32 mm [inner diameter]) bonded with DB5, a nonpolar phase similar to SE54 (J & W Scientific Inc., Rancho Cordova, Calif.). Column A was used simultaneously with the flame and thermoionic ionization detectors, and column B was used with the flame ionization detector only. The hydrogen carrier gas linear velocity was 50 cm/s. The oven temperature was programmed from 40 to 200°C at

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TABLE 1. Source of strains and odors produced

Strain studied[a]	Description of odors produced by TS cultures	
	Agar	Broth
Odoriferous strains		
Serratia odorifera 1073 (=ATCC 33077)	Potatolike, ammonia, nutty	Sprouted potato, nutty
Serratia ficaria 4024 (=ATCC 33105)	Potatolike, ammonia	Clear vegetable broth, tinned green-pea juice
Serratia rubidaea 393	Potatolike	Sprouted potato
Cedecea davisae 016 (=CDC 2296-78)	Potatolike, clear green-pea broth	Pungent with a potatolike odor, clear vegetable broth, pea pod
Control strains		
Serratia marcescens 504 (=ATCC 13880)	Fishy	Fishy, rotten, medicinal
Cedecea davisae 005 (=ATCC 33431)	Ammonia	Meat sauce, slightly nutty

[a] ATCC, American Type Culture Collection, Rockville, Md.; CDC, Centers for Disease Control, Atlanta, Ga.

2°C/min. The injector and detector temperatures were 200 and 230°C, respectively.

Before the nitrogen-containing compounds were studied in detail, the gas chromatographic effluents were sniffed to ascertain that no other compound possessed the potatolike odor.

Gas chromatography-mass spectrometry. A Girdel 31 gas chromatograph was equipped with a fused silica DB5 column (60 m by 0.32 mm [inner diameter]; J & W Scientific Inc.). Carrier gas velocity and temperature adjustments were the same as described above. The electron impact mass spectra were taken with a Nermag R10-10 quadripole mass spectrometer (Nermag, Rueil-Malmaison, France) in interface with a Sidar 111B data system (Nermag). The ionization voltage was 70 eV, and the source temperature was 150°C. Spectra were scanned from an m/z of 30 to 300 in 1.5 s.

Reference compounds or data. All identifications were made by comparison of mass spectra of unknown with that of reference compounds when available or with bibliographic data. Kovats retention indices of reference compounds (or published retention indices) were used to confirm these identifications. The following reference compounds were used: 2-ethyl-6-methylpyrazine; 2,3,5-trimethylpyrazine; 2,3,5,6-tetramethylpyrazine; 3-isopropyl-2-methoxypyrazine; 3-isopropyl-2-methoxy-5-methylpyrazine (Oril, Bolbec, France); 3-*sec*-butyl-2-methoxypyrazine; and 3-isobutyl-2-methoxypyrazine (K & K, ICN Pharmaceuticals, Plainview, N.Y.).

RESULTS

Odors of cultures. The odors that developed on plates and in flasks were somewhat different (Table 1). *C. davisae* 005, which served as a control because plate cultures did not produce any typical odor, was found to produce a pyrazinic odor when grown in flasks.

Identified pyrazines. The pyrazines detected in broth cultures are shown in Table 2. The extraction and chromatographic experiments done in triplicate yielded the same results. Methylpyrazine and 2,5-dimethylpyrazine, which were detected in inoculated and in uninoculated TS broth, were omitted from Table 2. All components (except compound 10) were identified against reference samples.

Mass spectral data for the pyrazines listed in Table 2 are shown in Table 3. It can be seen with the pair of compounds 6 and 9 and the pair of compounds 8 and 11 that addition of a methyl group to an alkyl-methoxypyrazine affected the m/z ratio of major fragments by adding 14 atomic mass units but did not affect the relative intensities of these ions. The same phenomenon occurred with 3-*sec*-butyl-2-methoxypyrazine (compound 7) and the unknown compound 10. Thus, compound 10 is probably 3-*sec*-butyl-2-methoxy-5(6)-methylpyrazine.

DISCUSSION

The evaluation of odors by sniffing requires some training from the experimenter and careful definition of experimental conditions. Odors in flasks and plate cultures were different

TABLE 2. Occurrence of pyrazines in some *Serratia* and *Cedecea* broth cultures

Compound no.	Pyrazine detected	Kovats retention index	Presence of pyrazines in cultures of[a]:					
			C. davisae 005	*C. davisae* O16	*S. marcescens* 504	*S. rubidaea* 393	*S. odorifera* 1073	*S. ficaria* 4024
	Alkylpyrazines							
1	2-Ethyl-6-methylpyrazine	1001	t	−	t	+ +	t	t
2	2,3,5-Trimethylpyrazine	1004	+ +	+ +	+ +	+ +	t	t
3	2,3,5,6-Tetramethylpyrazine	1088	t	t	t	−	−	t
4	2-Ethyl-3,6-dimethylpyrazine	1071	t	t	t	t	−	t
5	3-Isopropyl-2-methylpyrazine	1056	t	t	t	−	t	t
	Alkyl-methoxypyrazines							
6	3-Isopropyl-2-methoxypyrazine	1100	−	+	−	−	−	t
7	3-*sec*-Butyl-2-methoxypyrazine	1162	+	+ + +	−	−	−	t
8	3-Isobutyl-2-methoxypyrazine	1184	−	−	−	−	t	t
9	3-Isopropyl-2-methoxy-5-methylpyrazine	1149	t	t	t	+ + + +	+ + +	+ + +
10	3-*sec*-Butyl-2-methoxy-5(6)-methylpyrazine	1221	−	−	−	+ +	t	+
11	3-Isobutyl-2-methoxy-6-methylpyrazine	1239	−	−	−	−	+	−

[a] The following semiquantitative score was used (per liter of medium): t, less than 0.01 mg; +, 0.1 to 0.5 mg; + +, 1 to 3 mg; + + +, 8 to 12 mg; + + + +, 15 to 20 mg.

TABLE 3. Mass spectral data for the pyrazines found in *Serratia* and *Cedecea* cultures

Compound no.	Pyrazine	Mass spectrometric data (*m/z* [%])[a]						
1	2-Ethyl-6-methylpyrazine	122 (57)	121 (100)	94 (11)	56 (7)	41 (9)	39 (33)	27 (10)
2	2,3,5-Trimethylpyrazine	122 (57)	81 (11)	54 (5)	53 (4)	42 (100)	39 (24)	27 (8)
3	2,3,5,6-Tetramethylpyrazine	136 (100)	135 (22)	54 (99)	53 (21)	42 (71)	39 (39)	27 (42)
4	2-Ethyl-3,6-dimethylpyrazine	136 (70)	135 (100)	108 (16)	107 (15)	56 (13)	42 (53)	39 (39)
5	3-Isopropyl-2-methylpyrazine	136 (36)	121 (100)	108 (46)	42 (24)	41 (26)	39 (36)	27 (17)
6	3-Isopropyl-2-methoxypyrazine	152 (39)	137 (100)	124 (25)	105 (12)	41 (22)	39 (15)	27 (14)
7	3-*sec*-Butyl-2-methoxypyrazine	166 (1)	151 (45)	138 (100)	137 (28)	124 (83)	41 (27)	29 (26)
8	3-Isobutyl-2-methoxypyrazine	166 (2)	151 (15)	124 (100)	95 (14)	94 (22)	93 (12)	41 (16)
9	3-Isopropyl-2-methoxy-5-methylpyrazine	166 (40)	151 (100)	138 (32)	119 (15)	41 (14)	39 (20)	27 (13)
10	3-*sec*-Butyl-2-methoxy-5(6)-methylpyrazine	180 (0.3)	165 (45)	152 (100)	151 (33)	138 (85)	41 (25)	39 (29)
11	3-Isobutyl-2-methoxy-6-methylpyrazine	180 (0.3)	165 (13)	138 (100)	109 (12)	108 (18)	107 (10)	41 (16)

[a] Relative intensities, with the base peak taken as 100, are given in parentheses.

and evolved with time. A similar observation was made with *P. taetrolens* cultures (A. Gallois, D.Sc. thesis, Institut National Agronomique Paris-Grignon, Paris, France, 1984).

The typical potatolike odor of some *Serratia* and *Cedecea* cultures is not caused by the production of a single compound but by a complex mixture of pyrazines.

Alkylpyrazines, which were produced by all cultures, are known to have odor thresholds 100 to 1,000 times higher than those of alkyl-methoxypyrazines (17). *S. marcescens* 504 produced only traces of 3-isopropyl-2-methoxy-5-methyl-pyrazine (in addition to alkylpyrazines), and this did not result in any potatolike odor. No attempt was made to characterize the fishy odor classically attributed to the production of trimethylamine (6). *C. davisae* 005 produced 3-*sec*-butyl-2-methoxypyrazine and traces of 3-isopropyl-2-methoxy-5-methylpyrazine. The slightly nutty odor developed by this bacterium is more likely due to the larger amounts of 2,3,5-trimethylpyrazine (17). The four other strains studied, which had a potatolike odor characteristic of alkyl-methoxypyrazines, actually produced several of these compounds. The major pyrazine produced by odoriferous *Serratia* strains was 3-isopropyl-2-methoxy-5-methylpyrazine, whereas the major pyrazine produced by *C. davisae* 016 was 3-*sec*-butyl-2-methoxypyrazine. 3-Isopropyl-2-methoxypyrazine, which is the major pyrazine produced by *P. taetrolens* and "*P. perolens*" (18, 19; A. Gallois, D.Sc. thesis), occurred in cultures of *C. davisae* 016 and only at trace levels in cultures of *S. ficaria* and thus had a smaller contribution to the potatolike odor of these bacteria than previously thought (6).

Serratia is the first reported bacterial genus to produce 3-isobutyl-2-methoxypyrazine, 3-isopropyl-2-methoxy-5-methylpyrazine, 3-*sec*-butyl-2-methoxy-5(6)-methylpyrazine, and 3-isobutyl-2-methoxy-6-methylpyrazine. It should be mentioned that 3-isobutyl-2-methoxy-6-methylpyrazine had never been found in any natural product. Recently, 3-*sec*-butyl-2-methoxy-5-methylpyrazine was found in ginseng roots and synthesized (14). The spectral data reported by Iwabuchi et al. (14) for 3-*sec*-butyl-2-methoxy-5-methylpyrazine are very similar to those for our compound 10 (Table 3).

That pyrazines produced by odoriferous *Pseudomonas* (18, 19), *Cedecea*, and *Serratia* spp. are different might be of taxonomic significance. Furthermore, the finding that some pyrazines can be produced by some enterobacterial species might be of biotechnological significance.

LITERATURE CITED

1. **Buchanan, R. L., and W. M. Houston.** 1982. Production of blue fluorescent pyrazines by *Aspergillus parasiticus*. J. Food Sci. **47**:779–782.
2. **Demain, A. L., M. Jackson, and N. R. Trenner.** 1967. Thiamine-dependent accumulation of tetramethylpyrazine accompanying a mutation in the isoleucine-valine pathway. J. Bacteriol. **94**:323–326.
3. **Devys, M., J. F. Bousquet, A. Kollmann, and M. Barbier.** 1978. La Septorine, nouvelle pyrazine substituée, isolée du milieu de culture de *Septoria nodorum* Berk champignon phytophatogène. C.R. Acad. Sci. **286**:457–458.
4. **Dumont, J. P., R. Mourgues, and J. Adda.** 1983. Potato-like off flavour in smear coated cheese: a defect induced by bacteria, p. 424–428. *In* A. R. Williams and R. K. Atkin (ed.), Sensory quality in foods and beverages: definition, measurement and control. Ellis Horwood, Publisher, Chichester, United Kingdom.
5. **Gallois, A.** 1984. Les pyrazines présentes dans les aliment. Etat actuel de nos connaissances. Sci. Aliment **4**:145–166.
6. **Grimont, P. A. D., and F. Grimont.** 1984. Genus *Serratia* Bizio 1823, 288[AL], p. 477–484. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
7. **Grimont, P. A. D., F. Grimont, H. L. C. Dulong de Rosnay, and P. H. A. Sneath.** 1977. Taxonomy of the genus *Serratia*. J. Gen. Microbiol. **98**:39–66.
8. **Grimont, P. A. D., F. Grimont, J. J. Farmer III, and M. A. Asbury.** 1981. *Cedecea davisae* gen. nov., sp. nov. and *Cedecea lapagei* sp. nov., new *Enterobacteriaceae* from clinical specimens. Int. J. Syst. Bacteriol. **31**:317–326.
9. **Grimont, P. A. D., F. Grimont, C. Richard, B. R. Davis, A. G. Steigerwalt, and D. J. Brenner.** 1978. Deoxyribonucleic acid relatedness between *Serratia plymuthica* and other *Serratia* species, with a description of *Serratia odorifera* sp. nov. (type strain: ICPB 3995). Int. J. Syst. Bacteriol. **28**:453–463.
10. **Grimont, P. A. D., F. Grimont, and M. P. Starr.** 1979. *Serratia ficaria* sp. nov., a bacterial species associated with Smyrna figs and the fig wasp *Blastophaga psenes*. Curr. Microbiol. **2**:277–282.
11. **Grob, K., and G. Grob.** 1981. Capillary columns with immobilized stationary phases. I. A new simple preparation procedure. J. Chromatogr. **211**:243–246.
12. **Grob, K., and G. Grob.** 1981. Capillary columns with immobilized stationary phases. II. Practical advantages and details of procedures. J. Chromatogr. **213**:211–221.
13. **Grob, K., G. Grob, and K. Grob, Jr.** 1979. Deactivation of glass capillary column by silylation. Part I. Principle and basic technique. J. High Resolut. Chromatogr. Chromatogr. Commun. **2**:31–35.
14. **Iwabuchi, H., M. Yoshikura, S. Obata, and W. Kamisako.** 1984. Studies on the aroma constituents of crude drugs. I. On the aroma constituents of Ginseng radix. Yakugaku Zasshi **104**:951–958. (In Japanese.)
15. **Kosuge, T., and H. Kamiya.** 1962. Discovery of a pyrazine in a natural product: tetramethylpyrazine from cultures of a strain of *Bacillus subtilis*. Nature (London) **193**:776.
16. **Liardon, R., and S. Ledermann.** 1980. Volatile components of

fermented soya hydrolysate. Z. Lebensm. Unters. Forsch. **170**:208–213.

17. **Maga, J. A.** 1982. Pyrazines in food: an update. Crit. Rev. Food Sci. Nutr. **20**:1–48.
18. **Miller, A., III., R. A. Scanlan, J. S. Lee, L. M. Libbey, and M. E. Morgan.** 1973. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas perolens*. Appl. Microbiol. **25**:257–261.
19. **Morgan, M. E., L. M. Libbey, and R. A. Scanlan.** 1972. Identity of the musty-potato aroma compound in milk cultures of *Pseudomonas taetrolens*. J. Dairy Sci. **55**:666.
20. **Mottram, D. S., and L. S. Patterson.** 1984. 2,6-Dimethyl-3-methoxypyrazine: a microbiologically-produced compound with an obnoxious musty odour. Chem. Ind. **12**:448–449.
21. **Spanswick, M. P.** 1930. The cause of mustiness in eggs. Am. J. Public Health **20**:73–74.