Identification of Volatiles Derived from *Citrobacter freundii* Fermentation of a Trypticase Soy Broth

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Twenty-two compounds emanated as volatiles from an 8-day fermentation of trypticase soy broth by *Citrobacter freundii*, American Type Culture Collection (ATCC 8090), were identified by dynamic headspace analysis techniques. Compound identifications were made on the basis of a comparison of Kovats indices and GC/MS spectra for unknowns and authentic samples. Of the compounds identified, nine were pyrazines, four were alcohols, two were aldehydes, two were ketones, two were esters, two were sulfides, and one was phenol. In decreasing order, the five most abundant compounds in the volatiles were 3-methyl-1-butanol (91.1%), phenol (5.00%), 2,5-dimethylpyrazine and phenethyl alcohol (each 1.18%), and 2-methyl-1-propanol (0.38%). Low molecular weight amines (ammonia, methyl- and ethylamine) were not detected in the volatiles. Pyrazines identified in the bacteria-produced supernatant (BPS) were generated almost exclusively by heating (autoclaving) the broth; their formation did not seem to be influenced by bacterial action. Laboratory bioassay of 4- and 8-day BPS derived from *C. freundii* against mixed sexes of adult Mexican fruit flies, *Anastrepha ludens*, showed the supernatants were comparable in attractancy to a 30% NuLure solution.

Keywords: *Lure; bacterial metabolites; Mexican fruit fly; Anastrepha ludens; Citrobacter freundii; analysis of volatiles*

INTRODUCTION

The inextricably close relationship between bacteria and tephritid fruit flies has stimulated considerable research over the years to identify the roles that bacteria play in influencing the physiology, development, and behavior of both adult flies and their larval forms. The ability of bacterial isolates to elicit an olfactory attraction of tephritids has been well documented. For example, isolates from *Dacus* species in Australia were highly attractive (Courtice and Drew, 1984); enteric bacterial isolates from wild and laboratory-reared *Bactrocera dorsalis* Hendel attracted *B. dorsalis* adults (Jang and Nishijima, 1990); a bacteria similar to *Enterobacter agglomerans* attracted foraging *Rhagoletis pomonella* (MacCollum et al., 1992); a *Staphylococcus aureus* isolate from the mouth parts of *A. ludens* (Robacker et al., 1991) as well as their metabolites (Robacker et al., 1993) attracted adult *Anastrepha ludens*; isolates from the alimentary tract of wild *A. ludens* as well as from chapote amarillo, *Sargentia greggii* S. Watts, fruit were reportedly highly attractive to *A. ludens* in both laboratory and field studies (Martinez et al., 1994). In the Martinez et al. (1994) study, the invesigators showed that bacteria-produced supernatants (BPS) derived from two of these species (*Klebsiella pneumoniae* and *Citrobacter freundii*) captured as many adult *A. ludens* as the Torula yeast/borax standard and captured nearly twice as many females as males compared to equal numbers of males and females caught by Torula yeast/borax and NuLure (acid-hydrolyzed corn protein product).

As part of a long-term project to identify and develop new attractants for *A. ludens*, we identified in an earlier study (Lee et al., 1995) the volatiles emitted from a *K. pneumoniae* fermentation of a trypticase soy broth. Herein, we now report the chemical identity of volatiles purged from the headspace of a bacteria-produced supernatant (BPS) derived from *C. freundii*, a supernatant highly attractive to both sexes of adult *A. ludens* (Martinez et al., 1994).

MATERIALS AND METHODS

Materials. NuLure (Miller Chemical and Fertilizer Corp., Hanover, PA) was diluted with water to 30%, and this suspension was used as a standard for the laboratory bioassay. The BPS was obtained by culturing *C. freundii* (ATCC 8090) in trypticase soy broth (Baltimore Biological Laboratory, Baltimore, MD). The culture medium consisted of trypticase peptone (17 g), phytone peptone (3 g), sodium chloride (5 g), dipotassium phosphate (2.5 g), and dextrose (2.5 g). Unless otherwise specified, the BPS was processed as follows: The medium was suspended in water that had been subjected to reverse osmosis and carbon filtration, and 500−800 mL portions of the medium were transferred to 1 L Erlenmeyer flasks. The flasks were autoclaved at 121 °C for 20 min and then allowed to cool (unadjusted pH, 6.8). The medium was seeded with 1 mL of bacterium (1×10^9 cells) and then placed in a rotary shaker (250 rpm) for 4 or 8 days, depending on the experiment. Temperature was maintained at 30 °C. After fermentation, the flasks were removed and the whole beer was centrifuged at 10 000 rpm for 7 min. The supernatant was placed in 1 L Erlenmeyer flasks and autoclaved as before (unadjusted pH, 7.5). Flasks were cooled and refrigerated until used.

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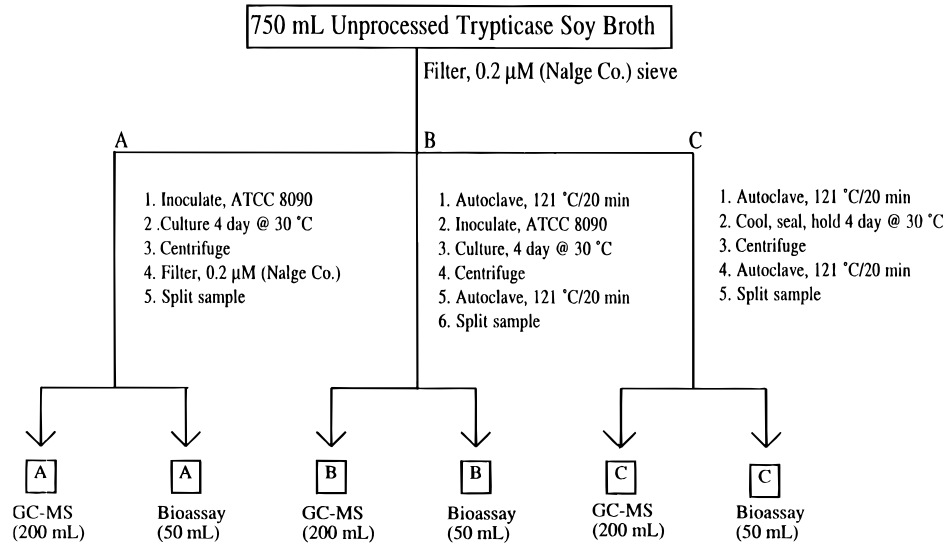

Figure 1. Experimental protocol followed to determine contribution of bacterial (ATCC 8090) action on trypticase soy broth to pyrazine formation.

Collection of Volatiles. A 50 mL aliquot of BPS was placed in a three-neck, round-bottom flask. As the solution was stirred at ambient temperature, prepurified nitrogen (i.e., passed through an activated charcoal bed) was swept at 300 mL/min over the BPS headspace, and volatiles were collected in a glass tube packed with 300 mg of activated charcoal (Darco, 20−40 mesh, Aldrich). Activated charcoal was used as the trapping agent because of its high efficiency to adsorb a variety of organic compounds (Heinz et al., 1966). The charcoal used in the collection trap was prepurified by continuous extraction (Soxhlet extractor) with methylene chloride and then benzene (caution: benzene and methylene chloride, cancer suspect agents, should be handled with care and adequate ventilation). After collecting volatiles for 15 h, the charcoal trap was removed and eluted with ca. 0.5 mL of methylene chloride. The methylene chloride was analyzed without concentration. The efficiency of the charcoal trap was determined by inserting a second charcoal trap (equal size and load) in the purge stream after the first trap. GC analysis of the eluate from the second trap showed complete absence of any volatiles associated with the supernatant.

Gas Chromatography (GC). A Shimadzu Model GC-14A (Shimadzu, Columbia, MD) equipped with a flame ionization detector (FID) and a bonded DB-1 (J&W Scientific, Folsom, CA) fused-silica capillary column (30 m × 0.248 mm i.d., 0.25 μm film thickness) was used to analyze volatile components. GC peak areas were quantified using a Shimadzu CR-501 Integrator. GC operating conditions were as follows: Injector/detector temperature, 250 °C; helium carrier, ca. 1 mL/min (1.5 kg/cm^2 head pressure); injector operated in split mode, 50:1; temperature program, 50 °C (5 min), ramped at 5 °C/min to 250 °C.

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS was performed on a Hewlett-Packard 5890A GC/MS equipped with a 5971A MSD and a HP5 (Hewlett-Packard, Avondale, PA) bonded fused-silica capillary column (25 m × 0.2 mm i.d., 0.11 μm film thickness). GC conditions used were the same as those described for GC analysis on the Shimadzu instrument except that the injection port was operated in the splitless mode. MS conditions (EI mode) used were as follows: ionization voltage, 70 eV; mass range, m/z 30−550; ion source temperature, 180 °C. The mass spectra of the unknown compounds were compared with those in the Wiley/NBS spectral data base. Identifications were also made by comparing Kovats indices (KI) of unknowns with those determined for authentic samples (Kovats, 1966).

Pyrazine Formation: Thermal Effect. Samples (250 mL) of filtered, uninoculated trypticase soy broth medium were autoclaved at 121 °C for periods of 20, 40, 60, and 100 min. Volatiles purged from ca. 50 mL portions of medium resulting from each of these heat treatments were collected onto charcoal traps. Following collection, the charcoal was washed with methylene chloride and the eluate analyzed by GC−GC/MS. Experimental conditions used for purging and trapping were the same as described under Collection of Volatiles.

Pyrazine Content: Bacterial Effect. To determine the effect that bacterial action had on pyrazine formation in the BPS, the experiment outlined in Figure 1 was conducted. Briefly, volatiles emitted from three differently processed trypticase soy broth media were collected by purging onto charcoal traps and eluates from these traps were analyzed by GC and GC/MS (methods described elsewhere in this section). Treatments consisted of the following variations: treatment A, medium inoculated but not heated; treatment B, medium inoculated and heated; treatment C, medium heated but not inoculated (see Figure 1 for details).

Bioassay. The laboratory bioassay was a modified version of a previously described cage-top bioassay (Robacker and Hart, 1984). Briefly, a 50−200 μL aliquot of BPS, or other test material, was applied to a 12.7 mm diameter filter paper disk attached to the bottom of a glass Petri dish. After application of the test sample, the dish (inverted) was placed on top of a wire-screened cage (2 m^3) containing 1000 mixed-sex adult *A. ludens* (3−7 days old) that were maintained, since emergence, on a 6% sucrose diet. The night before tests, the sucrose was removed and replaced with water. Tests were conducted in a room held at 25 °C and 70 ± 5% relative humidity. BPS samples were bioassayed in a test that compared them to a standard (30% NuLure), BPS check (sample processed identically to BPS but uninoculated), and water control. Five replicates of each of these treatments were placed on the cage in a randomized complete block design. Response determinations were made as follows: counts of flies found under the area of Petri dishes were taken every 5 min for a total of six recordings. Response means and standard errors of the means were calculated by using a SuperAnova (Accessible General Linear Modeling) program (Abacus Concepts, Inc., Berkeley, CA).

RESULTS AND DISCUSSION

To confirm attractancy of the BPS derived from *C. freundii* (ATCC 8090), 4- and 8-day fermentations (4-day BPS 8090 and 8-day BPS 8090, respectively) of trypticase soy broth were evaluated in a free-choice laboratory bioassay against 30% NuLure, water, and a 4-day BPS check (medium processed identically to 4-day BPS 8090 but not inoculated). Attractancy data, expressed in terms of mean fly response indices (RI), in Table 1 show that the standard, 30% NuLure, was most effective (RI = 5.066 ± 0.159) followed in decreasing

Table 1. Attractancy of BPS Derived from ATCC 8090 to Mixed Sexes of Adult *A. ludens*

treatment	mean (± SEM) fly response index[a]
water	1.000 ± 0.061 d
8-day BPS 8090	4.376 ± 0.213 b
4-day BPS 8090	4.891 ± 0.173 ab
BPS check[b] (4-day)	3.475 ± 0.183 c
30% NuLure[c]	5.066 ± 0.159 a

[a] Response index = observed number of fly responses for test material/observed number responses for water. Analysis used means of five tests (five replicates/treatment); total responses $n = 4531$. Means within column followed by the same letter are not significantly different ($F = 55.256$, df = 5, $P > 0.01$; Fisher's protected lsd $P = 0.05$). [b] BPS check processed same as 4-day BPS 8090 but was not inoculated. [c] Unbuffered, pH 4.1.

order by 4-day BPS 8090 (RI = 4.891 ± 0.173), 8-day BPS 8090 (RI = 4.376 ± 0.213), BPS check (RI = 3.475 ± 0.183), and finally water (RI = 1.000). Although attraction responses of an 8-day fermentation with *K. pneumoniae* (ATCC 13883) exceeded that for 30% NuLure (Lee et al., 1995), these data show that attractancy for supernatants derived from *C. freundii* nearly equaled (4-day BPS 8090) or were slightly lower (8-day BPS 8090) than the standard. Interestingly, the BPS check elicited a strong attractive response with an RI value 69% of that observed for 30% NuLure and 71% of that for the corresponding inoculated sample 4-day BPS 8090. These results are similar to those observed for the BPS check for 8-day 13883 (Lee et al., 1995).

Despite the fact that the 4-day fermentation appeared to be slightly more attractive than the 8-day fermentation (Table 1), we chose to investigate the volatiles produced by the 8-day fermentation. This was done so that direct comparisons could be made to volatiles previously identified in 8-day BPS 13883 (Lee et al., 1995).

Typical gas chromatographic profiles of the volatiles purged from 8-day BPS 8090 and its corresponding check are shown in parts A and B, respectively, of Figure 2. Chemical identities, Kovats indices (KI), and relative concentrations are listed in Table 2. Excluding benzene (a contaminant inadvertently introduced onto the charcoal), a total of 22 peaks appearing in the chromatogram of the volatiles of 8-day BPS 8090 (Figure 2A) were identified on the basis of a comparison of the KI values and GC/MS spectra with those obtained from authentic samples. Seven chemical classes are represented by the 22 identified compounds. The class and number of compounds corresponding to that class follow: pyrazines (9), alcohols (4), aldehydes (2), ketones (2), esters (2), sulfides (2), and phenols (1). In decreasing order, the five most abundant compounds in the volatiles were 3-methyl-1-butanol (91.1%), phenol (5.00%), 2,5-dimethylpyrazine and phenethyl alcohol (each 1.18%), and 2-methyl-1-propanol (0.38%). Except for phenol in this group, remarkably similar distribution and ranking were observed for the 8-day BPS 13883 (Lee et al., 1995). Fifteen of the volatiles identified in BPS 8090 were also identified as constituents in the volatiles of BPS 13883 (Lee et al., 1995).

The GC trace of the volatiles collected from the BPS check (Figure 2B) showed fewer peaks than the corresponding inoculated supernatant. Of those observed, 12 were identified by GC−GC/MS analyses, and their identities are given in Table 2. The five most abundant compounds in decreasing order were 3-methyl-1-butanal (46.0%), 2,5-dimethylpyrazine (31.5%), benzaldehyde (9.81%), pyrazine (3.88%), and 2-methylpyrazine (3.56%).

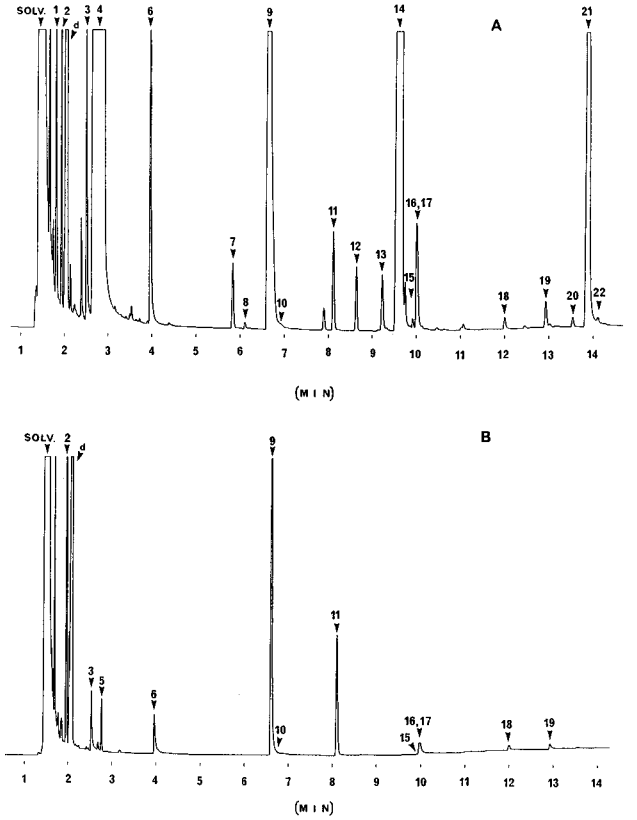

Figure 2. (A) Gas chromatogram from a temperature-programmed analysis of the volatile components from an 8-day bacteria (ATCC 8090)-produced supernatant (BPS). (B) Gas chromatogram [column/operating parameters identical to those in (A)] of volatile components from an 8-day BPS check (BPS check was processed identically to 8-day BPS except that it was not inoculated). See Table 2 for identity of chemicals corresponding to peak numbers.

Of significance and without exception is the fact that all pyrazines found in the inoculated sample were also found in the check. Moreover, the relative distributions of the pyrazines in both the check and the inoculated sample were strikingly similar.

The presence of other low molecular weight amines besides pyrazines in the volatiles, purged from an 8-day fermentation of trypticase soy broth by *K. pneumoniae*, was reported (Lee et al., 1995). However, ammonia or alkyl-substituted analogues (methylamine, ethylamine) could not be detected in the volatiles or liquid matrix of 8-day BPS 8090. Two different experiments were used to detect the amines. The first (for amines in volatiles) involved placing moistened ammonia test paper (Laboratory Supplies Co., Hicksville, NY) into the headspace purge stream, and the second (for amines in supernatant) involved a GC/MS analysis of a methylene chloride extract of the liquid matrix, the pH of which was appropriately adjusted to allow optimum extraction of amine components. The absence of ammonia or other low molecular weight amines in BPS 8090 contrasts with results from BPS 13883 (Lee et al., 1995) and is surprising in light of ammonia's importance to other proteinaceous baits (Bateman and Morton, 1981; Morton and Bateman, 1981; Mazor et al., 1987).

The presence of pyrazines in both BPS 8090 and corresponding check was not surprising since their detection paralleled earlier findings with BPS 13883 (Lee et al., 1995). Pyrazines have been well documented as being volatile constituents of proteinaceous lures (Buttery et al., 1983; Matsumoto et al., 1985; Flath et

Table 2. Volatiles Identified from 8-Day Fermentation of Trypticase Soy Broth by *C. freundii*

GC peak no.[a]	compound	mass spectral ions, *m/z* M+ (intensity)	mass spectral ions, *m/z* base	GC data for 8-day BPS 8090 KI[b]	GC data for 8-day BPS 8090 rel area %	GC data for 8-day BPS 8090 ref KI[c]	GC data of BPS check KI[b]	GC data of BPS check rel area %
1	2-methyl-1-propanol	74 (9)	43	609	0.378	609		
2	3-methyl-1-butanal	86 (8)	44	629	0.344	627	628	46.0
d	benzene[d]			646		645	645	
3	pyrazine	80 (100)	80	707	0.168	707	707	3.88
4	3-methyl-1-butanol	ND[j]	55	722	91.1	714		
5	dimethyl disulfide	94 (100)	94		*e*	722	722	2.25
6	2-methylpyrazine	94 (100)	94	798	0.252	796	798	3.56
7	3-methylbutyl acetate	ND	43	855	0.073	856		
8	2-heptanone	114 (5)	43	864	0.006	863		
9	2,5-dimethylpyrazine	108 (100)	108	882	1.18	881	881	31.5
10	2,3-dimethylpyrazine	108 (100)	108	891	0.009	889	891	0.256
11	benzaldehyde	106 (95)	77	925	0.072	925	925	9.81
12	dimethyl trisulfide	126 (100)	126	940	0.046	940		
13	3-methylbutyl propionate	ND	57	950	0.050	950		
14	phenol	94 (100)	94	959	5.00	954		
15	2-ethyl-6-methylpyrazine	122 (58)	121	970	0.009	969	970	0.164
16	2-ethyl-5-methylpyrazine	122 (73)	121	973[f]	*g*	972	973[f]	*h*
17	trimethylpyrazine	122 (62)	42	973[f]	*g*	973	973[f]	*h*
18	2-methyl-5-isopropylpyrazine	136 (39)	121	1028	0.010	*i*	1029	0.446
19	2,5-dimethyl-3-ethylpyrazine	136 (69)	135	1053	0.028	1053	1053	0.498
20	2-nonanone	142 (7)	58	1069	0.011	1069		
21	phenethyl alcohol	122 (33)	91	1080	1.18	1079		
22	2-nonanol	ND	45	1084	0.006	1084		

[a] Peak numbers correspond to those in Figure 2. [b] Kovats indices calculated from retention time data on a DB-1 capillary column. [c] Ref Kovats indices calculated from retention time data of authentic sample obtained on a DB-1 capillary column. [d] Introduced as impurity by washing charcoal with benzene. [e] Not calculated, peak 5 hidden under peak 4 (compound's presence was assumed since it was detected by GC/MS in the corresponding check). [f] Peaks 16 and 17 overlapped. [g] Relative area % of 16 and 17 = 0.119. [h] Relative area % of 16 and 17 = 1.64. [i] Authentic sample not available. [j] ND, not detected.

al., 1989), and their concentrations are pH dependent (Matsumoto et al., 1985). Their formation may be a result of thermal (Maga, 1992), chemical (Maga, 1992), or photochemical processes (Sheldon and Shibamoto, 1987) and often involves the Maillard reaction of α-dicarbonyls (sugar source) with amines produced from the Strecker degradation (de Rijke et al., 1981; Hwang et al., 1993).

The fact that the distribution and relative concentrations of pyrazines were similar in the volatiles of both the BPS and check suggested that heat may have played a major role in their formation, particularly since both media were autoclaved (121 °C) for a total of 40 min and pyrazines are known to rapidly form with prolonged heating at 120 °C (Koehler and Odell, 1970). To test this hypothesis, the pyrazine content in the volatiles collected from uninoculated trypticase soy broth heated for different time periods (20, 40, 60, 100 min) was analyzed. Data in Table 3 show, without exception, that the amount of pyrazine in the volatiles increased as the duration of heating increased. As expected, the content of benzaldehyde (artifact found in the unprocessed medium) remained fairly consistent with only a slight upward trend noted. In contrast, contents for the two remaining non-pyrazine type compounds, 3-methyl-1-butanal and dimethyl disulfide, increased to a maximum (ca. 5- and 10-fold, respectively) with 60 min of heating and subsequently decreased with continued heating.

Since pyrazine content increased with longer periods of heating (Table 3) and since BPS checks elicited moderately attractive responses to adult *A. ludens* (Table 1; Lee et al., 1995), we bioassayed the various heat-treated media to see if pyrazine content could be correlated with attraction. Results from bioassays indicated that mean fly responses (calculated average of two tests, five replicates per test) remained fairly constant throughout the four heat periods (20, 40, 60, 100 min), ranging from a mean of 17.334 ± 1.146 (SEM) for the 20-min period to a mean of 18.536 ± 1.119 for the 100-min heat period; mean responses for 30% NuLure and water were 30.466 ± 2.348 and 7.166 ± 0.463, respectively. Clearly, these data show that increasing pyrazine content beyond levels observed for the 20-min treatment had little effect, if any, on increasing attraction.

Table 3. Effect on Content of Volatiles by Heating Tryptic Soy Broth Medium[a] for Different Time Periods

volatile compound[c]	normalized relative GC peak area at indicated total heating time[b] 20 min	40 min	60 min	100 min
3-methyl-1-butanal	0.789	1.79	4.26	3.77
pyrazine	0.098	0.229	0.364	0.580
dimethyl disulfide	0.009	0.069	0.184	0.095
2-methylpyrazine	0.076	0.254	0.385	1.07
2,5-dimethylpyrazine	0.671	1.85	2.64	5.04
2,3-dimethylpyrazine	0.003	0.008	0.018	0.075
benzaldehyde	0.294	0.305	0.423	0.367
2-ethyl-6-methylpyrazine	trace	trace	0.012	0.037
2-ethyl-5-methylpyrazine and trimethylpyrazine	0.032[d]	0.099	0.197	0.485
2-methyl-5-isopropylpyrazine	0.011	0.016	0.025	0.029
2,5-dimethyl-3-ethylpyrazine	0.011	0.038	0.080	0.210

[a] Uninoculated sample. Except for inoculation and duration of heating, sample was processed identically to BPS. [b] Peak areas for all compounds were normalized to peak area observed for an internal standard (I.S.), 2,4-lutidine (retention time, 7.30 min). The I.S. was added to the broth at the end of the heating period. Tabulated values = peak area of compound/peak area I.S. [c] Identified by GC/MS. [d] Relative area for the two coeluting pyrazines.

To determine the extent to which the bacteria contributed to pyrazine formation, the trypticase soy broth medium was processed in three different ways (Figure 1): treatment A, fermented only; treatment B, fermented plus heated; treatment C, heated only. An aliquot from each of the treatments was analyzed by

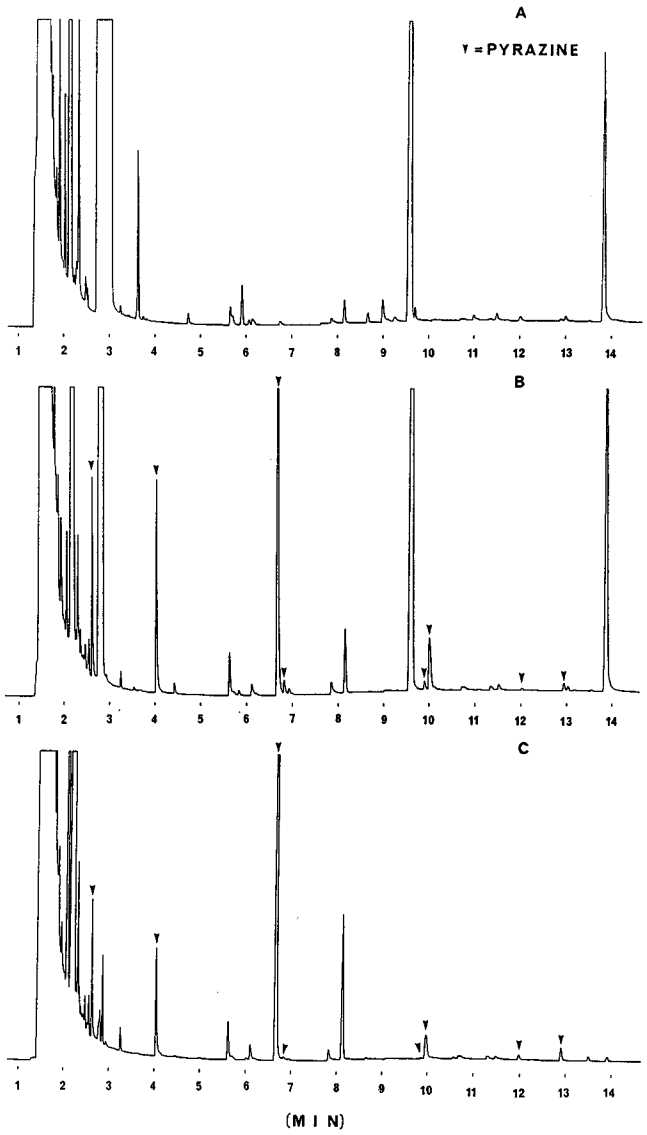

Figure 3. Gas chromatograms of volatile components observed from the following trypticase soy broth media treatments: (A) broth inoculated (4 days) but not autoclaved; (B) broth inoculated (4 days) and autoclaved (4-day BPS 8090); (C) broth autoclaved but not inoculated (4-day BPS check). Labels A−C in Figure 3 correspond to treatments A−C described in Figure 1. Peaks with arrowhead markings above correspond to pyrazines (see retention time data in Table 2 for their identity).

GC−GC/MS to identify and quantify the volatiles and another aliquot subjected to bioassay. Gas chromatographic traces of the volatiles corresponding to treatments A−C are shown in Figure 3. A comparison of the relative peak heights of pyrazines resulting from the three treatments supported two conclusions: (1) pyrazines form nearly exclusively from the heat treatment (i.e., the identity and relative distribution of pyrazines from treatments B and C were strikingly similar); (2) bacteria appeared to lack a significant role in their formation. In support of the latter was the fact that pyrazines resulting from treatments B and C, particularly those present in appreciable quantity [i.e., 2-methylpyrazine (3.96 min), 2,5-dimethylpyrazine (6.60 min)], were absent in the GC trace of the volatiles corresponding to treatment A.

Attractiveness of the differently treated media was determined against mixed sexes of adult *A. ludens* in a laboratory bioassay (Table 4). Data show that while

Table 4. Relative Attractancy of Differently Processed Trypticase Soy Broth Media to Mixed Sexes of Adult *A. ludens*

treatment[a]	mean (±SEM) fly response index[b]
water	1.000 ± 0.063 c
A	3.556 ± 0.291 b
B	3.472 ± 0.432 b
C	3.000 ± 0.329 b
30% NuLure	4.836 ± 0.225 a

[a] See Figure 1 for details of treatments and Figure 3 for GC chromatograms of volatile components derived from the treatments; treatments A−C are identical to those in Figures 1 and 3: (A) broth inoculated and fermented for 4 days but not autoclaved; (B) broth inoculated, fermented for 4 days, and autoclaved (4-day BPS 8090); (C) broth autoclaved, held for 4 days at 30 °C, but not inoculated. [b] Analysis used means of three tests (461 responses per test), 5 replicates per treatment. Means within column followed by the same letter are not significantly different (F = 17.05, df = 5, P = 0.0001, Fisher's protected lsd P = 0.05).

mean response indices were essentially equal for media corresponding to treatments A−C, the mean response index for 30% NuLure was significantly higher. It is interesting that the medium from treatment A, the volatiles of which were void of pyrazines (or contained only trace amounts), was equally as attractive as media derived from treatments B and C, both of which contained appreciable quantities.

In summary, we have identified 22 compounds present in the volatiles derived from a bacterial fermentation (*C. freundii*) of a proteinaceous soy broth. Nine alkyl-substituted pyrazines were identified in the volatiles. Our studies showed that autoclaving of the supernatant was the principal source for their formation. Data suggested that bacteria did not play a major role in pyrazine formation. New studies are in progress to fully evaluate the attractancy of the volatile components, separately or in selected mixtures. We are hopeful that information derived from these and future studies will contribute to the development of more potent, persistent, and convenient to deploy lures than those currently in use for detection and population monitoring of *A. ludens* and related tephritids.

ACKNOWLEDGMENT

We thank Robert A. Flath, USDA-ARS, Albany, CA, for providing authentic samples of 2,5-dimethyl-3-ethylpyrazine and 2,6-dimethyl-3-ethylpyrazine. We also thank Ismael Saenz for conducting bioassays and Armando Loya and Eduardo Pinon for doing fermentations.

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Received for review August 8, 1995. Accepted November 21, 1995.[⊗] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

JF950525O

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1996.