Volatile Compounds from *Escherichia coli* O157:H7 and Their Absorption by Strawberry Fruit

Keshun Yu,[†] Thomas R. Hamilton-Kemp,*[,†] Douglas D. Archbold,[†] Randall W. Collins,[†] and Melissa C. Newman[‡]

Departments of Horticulture and Landscape Architecture and of Animal Science, University of Kentucky, Lexington, Kentucky 40546-0091

Volatile compounds emitted by cultures of two strains of the pathogenic bacterium *Escherichia coli* O157:H7 and a nonpathogenic strain of *E. coli* were trapped on Super-Q porous polymer and identified by GC−MS. The predominant compound produced by all three strains was indole with lesser amounts of other components including methyl ketones, 2-heptanone, 2-nonanone, 2-undecanone, and 2-tridecanone. The vapor-phase profiles of these strains were similar for most chemicals identified but differed with regard to ketones. Strawberry fruit was shown to be a suitable host for *E. coli* O157:H7 with the population of the bacterium either increasing or remaining stable after 3 days depending on inoculation level. Headspace analysis of the volatile compounds from inoculated fruit yielded no detectable quantity of indole. Strawberry fruit readily absorbed indole and other volatile compounds produced by the bacteria and in some cases metabolized the compounds to new volatile products. Thus, headspace "marker" compounds indicating possible bacterial contamination of fruit were largely removed from the vapor phase by the strawberries.

Keywords: *Indole; 2-heptanone; aroma; headspace; food safety; human pathogen*

INTRODUCTION

The appearance of a new food safety pathogen, *Escherichia coli* O157:H7 (Riley et al., 1983; Armstrong et al., 1996; WHO, 1996), has raised the specter of contamination of previously nonhost foods, namely fruits and vegetables. The acid tolerance characteristic of this organism (Buchanan and Doyle, 1997) with serious health threat potential is one factor that has permitted it to move into plant-derived foods. Well-known examples of outbreaks associated with plant-derived food products include the case of contaminated apple juice or cider which resulted in widespread illness and one death in the United States in recent years (CDC, 1996, 1997a).

In considering properties of food borne pathogenic bacteria which might be used in preliminary nondestructive screening of fresh produce to detect contamination, one characteristic of *E. coli* O157:H7 which might signal its presence is the profile of volatile compounds synthesized and emitted by the organism. It is well-known that many bacteria emit volatile compounds, and thus it was considered worthwhile to examine the compounds produced by this serious pathogen.

In addition, experiments were conducted to determine if *E. coli* O157:H7 could successfully colonize strawberry which is one of the most widely consumed fruits in developed countries and is usually eaten raw. It was felt that if *E. coli* O157:H7 could survive and grow on strawberries, and if the bacteria made a series of volatile compounds, these compounds might be used in preliminary screenings to determine if strawberries in large quantities after field harvest, in storage, or during transport were contaminated by this food safety organism.

Also, based on previous studies on the absorption of volatile compounds tested as fumigants for strawberry fruit (Hamilton-Kemp et al., 1996), we were interested in the possibility of the fruit absorbing the bacterial-emitted compounds. Therefore, studies were conducted on the capacity of strawberry fruit to absorb and/or metabolize volatile compounds produced and emitted by the bacteria under study. Thus the objectives of this work were to see if *E. coli* O157:H7 emitted volatile compounds, if strawberry served as a suitable host for this pathogen, and subsequently if "marker" compounds from bacteria could be detected in the vapor from inoculated strawberries and if strawberries absorbed and/or metabolized bacterial compounds.

EXPERIMENTAL METHODS

E. coli O157:H7 ATCC 43895 obtained from American Type Culture Collection, Rockville, MD, was used for fruit inoculation experiments. *E. coli* O157:H7 ATCC 35150 and the nonpathogenic *E. coli* ATCC 15597 obtained from the same source were used for comparison of culture volatile compounds. Bacteriological media were purchased from Difco Laboratories, Detroit, MI. Fresh strawberries were purchased from a supermarket. Authentic samples of volatile compounds were obtained from Aldrich Chemical Co., Milwaukee, WI, or were gifts from Bedoukian Chemical Co., Danbury, CT.

Volatile Compounds from *E. coli* O157:H7 and Nonpathogenic *E. coli.* *E. coli* O157:H7 ATCC 43895 and ATCC 35150 and *E. coli* ATCC 15597 were stored in a refrigerator at 4 °C on a trypticase soy agar (TSA) slant and transferred to a new slant biweekly for preservation. Prior to use, three consecutive transfers of the bacteria to brain and heart infusion (BHI) broth were made. The broth was incubated at 35 °C for 24 h after each transfer. Samples from BHI broth

[†] Department of Horticulture and Landscape Architecture.
[‡] Department of Animal Science.

10.1021/jf990576b CCC: $19.00
Published on Web 01/12/2000

Table 1. Volatile Compounds Produced by *E. coli* O157:H7 and Nonpathogenic *E. coli*

compounds	retention time (min)	*E. coli* O157:H7 (ng)[a]		*E. coli* (ng)[a]	
		ATCC 43895	ATCC 35150	ATCC 15597	plate count agar (ng)[a]
2,5-dimethyltetrahydrofuran	13.36 and 14.01[b]	216 ± 124 NS[c]	152 ± 17	152 ± 70	92 ± 13*[d]
dimethyl disulfide	16.54	54 ± 56 NS	25 ± 7	12 ± 6	2 ± 2.4 NS
2-heptanone	28.32	60 ± 19 A	52 ± 4 A	8 ± 1 B	0 ± 0*
2,5-dimethylpyrazine	30.27	297 ± 31 NS	515 ± 31	389 ± 326	210 ± 87*
benzaldehyde	35.12	398 ± 127 NS	483 ± 67	130 ± 6	1693 ± 796*
dimethyl trisulfide	36.40	112 ± 133 NS	23 ± 1	29 ± 2	0 ± 0 NS
2-nonanone	46.31	147 ± 46 A	133 ± 18 A	9 ± 12 B	0 ± 0*
nonanal	47.43	109 ± 36 NS	71 ± 34	72 ± 52	120 ± 57 NS
decanal	55.86	58 ± 16 NS	53 ± 22	34 ± 6	61 ± 19 NS
2-undecanone	62.70	41 ± 9 NS	33 ± 6	31 ± 1	0 ± 0*
indole	63.55	20248 ± 1622 NS	19249 ± 473	19427 ± 4238	40 ± 31*
unknown	75.76	83 ± 15 B	72 ± 7 B	132 ± 4 A	0 ± 0*
2-tridecanone	77.22	24 ± 7 B	19 ± 2 B	44 ± 1 A	0 ± 0*

[a] Amount of compound ± standard deviation emitted per 5 plates of culture medium. [b] Two isomers of compound detected. [c] Means within *E. coli* strains followed by different letters are significantly different by least-squares difference (LSD) at $P = 0.05$ or are not significantly different (NS). [d] Plate count agar means significantly differs (*) from *E. coli* mean across strains by a single degree of freedom contrast at $P = 0.05$ within the analysis of variance or does not differ (NS).

cultures were spread on plate count agar (PCA) in 60-mm × 15-mm glass Petri dishes and incubated at 35 °C for 16 h. Following incubation five Petri dishes with bacteria were inverted inside a 1-L glass jar which was then sealed with a plastic screw cap containing air inlet and outlet lines. Prior to collecting headspace volatile compounds, the jar was placed in a 35 °C water bath. The controls were set up in the same way as treatments except PCA plates were inoculated with BHI broth alone.

To collect headspace samples, air from a commercial cylinder was passed by means of Teflon tubing through each jar at a flow rate of 60 mL/min. A 0.4-cm diameter glass trap packed with 50−60 mg of Super-Q absorbent (Alltech, Deerfield, IL) was connected to the outlet line from each jar to collect the volatile compounds entrained by the air for an 18-h period.

The volatile compounds trapped were eluted with 300 μL of hexane and methylene chloride solution (4:1 v/v) and cumene was added as an internal standard. Approximately 2 μL of the solution of volatile compounds was injected into a Hewlett-Packard 5890A gas chromatograph (GC) containing a 60-m × 0.32-mm fused silica DB-5 column (J & W Scientific, Folsom, CA) with a 1.0-μm film thickness. The following chromatographic conditions were used: inlet, 220 °C; column 50 °C for 5 min and then programmed at 2 °C min^{-1} to 220 °C; FID, 240 °C; He carrier linear flow rate, 30 cm s^{-1}. Mass spectral analyses were carried out with a Hewlett-Packard GCD 1800B instrument equipped with a 25-m × 0.25-mm DB-5 column with a 0.25-μm film thickness operated using the following conditions: inlet, 250 °C; column 40 °C for 5 min and then programmed at 2 °C min^{-1} to 200 °C. The scan mass range was from *m*/*z* 30 to 450, and the scan time was 0.1 s. Mass spectra of volatile components were compared to those obtained from the NIST library, and identifications were confirmed by co-chromatography of the trapped volatile compounds with authentic compounds where commercially available.

Test of the Ability of *E. coli* O157:H7 To Survive and Grow on Strawberry Fruit. BHI broth cultures of *E. coli* O157:H7 which contained approximately 10^9 cells/mL were prepared. Dilutions corresponding to 10^7, 10^6, and 10^5 cells/mL of the *E. coli* O157:H7 inoculum were made with phosphate buffer (pH 7.2). Fresh strawberry fruits were submerged for several seconds in the inoculum and then placed on a metal mesh screen and allowed to dry for 0.5−1 h. Fruits (approximately 150 g) were either used immediately for the day 0 plating or placed at room temperature in 9-cm × 9-cm × 5-cm plastic cartons enclosed in plastic bags for 1 or 3 days.

A model 400 Stomacher was used to homogenize the inoculated strawberry samples for 1 min. A 10-fold dilution of each sample with 0.1% peptone solution was also homogenized for 1 min, and subsequently 10-fold serial dilutions with phosphate buffer (pH 7.2) were made for plating. Prepoured sorbitol MacConkey agar plates were used to recover *E. coli* O157:H7 using a surface spread method. A sample of 0.1 mL of solution was spread on each plate, and the plates were inverted. After incubation at 35 °C overnight, *E. coli* O157:H7 colonies were counted using a Darkfield Quebec colony counter. Typical colonies of *E. coli* O157:H7 were confirmed by a positive "snow flake" test using Bacto *E. coli* O antiserum O157, (Difco Laboratories, Detroit, MI). Weighed portions of homogenate of each sample were placed into aluminum dishes and dried in an oven at 100 °C for 24 h for measuring moisture content. The colony forming units (CFUs) for each sample were expressed on fruit dry weight basis.

Volatile Compounds from *E. coli* O157:H7-Inoculated Strawberry Fruit. Fruit (approximately 350 g) were inoculated with the BHI broth culture of *E. coli* O157:H7 ATCC 43895 (10^7 cells/mL) as described above. Control fruit were inoculated with sterile BHI broth. The fruit were allowed to dry for 1 h before placing them into the 1-L glass jars with lids containing air inlet and outlet lines. Headspace volatile compounds were collected using Super-Q absorbent at room temperature for periods up to 72 h and analyzed by the GC methods described above.

Absorption of Bacterial Volatile Compounds by Strawberries. Approximately 45 g of fruit and 5 mg of indole in a 2-mL glass beaker were placed inside a 1-L glass jar sealed with a plastic screw-cap and maintained in a 20 °C incubator for 18 h. The controls were set up in the same way except that there was only indole or fruit inside the jars. The vapor-phase composition within each jar was analyzed by direct sampling using a 500-mL gastight syringe. The samples were withdrawn through a 0.5-cm half-hole rubber septum in the lid of each jar. A similar procedure was used for the other *E. coli* components tested except that the amount of test compound placed in the system ranged from 1 to 10 mg depending on the volatility of the compound. The GC column and conditions used for analyses were the same as described above.

RESULTS AND DISCUSSION

Volatile Compounds from Bacteria. Results of the studies of headspace compounds emitted by two strains of *E. coli* O157:H7 and the nonpathogenic *E. coli* are shown in Table 1. The volatile profiles of the strains reveal the predominant compound was indole, which accounted for more than 90% of the total volatile compounds emitted by the bacterial cultures. In addition to this nitrogen heterocycle, the bacteria synthesized a group of odd-carbon number methyl ketones ranging from 2-heptanone to 2-tridecanone. An unknown compound with mass spectral ions with decreasing intensity at *m*/*z* 43, 54, 67, 68, 81, 96, 55, 41, 82, and 110 was also emitted. Several compounds were also detected

Table 2. Population of *E. coli* O157:H7 after Inoculation on Strawberry Fruit (log[CFU/g])[a,b]

days of storage at room temp	inoculum concentration (cell/mL)		
	10^5	10^6	10^7
0	4.54 ± 0.14 B	5.60 ± 0.17 NS	6.56 ± 0.06 NS
1	4.73 ± 0.10 B	5.46 ± 0.12	6.17 ± 0.17
3	6.46 ± 0.19 A	5.35 ± 0.95	6.39 ± 0.25

[a] Data are means ± standard deviation on a fruit dry weight basis. [b] Data within columns (inoculum concentrations) followed by different letters are significantly different by least-significant difference (LSD) at $P = 0.05$ or not significantly different (NS).

from the media (plate count agar); however, the quantity of benzaldehyde was less with bacteria indicating the possible metabolism by the bacteria of a media component. In contrast, the amounts of dimethyl disulfide were so variable that no statistical difference was observed between the cultures and the media control.

The results show that the two strains of *E. coli* O157:H7 and the nonpathogenic *E. coli* yielded similar volatile profiles. However, among the four ketones produced by the organisms, the nonpathogenic *E. coli* produced significantly more 2-tridecanone but significantly less 2-heptanone and 2-nonanone than did *E. coli* O157:H7. In addition, there was a lesser amount of the unknown compound in *E. coli* O157:H7. It would be interesting in future studies to determine if additional strains of *E. coli* O157:H7 yield similar patterns of ketone production.

Other bacteria including some food borne pathogens are known to produce various types of these volatile compounds. For example, *Pseudomonas aeruginosa* produced a characteristic profile of methyl ketones (excluding 2-tridecanone) and 1-undecene as a major component; however, no indole was found in this organism (Zechman and Labows, 1985; Zechman et al., 1986). Recently, Arnold and Senter (1998), as part of the development of digital aroma technology, studied headspace compounds from several bacteria including *P. aeruginosa* and *E. coli,* and alcohols including ethanol were among the primary products isolated. The predominance of alcohols in the latter case may be associated with the use of unagitated broth cultures.

Studies have shown that indole is frequently produced by coliform bacteria which are associated with fecal material (Krieg and Holt, 1984). Fruit such as strawberries do not produce indole (Nijssen et al., 1996), and the detection of this compound in the vapor phase surrounding fruit would indicate a need for further investigations for the presence of microbial contamination.

Growth of *E. coli* O157:H7 on Strawberry Fruit. Inoculations of strawberry fruit with *E. coli* O157:H7 resulted in proliferation or maintenance of the population as shown in Table 2. At the lower inoculation level (10^5 cells/mL) the population remained stable after 1 day; however, *E. coli* O157:H7 had increased significantly after 3 days from 4.54 to 6.46 CFU/g dry wt of fruit. At the higher inoculum levels of 10^6 and 10^7 cells/mL the *E. coli* O157:H7 population remained stable after the 3-day interval. These results showed that fresh strawberries were a suitable host for *E. coli* O157:H7. It has been shown that other plant-derived foods, for example, lettuce and radish sprouts, can support this organism (Abdul-Raouf et al., 1993; Diaz and Hotchkiss, 1996; Itoh et al., 1998). With regard to fruit products, the primary examples of a host are apple juice or cider, as noted, which are associated with serious outbreaks

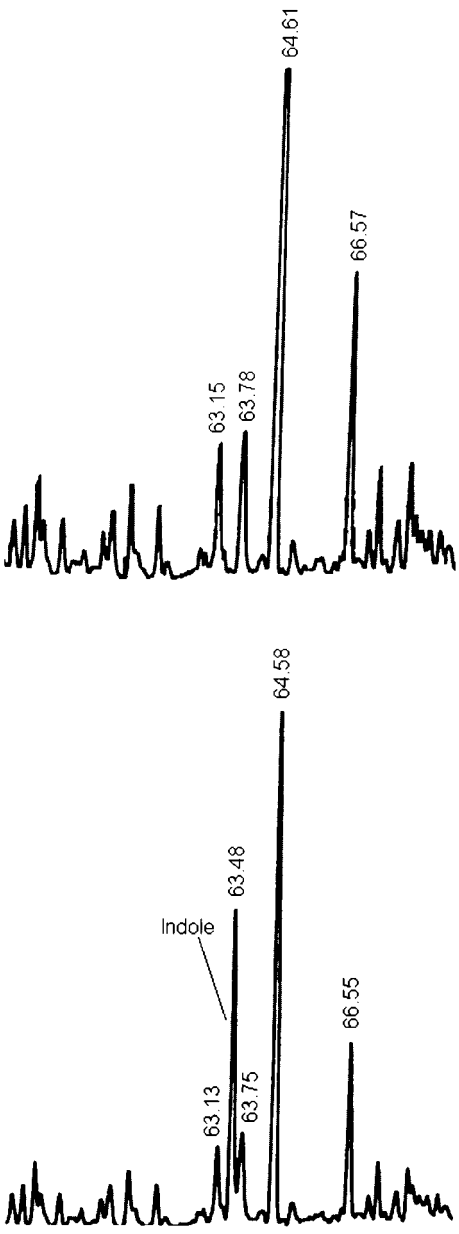

Figure 1. Portion of gas chromatogram of headspace vapors trapped from *E. coli* O157:H7-inoculated strawberry fruit revealing the lack of a detectable level of indole: top, vapors from bacteria-inoculated fruit; bottom, same sample as above spiked with an authentic sample of indole.

of *E. coli* O157:H7-caused disease (CDC, 1996, 1997a). To date, no reports of *E. coli* O157:H7-associated illness has been traced to berries although instances of Cylospora associated with imported raspberries causing food borne illness have been reported (CDC, 1997b).

Examination of Headspace from Inoculated Strawberry Fruit for Extraneous Volatile Compounds. Since *E. coli* O157:H7 was found to synthesize an array of volatile compounds, principally indole, and strawberry fruit was shown to sustain this pathogen, it was decided to examine the headspace from inoculated strawberries for bacterial volatile compounds, primarily indole. Headspace trapping, with the porous polymer, of vapors from *E. coli* O157:H7-inoculated strawberries revealed relatively large quantities of aroma compounds from the fruit, but the chromatographic area where indole eluted showed no detectable peak corresponding to the bacterial component. The area of the chromatogram where authentic indole eluted was relatively free

Table 3. Uptake and Metabolism of Synthetic Samples of Bacterial Volatile Compounds by Strawberry Fruit

		vapor phase concentration (μg/L)		
test compd	volatile metabolites	no fruit	fruit	% decrease of starting compd
indole		10.3 ± 0.1[a]	0.3 ± 0.1*[b]	97.1
2-heptanone		55.7 ± 1.9	7.6 ± 0.5*	86.4
	2-heptanol		1.1 ± 0.2	
	2-heptyl acetate		4.6 ± 0.1	
	2-heptyl butyrate		2.0 ± 0.2	
2-nonanone		36.9 ± 0.7	4.2 ± 0.3*	88.6
	2-nonyl acetate		0.2 ± <0.1	
	2-nonyl butyrate		0.8 ± 0.1	
2-undecanone		2.8 ± 0.3	0.3 ± <0.1*	88.9
2-tridecanone		3.4 ± 0.9	0.5 ± 0.1*	86.5
dimethyl trisulfide		28.3 ± <0.1	0.3 ± <0.1*	99.1

[a] Data are means ± standard deviation. [b] Fruit and no fruit means significantly differ (*) at $P = 0.05$ by t-test.

of aroma components that would interfere with detection of indole (Figure 1). In addition, no mass spectral evidence was seen for other compounds emitted by *E. coli* O157:H7 during an analysis of the aroma compounds produced by the inoculated strawberry fruit. The predominant compounds detected in the fruit emissions were well-known aliphatic esters associated with strawberry aroma (Nijssen et al., 1996).

Absorption of Bacterial Volatile Compounds by Strawberries. Based on work showing that strawberry fruit absorbed and metabolized certain volatile compounds tested as antifungal fumigants, especially aldehydes and alcohols (Hamilton-Kemp et al., 1996), it was decided to determine whether indole and other volatile compounds released by bacteria in the present study were absorbed by strawberry. Tests with indole alone showed that strawberry fruit readily absorbed this volatile nitrogen heterocycle yielding a 97.1% decrease of the vapor-phase concentration during the 18-h test period (Table 3). No volatile metabolites of indole were detected in the air surrounding the fruit as had been found with oxygenated test fumigants, namely, alcohols and aldehydes, studied earlier (Hamilton-Kemp et al., 1996).

Since methyl ketones have been reported as volatile compounds emitted from several bacteria (Marsili, 1999; Zechman and Labows, 1985; Zechman et al., 1986) including food borne pathogens, and a series of these metabolites were produced by bacteria in the present work, it was decided to determine the capacity of strawberries to absorb these bacterial products. 2-Heptanone was readily absorbed (86.4% loss) during the 18-h test period, and the compound was converted by the fruit to its corresponding alcohol, 2-heptanol, and the esters, 2-heptyl acetate and 2-heptyl butyrate, which were prominent in the vapor phase. Similar tests with 2-nonanone showed that this compound was absorbed (88.6% loss) and metabolized to 2-nonyl acetate and 2-nonyl butyrate; however, the alcohol, 2-nonanol, was not detected perhaps due to its relatively high boiling point. The remaining two methyl ketones, 2-undecanone and 2-tridecanone, were absorbed at 88.9% and 86.5%; however, no volatile products were detected from these compounds perhaps due to high boiling points of metabolites. Tests with the sulfur component, dimethyl trisulfide, showed that like the other compounds evaluated it was readily absorbed by the fruit (99.1% loss). Interestingly, at higher test levels it promoted emission of greater amounts of ethyl esters, such as ethyl acetate, from the fruit (data not shown). However, this response was not observed with bacteria-infected fruit. The principal observation from these studies was that strawberries caused a statistically significant reduction of indole and other bacterial metabolites from the vapor phase surrounding the fruit.

CONCLUSIONS

These studies showed that strains of *E. coli* O157:H7 and nonpathogenic *E. coli* emitted a profile of volatile compounds comprising predominantly indole and in addition methyl ketones and other components. Strawberry was found to be a suitable host for the acid-tolerant food borne pathogen *E. coli* O157:H7 which either proliferated at relatively low inoculum levels or remained at a stable population at higher inoculum levels. Inoculation of strawberry fruit with *E. coli* O157:H7 did not result in detectable amounts of indole in headspace vapor samples collected from the fruit although there were large quantities of aroma compounds trapped. Tests showed that strawberries absorbed relatively large quantities of indole and other bacterial volatile compounds from the vapor phase. In the case of certain compounds such as methyl ketones, the fruit produced volatile metabolites from the substrates which were released into the air.

These results show that *E. coli* O157:H7 produces volatile compounds which have the potential of being detected in initial screenings of food to indicate, in a nondestructive analysis, that food items are contaminated with bacteria and thus require further microbiological testing for confirmation and typing. It was observed that the nonpathogenic strain of *E. coli* produced a similar profile of volatile compounds. Coliform bacteria, such as the latter, are frequently associated with fecal matter, and any sign of their presence on food would also call for further testing for food contamination. Recent studies (Nakai et al., 1999) have indicated that volatile compound profiles can be used to detect *E. coli* O157:H7 in hamburger and salmon. The present study showed that strawberry rapidly absorbs bacterial products including the major compound indole and other volatile compounds such as methyl ketones and this would reduce the levels of potential bacterial "markers" associated with contamination. However, other types of plant-derived foods, for example, grapes, did not take up extraneous vapors nearly as readily as strawberries in previous studies with potential fumigants (Archbold et al., 1997), and thus bacterial contaminants might well be detected on these foods.

This field of study is rapidly becoming more relevant due to the development of electronic noses for the detection of vapor-phase profiles, based on an array of sensors and detectors, which can be designed for mul-

tiple practical applications. For example, a recent system described by Marsili (1999) using GC−MS/multivariate analysis to study off-flavors in milk indicates the potential power and specificity of an electronic nose in sensing applications. In the future, these systems might allow the detection of volatile compounds from microbial contaminants on types of fresh produce which do not readily absorb or metabolize marker compounds.

ACKNOWLEDGMENT

We thank Pam Wingate for manuscript preparation and Dr. John Loughrin and Katherine Akers for technical advice.

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Received for review June 1, 1999. Revised manuscript received August 9, 1999. Accepted November 22, 1999. K.Y. thanks the University of Kentucky Nutritional Sciences Program for financial assistance.

JF990576B