A New Type of Flat Sour Spoilage of Commercial Canned Coffee*

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INTRODUCTION

In a previous paper[1], the authors reported that a new type of spoilage similar to flat sour spoilage develops in canned coffee samples produced and kept heated in vending machines in Japan. This spoilage is characterized by flat can ends (no swelling) and acid contents. However, in contrast to the flat sour spoilage known to be caused by the thermophilic facultative anaerobes, *Bacillus stearothermophilus* or *Bacillus coagulans*[2], it is caused by thermophilic obligate anaerobes.

In the previous studies, pure cultures were obtained from only 15 out of 40 spoiled cans, which were found after incubation of 10,800 cans of the commercial canned drink at 55°C. This low ratio (15/40) of isolation of the bacteria from the spoiled cans was due to delay in the isolation of the obligate anaerobes, based on the conventional view that the causative bacteria of flat sour spoilage are thermophilic facultative anaerobes.

In this study, with due regard to the previous findings, another investigation was made on commercial canned coffee taken from lots which had suffered from flat sour spoilage.

MATERIALS AND METHODS

Samples of canned coffee

Two kinds of commercial canned coffee samples were taken from the lots which had suffered from flat sour spoilage. As shown in Table 1, these were products packed on different dates by the manufacturer J. Each sample consisted of 20 cases (600 cans).

Incubation test

Fifteen cases of each kind were incubated at 55°C and 5 cases of each kind were tested every ten days. The remaining 5 cases were incubated at room temperature and tested after 30 days.

Table 1. Samples used for incubation

Manufacturer	Sample No.		Contents		Cans
J	26	250 g	Coffee *1	8704 *2	30 x 20
	27	250 g	Coffee	8705	30 x 20

*1 With milk and sugar.
*2 Japanese code for the date of production: First, second, third and fourth numbers show the year, month and day, respectively.

* A New Type of Flat Sour Spoilage – II.

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The level of vacuum and pH values of 40% of the cans were measured.

Hoever. the remaining 60% of the cans was opened under aseptic conditions and the contents were subjected to pH measurement. Microbiological tests were carried out when the contents had pH values of below 6.0. The contents of these cans showed deterioration in color and flavor.

Isolation of bacteria from spoiled cans

For the detection of bacteria from the spoiled cans, modified fluid thioglycollate medium (mTGC)[3], standard methods agar (SMA)[4], iron sulfite agar (ISA)[5], iron sulfate agar in which sodium sulfite was replaced with sodium sulfate (ISaA), TF medium (TF)[1], TSiF medium (TSiF)[1], TSaF medium (TSaF)[1] and dextrose tryptone agar (DTA)[6, 7] were used. The contents of the spoiled cans were inoculated by the method described in the previous paper[1]. mTGC and SMA were incubated at 35°C for 10 days. DTA and SMA were incubated at 55°C for 5 days, and the others were incubated for 10 days. The bacteria were isolated from the spoiled cans and purified by the methods described previously[1] except for the replacement of Tomy's anaerobic jar[8] with a BBL Gas Pak anaerobic system.

Biochemical examination, preparation of spore suspensions of the isolated bacteria, and determination of D values of the spores at 120°C

These experiments were carried out by the methods described previously[1].

Reduction of sulfite and sulfate

To test for the reduction of sulfite and sulfate by the isolates, ISA, ISaA, TF, TSiF and TSaF were used. The reduction of sulfite and sulfate was judged by the blackening of the media, as described previously[1].

RESULTS AND DISCUSSION

Incubation test

No swollen cans were found under any of the conditions of incubation.

The pH values and vacuum levels of normal cans are shown in Table 2. The pH values of normal cans incubated at 55°C ranged from 6.2 to 6.4, and the average was 6.3. Those of normal cans

Table 2. Values of pH and vacuum level of normal cans

Sample No.	Incubation		pH			Vacuum*		
	Temp.	Days.	Min.	Mean	Max.	Min.	Mean	Max.
26	55°C	10	6.2	6.3	6.4	29	32	34
		20	6.2	6.3	6.3	32	34	39
		30	6.2	6.3	6.4	34	34	38
	Room temp.	30	6.2	6.4	6.4	34	37	41
27	55°C	10	6.2	6.3	6.4	32	34	38
		20	6.2	6.3	6.4	34	36	40
		30	6.2	6.3	6.3	34	38	42
	Room temp.	30	6.3	6.4	6.4	38	39	41

* cmHg

incubated at room temperature ranged from 6.2 to 6.4, and the average was 6.4.

The vacuum levels of normal cans incubated at 55°C ranged from 29 cmHg to 42 cmHg, and the average was about 35 cmHg. Those of normal cans incubated at room temperature ranged from 34 cmHg to 41 cmHg, and the average was about 38 cmHg.

From Table 2, it seems that incubation at 55°C caused slight decreases of pH and vacuum level.

The number of spoiled cans and the spoilage rate of each sample are shown in Table 3. No spoiled cans were found among those incubated at room temperature for 30 days.

Table 3. Numbers of spoiled cans and spoilage rates

Sample No.	Incubation		Number of spoiled cans	Spoilage rate
	Temp.	Days		
26	55°C	10	12	8.0%
		20	16	10.7%
		30	12	8.0%
		(Total spoilage rate ..... 8.9%)		
	Room temp.	30	0	0 %
27	55°C	10	5	3.3%
		20	9	6.0%
		30	2	1.3%
		(Total spoilage rate ..... 3.6%)		
	Room temp.	30	0	0 %

The numbers of spoiled cans in Sample No. 26 incubated at 55°C for 10, 20 and 30 days were 12, 16 and 12, respectively. The spoilage rates of the sample after 10, 20 and 30 days were 8.0%, 10.7% and 8.0%, respectively, and the total spoilage rate was 8.9%.

The numbers of spoiled cans in Sample No. 27 incubated at 55°C for 10, 20 and 30 days were 5, 9 and 2, respectively. The spoilage rates of the sample after 10, 20 and 30 days were 3.3%, 6.0% and 1.3%, respectively, and the total spoilage rate was 3.6%.

The appearance and the change of flavor of the spoiled contents were the same as those described in the previous paper[1].

On comparing the results at room temperature and at 55°C, it is clear that the spoilage was caused by thermophiles.

Table 4 shows the pH values and vacuum levels of the spoiled cans. The pH values of the spoiled cans ranged from 5.2 to 6.0. Those of almost all the spoiled cans were about 1.0 unit lower than those of normal cans. The vacuum levels of the spoiled cans ranged from 20 cmHg to 30 cmHg. The levels of almost all the spoiled cans were about 10 cmHg less than those of normal ones. The decrease of pH values and vacuum levels of the spoiled cans is consistent with the results reported in the previous paper[1]. It is clear that the spoilage decreases the pH value and the vacuum level of canned coffee.

Isolation of bacteria from spoiled cans

Table 5 shows the results of bacteria detection tests of coffee from the spoiled cans with 8 kinds of media, both at 35°C and 55°C. No bacteria were detected from any of the spoiled cans with mTGC or SMA incubated at 35°C, or with DTA or SMA incubated at 55°C. Some bacteria

Table 4. Values of pH and vacuum level of spoiled cans

Sample No.	Day of incubation	Can No.	Vacuum*	pH
26	10	26-1- 12	20	5.6
		26-1- 22	22	5.7
		26-1- 23	20	5.6
		26-1- 34	20	5.5
		26-1- 50	21	5.5
		26-1- 53	22	5.6
		26-1-104		5.6
		26-1-125		5.5
		26-1-137		5.5
		26-1-138		5.6
		26-1-144		5.6
		26-1-149		5.6
	20	26-2- 1	24	5.5
		26-2- 5	24	5.5
		26-2- 21	24	5.4
		26-2- 23	23	5.4
		26-2- 33	24	5.4
		26-2- 39	23	5.3
		26-2- 44	24	5.3
		26-2- 68		5.4
		26-2-110		5.4
		26-2-112		5.4
		26-2-113		6.0
		26-2-115		5.4
		26-2-127		5.4
		26-2-131		5.3
		26-2-135		5.7
		26-2-149		5.4
26	30	26-3- 10	26	5.3
		26-3- 17	26	5.4
		26-3- 39	25	5.3
		26-3- 49	24	5.4
		26-3- 55	24	5.5
		26-3- 57	24	5.4
		26-3- 67		5.4
		26-3- 71		5.4
		26-3- 75		5.3
		26-3- 81		5.4
		26-3- 87		5.3
		26-3-145		5.4
27	10	27-1- 47	24	5.6
		27-1- 55	25	5.4
		27-1- 56	24	5.5
		27-1- 61		5.5
		27-1-138		5.6
	20	27-2- 13	27	5.2
		27-2- 15	24	5.2
		27-2- 28	26	5.3
		27-2- 70		5.9
		27-2- 80		5.5
		27-2- 88		5.2
		27-2- 96		5.3
		27-2-117		5.3
		27-2-146		5.2
	30	27-3- 14	30	5.4
		27-3-129		5.4

* cmHg

grew in mTGC incubated at 55°C, except in the case of can No. 26-2-135.

Blackening was not observed in ISaA, TF or TSaF. In almost all cases of ISA and TSiF media, blackening was found. Thus, it is considered that the bacteria causing the spoilage are thermophilic, anaerobic and sulfite-reducing, but not sulfate-reducing.

Some spore-forming obligate anaerobes were isolated from 27 out of 30 spoiled cans by the method described in the previous paper[1]. These 27 cans are listed in Table 6. The can numbers are simplified in this table. A strain was selected at random from isolates of each can and the strains were given the same numbers as the cans from which they had been isolated. Twenty-seven strains were obtained.

Table 7 shows the results of incubation of all the strains under the conditions described in Table 5. All the strains isolated were found to be the causative bacteria of the spoilage. From Tables 5 and 7, it appears that TSiF is better than ISA for the growth of the causative bacteria.

Thus, prompt isolation of the obligate anaerobes resulted in a high isolation ratio (27/30) of the causative bacteria from the spoiled cans by the methods described in the previous paper[1].

Table 5. Detection of bacteria from spoiled cans[*1]

Incubation temp.	35°C		55°C							
Can No. \ Media	mTGC[*2]	SMA[*3]	mTGC[*2]	ISA[*4]	ISaA[*4]	TF[*4]	TSiF[*4]	TSaF[*4]	DTA[*3]	SMA[*3]
26-1-104	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-1-125	−,−	0,0	+,+	−,+	−,−	−,−	+,+	−,−	0,0	0,0
26-1-137	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-1-138	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-1-144	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-1-149	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2- 68	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-110	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-112	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-113	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-115	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-127	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-131	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-2-135	−,−	0,0	−,−	−,−	−,−	−,−	−,−	−,−	0,0	0,0
26-2-149	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-3- 67	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-3- 71	−,−	0,0	+,+	−,−	−,−	−,−	−,+	−,−	0,0	0,0
26-3- 75	−,−	0,0	+,+	+,+	−,−	−,−	−,+	−,−	0,0	0,0
26-3- 81	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-3- 87	−,−	0,0	+,+	−,−	−,−	−,−	−,+	−,−	0,0	0,0
26-3-145	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27-1- 61	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27-1-138	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27-2- 70	−,−	0,0	+,+	+,+	−,−	−,−	−,−	−,−	0,0	0,0
27-2- 80	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27-2- 88	−,−	0,0	+,+	−,+	−,−	−,−	+,+	−,−	0,0	0,0
27-2- 96	−,−	0,0	+,+	+,−	−,−	−,−	+,+	−,−	0,0	0,0
27-2-117	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27-2-146	−,−	0,0	+,+	+,−	−,−	−,−	+,+	−,−	0,0	0,0
27-3-129	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0

*1 Duplicate examinations.
*2 + : growth of bacteria was indicated by the turbidity of the media, − : no growth.
*3 Numbers of colonies.
*4 Blackening of the media was observed in some cases, + : blackened, − : not blackened.

Table 6. Cans from which strains were isolated

Can No.	Simplified No.	Can No.	Simplified No.
26-1-104	26- 1	26-3- 67	26-16
26-1-125	26- 2	26-3- 71	26-17
26-1-137	26- 3	26-3- 75	26-18
26-1-138	26- 4	26-3- 81	26-19
26-1-144	26- 5	26-3- 87	26-20
26-1-149	26- 6	26-3-145	26-21
26-2- 68	26- 7	27-1- 61	27- 1
26-2-110	26- 8	27-1-138	27- 2
26-2-112	26- 9	*	
*		27-2- 80	27- 4
26-2-115	26-11	27-2- 88	27- 5
26-2-127	26-12	27-2- 96	27- 6
26-2-131	26-13	27-2-117	27- 7
*		27-2-146	27- 8
26-2-149	26-15	27-3-129	27- 9

* No strains were isolated from 26-2-113, 26-2-135 and 27-2-70.

Table 7. Incubation of isolates under the conditions used for the detection of bacteria from the spoiled cans[*1]

Incubation temp.	35°C		55°C							
Strain No. Media	mTGC	SMA	mTGC	ISA	ISaA	TF	TsiF	TSaF	DTA	SMA
					[*2]					
26- 1	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26- 2	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26- 3	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26- 4	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26- 5	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26- 6	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26- 7	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26- 8	−,−	0,0	+,+	+,−	−,−	−,−	+,+	−,−	0,0	0,0
26- 9	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-11	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-12	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-13	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-15	−,−	0,0	+,+	+,−	−,−	−,−	+,+	−,−	0,0	0,0
26-16	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-17	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
26-18	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-19	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-20	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
26-21	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27- 1	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27- 2	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27- 4	−,−	0,0	+,+	+,−	−,−	−,−	+,+	−,−	0,0	0,0
27- 5	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
27- 6	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
27- 7	−,−	0,0	+,+	+,+	−,−	−,−	+,+	−,−	0,0	0,0
27- 8	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0
27- 9	−,−	0,0	+,+	−,−	−,−	−,−	+,+	−,−	0,0	0,0

*1 See Table 5.
*2 Symbols are the same as in Table 5.

Table 8. Characteristics of isolated strains

Strain No.	26- 1, 26- 3, 26- 4, 26- 6, 26- 7, 26- 8, 26- 9, 26-11, 26-12, 26-13, 26-15, 26-16, 26-17, 26-18, 26-19, 26-20, 26-21, 27- 1, 27- 2, 27- 4, 27- 5, 27- 6, 27- 7, 27- 9	26- 2, 26- 5, 27- 8
Gram stain	−	−
Acid from		
glucose	+	+
lactose	−	−
salicin	−	−
sucrose	−	−
Production of		
indole	−	−
H_2S	+	+
Coagulation of		
milk	−	−
Digestion of		
albumin	−	−
meat	−	−
Nitrite from		
nitrate	+	−
Hydrolysis of		
gelatin	−	−
D_{120} value	26- 9 : 17, 26-11 : 23	27- 8 : 25

Characteristics of the isolates

Table 8 shows the characteristics of the strains. All of them were Gram-negative and produced acids from glucose but not from lactose, sucrose or salicin. They did not produce indole, but did produce hydrogen sulfide. The tests for coagulation of milk, digestion of albumin and meat, and hydrolysis of gelatin all gave negative results.

However, the isolates could be divided into two groups as regards the ability to reduce nitrate. One group contained 24 strains which reduced nitrate. The other contained 3 strains which did not reduce nitrate.

The D values at 120°C of representative strains of each group were 17 and 23, and 25, respectively.

These characteristics of the strains are the same as those found in the previous paper[1].

The flat sour spoilage of the type reported in this paper has not been known as yet in Japan. Some principal characteristics of the strains, which are thermophilic, obligate anaerobic, spore-forming, Gram-negative and hydrogen sulfide-producing, are consistent with those of *Desulfotomaculum nigrificans*. However, the lack of sulfate reduction capability complicates the identification of the strains by means of Bergey's Manual of Determinative Bacteriology (Eighth Edition).

It seems likely that the causative bacteria originate from the coffee beans and/or sugar. Studies are continuing in our laboratories.

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