Short Paper

Anaerobic Microflora of "Kusaya" Gravy

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The unique flavor of *kusaya*, a specially brined and dried fish produced in the Izu Islands, is popular among the Japanese. Though some of the characteristic flavor compounds have been demonstrated,¹⁾ the causative microorganisms are not well known. It was reported by Fujii *et al.*²⁾ that obligately anaerobic bacteria occur in *kusaya* gravy at approximately the same population level of 10⁷/ml as aerobes, and these are thought to contribute to the formation of the unique flavor of *kusaya*. Of 19 isolates which were confirmed to be obligate anaerobes, 5 strains were identified to genus *Peptostreptococcus* and 3 to *Sarcina*. These isolates, however, were restricted to the predominant ones, as only a few non-selective media were employed for isolation. The present study, therefore, was undertaken to investigate the anaerobic flora of *kusaya* gravy by applying selective media according to the method of Mitsuoka *et al.*^{3,4)}

A *kusaya* gravy sample obtained from a manufacturer on Niiijima Island, south of Tokyo, in March 1992 was kept in a screw-capped plastic bottle and used for the experiment within a few days of sampling. Tenfold serial dilutions of the gravy were made in diluent solution A,⁴⁾ and 0.05 ml of appropriate dilutions were spread on the following agar media. The plating media^{3,4)} used in this study were TS blood agar, EG agar (Nissui), and BL agar (Nissui) as non-selective media, and DHL agar (Eiken), TATAC agar, PEES agar, NBGT1/5 agar, BS agar, ES agar, and NN agar as selective media. Inoculated DHL, TS, TATAC, and PEES agar were incubated aerobically, and EG, BL, NBGT1/5, BS, ES, and NN agar were incubated anaerobically (GasPak system, BBL). After 3 days'

incubation at 25°C the bacterial colonies were counted and different types of colonies which had developed on each plate were isolated to classify them on the basis of Gram stain, cellular morphology and arrangement, motility, spore formation, and the ability to grow aerobically. The isolates were identified at generic levels in accordance with the protocol shown by Mitsuoka.⁴⁾

Aerobic and anaerobic bacterial counts developed on each medium and the estimated bacterial groups are shown in Table 1. The total viable count (log cfu/ml) as the summation of the viable counts of all bacterial genera was 7.3, which was approximately the same population level as the anaerobic viable count previously reported.²⁾ Bacterial groups and the log of viable cells/ml detected in *kusaya* gravy in this experiment were Peptococcaceae 7.1 and *Clostridium* 3.4 as obligate anaerobes, and Enterobacteriaceae 6.4, *Streptococcus* 5.3, and *Staphylococcus* 3.7 as facultative anaerobes. The bacterial group estimated in this study to be Peptococcaceae is considered to be identical to genus *Peptostreptococcus*, dominant anaerobes in the gravy as reported in the previous paper.²⁾ Among the members of these Peptococcaceae, the isolates from BL agar, of which the population level (log cfu/ml) in the gravy was 7.0, showed a characteristic *kusaya*-like odor and were suggested to be responsible for the flavor formation of *kusaya*. Further studies concerning this issue are now in progress.

References

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- 3) T. Mitsuoka, K. Ohno, Y. Benno, K. Suzuki, and K. Namba: *Zbl. Bakt. Hyg., I. Abt. Orig.*, **A234**, 219-233 (1976).
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Table 1. Estimated bacterial groups and viable counts of *kusaya* gravy by using several kinds of non-selective/selective media (log cfu/ml)

Bacterial groups	Aerobic incubation				Anaerobic incubation						Viable counts
	DHL	TS	TATAC	PEES	BL	EG	BS	ES	NN	NBGT1/5	
Peptococcaceae											
<i>Kusaya</i> -like odor	+				7.0	7.1					7.1
	-				6.3	6.0			6.4		6.4
<i>Clostridium</i>							3.4	3.2		3.4	3.4
<i>Streptococcus</i>			5.3						4.6		5.3
<i>Staphylococcus</i>				3.7							3.7
Enterobacteriaceae		6.4									6.4
Not identified* ¹											
<i>Kusaya</i> -like odor	+	5.3									5.3
	-	6.6* ²									6.6
Total viable counts											7.3

*¹ Did not grow anaerobically on GAM agar.*² May belong to Enterobacteriaceae.

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