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Studies in the Properties of Oral Bacteria. I. Growth Inhibition by Surface Active Agent

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青井　陽：横田正義：中田秀彦：口腔微生物の研究 Ⅰ．界面活性剤による発育阻止

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Abstract

The effect of two types of detergent on oral bacteria was investigated in vitro. 0.01 % Sodium Laurylsulfate (SDS) prevented the growth of normal and lactic bacteria in the oral cavity and inhdbited acid formation. The effect of Triton X-100 was similar to that of SDS ; its grade, however, inferior to SDS.

Introduction

The surface active agent, which is divided into four groups —— cationic, anionic, amphoteric and non-ionic —— is known to have various affects on bacteria [1,3]. Cationic detergents have a strong bactericidal affect on many bacterial species, and anionic ones inhibit the growth of gram positive bacteria [3]. On the other hand *they* have a rinsing effect and anionic ones are contained in tooth paste as rinsing material. The purpose of the present study was to examine the affect of surface active agent on oral bacteria.

Materials and methods

Media and chemicals —— Tryptosoy agar (Eiken., Co. Ltd., Tokyo). Tomato Juice agar (Eiken., Co. Ltd., Tokyo). BCP plate count agar (Eiken., Co. Ltd., Tokyo). SDS (Sodium Laurylsulfate : Nakarai Chemicals Co. Ltd., Kyoto). Triton X–100 (Wako Chemicals Co.

Ltd., Osaka).

The isolation of bacteria from oral cavity —— A student voluntered to be the subject. She had natural teeth and good oral hygiene. After taking a meal, she brushed her teeth for three minutes and rinsed out her mouth with 30 ml sterilized saline. The rinsing saline was centrifuged at 2000 rpm for five minutes and the supernatant was used for subsequent experiments.

Detergent containing media —— Detergent solution sterilized by micropore filter (Membrane Filter TM-2 0.45μ : Toko Roshi Co. Ltd., Tokyo) was added to the autoclaved media which had been cooled to 50 ℃. The detergent concentration in the basal medium was represented by W/V % in SDS and by V/V % in Triton X-100. Maximum concentration of the detergent was 0.1 % and minimum was 0.00001 % to the medium. The pH of the media was not alternated after the addition of detergent.

Incubation of oral bacteria —— The experimental material was diluted in 9 ml saline by a serial 10 fold dilution. One ml of each dilution was put into a petri dish and added to the 20 ml of detergent containing media. The incubation period was 72 hrs for Tomato Juice agar, 48 hrs for BCP plate count agar and 24 hrs for Triptosoy agar. Each medium was incubated at 37 ℃ aerobically. The numbers of the viable colony were counted

Results

1. Growth inhibition of oral bacteria in Tryptosoy agar by SDS and Triton X-100.

Table 1 shows the growth inhibition of oral bacteria by SDS and Triton X-100 in Tryptosoy agar. 0.01 % SDS completely prevented the growth of oral bacteria. Slight inhibition was shown by 0.001 % SDS but no inhibition was observed with a lower concentration. The inhibition rate of Triton X-100 on the growth of oral bacteria was lower than that of SDS, 99 % at 0.1 % Triton X-100 and 98 % at 0.1 %.

2. The growth inhibition of oral bacteria in Tomato Juice agar by SDS and Triton X-100.

Table1. Growth inhibition of oral bacteria by SDS and Triton X-100 in Tryptosoy agar.

	SDS (n = 3 experiments)	Triton X-100 (n = 3 experiments)
Control	$4.4\pm0.45\times10^{5}$ #2	$2.9\pm0.31\times10^{5}$
0.00001% #1	$3.8\pm0.42\times10^{5}$	$2.9\pm0.30\times10^{5}$
0.0001%	$3.9\pm0.49\times10^{5}$	$2.7\pm0.25\times10^{5}$
0.001%	$2.6\pm0.28\times10^{5}$	$2.6\pm0.25\times10^{5}$
0.01%	0	$3.9\pm0.22\times10^{3}$
0.1%	0	$6.2\pm0.17\times10^{2}$

(Mean ± S.E.)

#1 The concentration of detergents in Tryptosoy agar.

#2 The numbers show the colony counts grown in the given detergent media, represented in terms of colonies per ml.

Tomato Juice agar was devised for isolation of lactic bacteria.

0.01 % SDS again inhibited the growth of oral lactic bacteria completely. 51 % of the bacteria was inhibited in 0.001 % SDS. However, no inhibition was observed at lower concentrations (Table 2). Triton X-100 inhibited the growth of 94 % bacteria at 0.01 % and 99 % at 0.1 % concentrations, but the degree of the inhibition was far weaker than that of SDS.

The inhibition pattern of oral bacteria by the detergent in Tryptosoy agar and Tomato Juice agar resembled each other.

3. The growth and acid forming inhibition of oral bacteria in BCP plate count agar by SDS and Triton X-100.

0.1 % SDS completely prevented the growth of oral bacteria in BCP plate count agar, and at 0.01 % a slight inhibition was observed (Table 3). The growth inhibition of SDS in BCP plate count agar was not so strong as in the other two media. However, acid formation was prevented by SDS. The acid formation by the colonies grown in BCP plate count agar was displayed by the change of the colour, blue to yellow, around the colonies. The percentage of yellow colonies was determined in relation to the total number of bacteria grown in the

Table2. Growth inhibition of oral bacteria by SDS and Triton X-100 in Tomato Juice agar.

	SDS (n = 5 experiments)	Triton X-100 (n = 4 experiments)
Control	$1.3\pm0.20\times10^{5}$ #2	$1.4\pm0.18\times10^{5}$
0.00001% #1	$1.5\pm0.21\times10^{5}$	N.D.
0.0001%	$1.3\pm0.21\times10^{5}$	$2.4\pm0.14\times10^{5}$
0.001%	$6.2\pm0.10\times10^{4}$	$2.3\pm0.16\times10^{5}$
0.01%	0	$8.4\pm0.13\times10^{3}$
0.1%	0	$4.0\pm0.52\times10^{2}$

(Mean ± S.E. N.D. = Not determined.)

#1 The concentration of detergents in Tryptosoy agar.

#2 The numbers shows the colony counts grown in the given detergent media, represented in terms of colonies per ml.

Table3. Growth and acid formation inhibition of oral bacteria by SDS and Triton X-100 in BCP plate count agar.

	SDS (n = 3 experiments)		Triton X-100 (n = 3 experiments)	
Control	$2.0\pm0.26\times10^{6}$ /	$2.6\pm0.32\times10^{6}$ #2	$1.3\pm0.73\times10^{6}$ /	$2.0\pm0.67\times10^{6}$
0.001% #1	$2.1\pm0.18\times10^{6}$ /	$3.3\pm0.21\times10^{6}$	$1.5\pm0.11\times10^{6}$ /	$2.2\pm0.18\times10^{6}$
0.01%	$9.3\pm0.44\times10^{5}$ /	$1.8\pm0.13\times10^{6}$	$9.0\pm0.11\times10^{5}$ /	$2.1\pm0.13\times10^{6}$
0.1%	0 /	0	$4.1\pm0.27\times10^{5}$ /	$4.2\pm0.23\times10^{5}$

(Mean ± S.E.)

#1 The concentration of detergents in BCP plate count agar.

#2 Denominators indicate the colony count grown in the given detergents media, represented in terms of colonies per ml. Numerators indicate the acid forming colonies under the same condition.

medium. About 70 % of the bacteria in the oral cavity formed acid after incubation at 37 C for 18 hrs (Data not shown). The proportion of the acid forming bacteria to the total bacteria grown in BCP plate count agar was lower in the medium containing SDS when compared with control. The proportion of the acid forming colonies to total colonies is 64 % at 0.001 % SDS and 52 % at 0.01 %. On the other hand it is 77% in media containing no detergent. The inhibition of acid formation was especially strong at 0.01 % SDS. Acid was produced in control and at 0.001 % SDS after 18hrs incubation, but not at 0.01 % SDS.

The growth inhibition by Triton X-100 was weaker than with SDS —— only 80 % of the colonies was inhibited by Triton X-100. And acid formation was not inhibited at all even with the same concentration.

Discussion

The anionic surface active agents SDS and the non-ionic surfactant Triton X-100 are widely used in biological studies. The HLB (Hydrophile Lipophile Balance) rate of the former is 40.0 and the latter is 13.5. Though cationic detergent has a strong bactericidal affect on many species of bacteria and has been used for sterilization, the other types do not have such a strong affect. SDS and Triton X-100, at comparatively high concentrations [1,3,4], however, are known to inhibit the growth of some bacteria.

Our experiments showed 0.01 % SDS inhibited the growth of oral bacteria in Tryptosoy agar and Tomato Juice agar aerobically. The tooth paste contains SDS of about 2 % concentration. When used as tooth paste, the SDS concentration in the oral cavity is far denser than 0.01 %. So SDS contained in tooth pastes may have a bacteristatic affect as well as a rinsing affect against the bacteria living in large number within the oral cavity.

Triton X-100 has weak a growth inhibition affect on oral bacteria compared with SDS. This may be caused by the lower HLB rate or by the non-ionoc nature of Triton X-100. It has been considered that Triton X-100 had little toxic affect on bacteria and it has been reported that 0.1 % Triton X-100 was most effective for the reagglegation of skin surface bacteria [2. 6]. However, interestingly enough, 0.01 % Triton X-100 strongly inhibited the growth of oral bacteria.

It is interesting to note that the growth of oral bacteria was inhibited or prevented by SDS or by Triton X-100 in Tomato Juice agar. The bacteria grown in this media are lactic : they produce acid and are tolerant of a high degree of acidity. Streptococcus mutans, which produces dental caries and ferment the sucrose or other sugars into lactic acid, may have been inhibited. This may indicate that the SDS plays an important role in protection against dental caries.

70 % colonies counted in BCP plate count agar produced acid, and no colonies produced alkali. Streptococci, which are dominant inhabitants in oral bacterial flora [5], produce acid, so that the bacteria showing acid formation in BCPplate count agar may be mainly streptococai. Acid formation, the primary factor in the initiation of dental caries, was strongly inhibited by SDS. This may to some extent show the possible role of SDS in the prevention of dental caries

as well as in the inhibition of the growth.

The activity of detergent to inhibit the growth and the acid formation of oral bacteria is not clearly realized. That the low surface tension, a formation of micelle by detergent, causes the enhancement of solubilization, and so may result in the death or growth inhibition of oral bacteria. The activity of enzymes embedded in the cytoplasmic membrane may be inhibited by detergent. The fact that oral bacteria was inhibited by Triton X-100, the comparatively mild detergent, shows that oral bacteria differ from other bacteria, such as skin surface bacteria, which is not inhibited by Triton X-100 [2,6]. The results on BCP plate count agar may show that the enzymatic inhibition occurs more easily than the growth inhibition. In the detergent containing the medium the number of acid forming colonies became smaller than the total colonies.

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