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Comparative antimutagenicity of saliva and oral bacteria against mutagens

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Abstract The purpose of this study was to investigate the mutagenic and antimutagenic interactions between several fractions of saliva and the habitual oral bacteria. Antimutagenicity was examined against the known mutagenic substances, 4-nitroquinoline-1-oxide (4NQO), AF-2 and benzo[a]pyrene (B[a]P)), using *Salmonella typhimurium* strains TA98 and TA100 in Ames test and Rec$^+$ and Rec$^-$ strains of *Bacillus subtilis* of Rec-assay. Four fractions from human saliva, whole saliva, supernatant saliva (Sup), precipitation in saliva (PPT) and heated saliva (Heat), were examined against 4NQO, AF-2 and B[a]P-induced mutagenicity. The whole saliva exhibited the strongest antimutagenic activity amongst all fractions applied against 4NQO and AF-2 in Ames test with more than an 80% inhibition rate. However, the whole saliva had an activity of only 50% inhibition against BP. The potency of the antimutagenicity was in the following order: whole saliva>Sup>PPT>Heat, in Ames test. Rec-assay also exhibited a pattern of antimutagenicity similar to that of Ames test. The cultured supernatants of the oral anaerobic bacteria exhibited a weak mutagenic potency. The cell wall skeletons of the oral anaerobic bacteria exhibited stronger antimutagenic activity against each mutagen than that of the oral aerobic bacteria.

Key words
Ames test,
Antimutagenicity,
Human saliva,
Oral anaerobic bacteria

Introduction

It has been reported that the mutagenic toxicity of known carcinogens has been decreased by the human saliva[1)]. The antimutagenic reaction has been considered to be dependent on the enzymes and vitamins in the saliva. However, the antimutagenic activities of the human saliva have not yet been fully identified. In addition, studies on the habitual oral bacteria in saliva, as antimutagens, have not been reported. Conversely, the correlation of genotoxicity and disease caused by environmental mutagens has facilitated interest in the further study of antimutagen[2)]. It is thought that a possible major cause of human cancer is from environmental factors and, although it may conflict with current lifestyles, avoiding these factors may be the best method for cancer prevention[3,4)]. Another means for preventing mutagenesis would be the consumption of antimutagen[5,6)]. Therefore, significant attention has been focused on the antimutagenic factors that reduce the spontaneous and induced mutagenesis. Recent studies suggest that the polyphenols in fruits and vegetables are active antioxidants with anti-mutagenic and anticarcinogenic properties[5–8)]. Hour *et al.*[9)] reported that various tea extracts and their components exhibited antimutagenic activities against the mutagenicity induced 2-acetylamino-fluorene and N-methyl-N'-nitro-N-nitrosoguanidine in Ames test. We previously reported that the bamboo extract, lignin[10)] and royal jelly[11,12)] exhibited inhibitory

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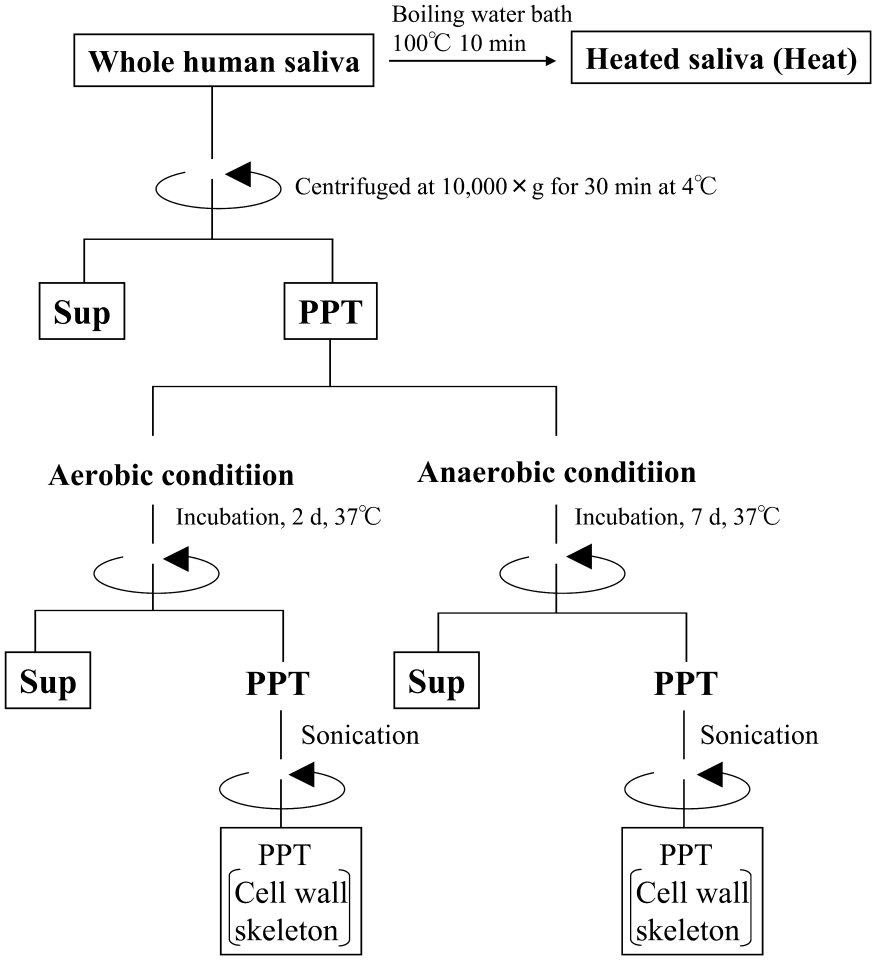


Fig. 1 The saliva, anaerobic and aerobic oral bacteria fractionation processes

effects against carcinogenicity *in vitro*, as well as, *in vivo*. We also found that various analgesics and their derivatives, and the anti-inflammatory drugs showed mutagenic activity by Ames test and also DNA-damaging properties using Rec-assay[13,14].

In the present study, the antimutagenic activities of several fractions of saliva, cultured supernatants and cell wall skeletons of the oral anaerobic and aerobic bacteria against the various mutagens have been observed using *Salmonella typhimurium* strains TA98 and TA100 mutagenicity assay, Ames test and the DNA-damaging capacity by Rec-assay, cold-incubation method, using *Bacillus subtilis*.

Material and Methods

Chemicals and bacterial strains

The mutagens, 4-nitroquinoline-1-oxide (4NQO) and benzo[a]pyrene (B[a]P) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Mitomycin C (MMC; Kyowa Hakko Co., Tokyo, Japan), and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) were kindly supplied by Dr. T. Kada, National Institute of Genetics (Mishima, Japan). NADH, NADPH, ATP and G-6-P were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The known positive mutagens, MMC, 4NQO, AF-2 and B[a]P were dissolved in dimethyl sulfoxide (DMS). All other chemicals were purchased from Wako Pure Chemical Industries.

The reverse mutation assay (Ames test), was performed using *Salmonella typhimurium* TA98 strain for the detection of frame-shift mutations and TA100 strain for the base-pair substitutions[15,16]. DNA repair test (Rec-assay) and DNA-damaging potential, was conducted using the recombination-positive wild H17 (Rec^+) and recombination-deficient M45 (Rec^-) strains of *Bacillus subtilis*. These were done in

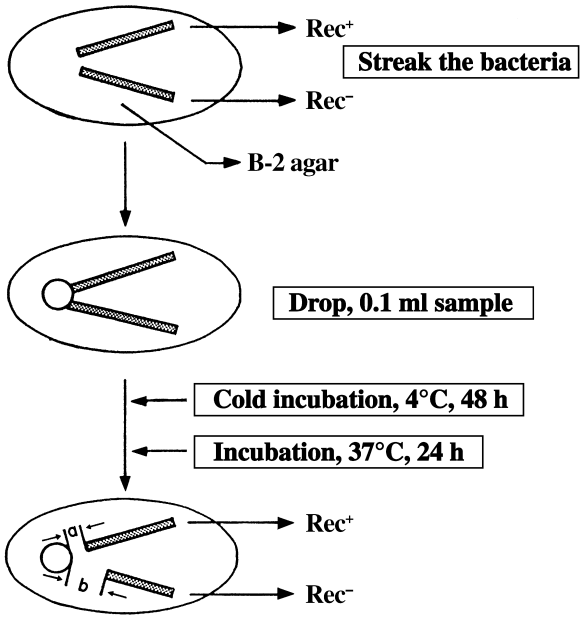
order to identify their DNA-damaging capacity and mutagenicity[17]. All test strains were kindly provided by Dr. T. Kada. All of the test strains were cultured in a nutrient broth for 16 h at 37°C, and harvested by centrifugation at 1,500 × g for 20 min at 4°C.

Preparation of the saliva

Whole human saliva was obtained from nine healthy, non-smoking, 22–56 year old (three periodontitis), male volunteers, without any stimulation. Approximately 10 m*l* of whole saliva was collected from each subject. Fractioning of the whole saliva was performed as follows: Whole saliva treated with dispersion by vortexing for 10 s and then disrupted by sonication at 200 W for 10 min at 4°C. The saliva was divided into supernatant and precipitates by centrifugation at 10,000 × g for 30 min at 4°C. The supernatant was used for the assay. The precipitates were disrupted by sonication at 200 W for 10 min, and then filtered through a 0.22 μm sterile membrane filter. The precipitates were washed three times with 0.02 M phosphate buffer saline (PBS, pH 7.4), and were condensed by being freeze-dried. One milliliter of whole saliva contained approximately 1 mg of the precipitates. For the heated saliva, the fresh whole saliva was heated for 10 min in a boiling water-bath. Thus, the whole saliva was divided into the four fractions: whole saliva, the supernatant saliva (Sup), freeze-dried precipitates (PPT) and heated saliva (Heat) and kept at −70°C until use. Whole saliva, Sup and Heat were thawed and the PPT diluted to 1×10^{-3}–10^{0} with 0.02 M PBS. The PPT, which was in a range from 0.01 to 10.0 mg/plate of PPT, was re-suspended in 0.02 M PBS (Fig. 1).

Preparation of the oral bacteria in the human saliva

Oral bacteria in approximately 20 m*l* of whole saliva were obtained as a pellet after centrifugation at 10,000 × g for 30 min at 4°C. The obtained oral bacteria were anaerobically grown under 5%CO_2-10%H_2-85%N_2 on supplemented Trypticase soy broth base with 0.1% yeast extract, 5 μg/m*l* of hemin, 0.5 μg/m*l* of menadione, and 5% defibrinated sheep blood. The bacteria were incubated for 7 d at 37°C with a rotary shaker. The pellet of centrifuged saliva was grown at 37°C nutrient medium supplemented with yeast extract under aerobic conditions. The oral anaerobic and aerobic bacteria were identified by Gram staining. The cells were harvested by centrifugation at 10,000 × g for 30 min at 4°C and washed three times with 2 m*l* of 0.02 M PBS. The cells were then disrupted by sonication at 200 W for 10 min. The precipitates were divided into cellular cytosol and cell wall skeletons by centrifugation at 10,000 × g for 30 min at 4°C. Harvested cell wall skeletons were freeze-dried and diluted 0.01 to 10.0 mg/plate with 0.02 M PBS. The cultured supernatants of the anaerobic and aerobic bacteria were collected, and diluted to 10^{-3}–10^{0} with 0.02 M PBS (Fig. 1).



a, b; Length of inhibition zone

Criterion

b−a (Rec⁻−Rec⁺), mm	DNA damage
< 2	−
≧ 2	+

Fig. 2 Procedure for the Rec-assay (cold-incubation method) using the *Bacillus subtilis* H17(Rec$^+$) and M45 (Rec$^-$) strains

Antimutagenic activity by Ames test

The antimutagenic activity was performed by *Salmonella*/microsome mutagenicity test reported by Ames *et al.*[15], with a slight modification[16]. The antimutagenic activity was evaluated using the test strains TA98 and TA100 against the known mutagens, 4NQO, AF-2 and B[a]P[18,19]. The B[a]P required metabolic activation in order to induce mutation in TA98 and TA100. Various concentrations of each test sample, containing 0.5 m*l* and 0.1 m*l* of bacterial

suspension (approximately $1–2\times10^9$ cells/m*l*), were placed into each tube. Next, 0.1 m*l* of each mutagen (dissolved in 50μl DMSO), and 0.5 m*l* 0.15 M PBS (pH 7.4) or 0.5 m*l* polychlorobiphenyl induced rat liver microsome faction (S9) were added to the mixture. For metabolic activation, the S9 and S9 mix (contained 10% S9 enzyme co-factors) were prepared according to the procedures of Ames *et al.*[15] The test compounds were considered mutagenic when the observed number of His^+ revertant colonies was at least 2-fold over the spontaneous mutation. Antimutagenicity was expressed as a percentage of the mutagenicity inhibition by using the following formula:

$$\text{Antimutagenicity (\%)} = \frac{B-A}{B}\times 100$$

Where, A is the number of His^+ revertant colonies by the mutagens (4NQO, AF-2, B[a]P) presence in the saliva plate and B is the number of His^+ revertant colonies by the mutagens in the plate without saliva.

Antimutagenic activity by Rec-assay

The experimental procedures for Rec-assay using the cold-incubation method were followed to those as described by Kada *et al.*[17], with a slight modification[20] (Fig. 2). The modified assay method was approximately 2–3 times more sensitive than that of the original Rec-assay. The mixture of 0.1 m*l* of the mutagen, 4NQO or AF-2, and 0.1 m*l* of the test sample was pre-incubated at 37°C for 30 min. The reaction mixture (100μl) was deposited onto a paper disc (diameter 8 mm), which had been placed at the starting point of the streaks. All of the plates were kept for 48 h at 4°C, and then incubated for 24 h at 37°C. The length of the inhibition zone was then measured. When the difference between the growth inhibition zones for Rec^+ and Rec^- strains was greater than 2 mm, it was considered that the test sample had a damaging effect on the DNA. The MMC was used as a positive control[20,21]. Inhibition of the DNA-damaging was expressed as a percentage of the DNA-damaging inhibition by using the following formula:

$$\text{Inhibition of DNA-damaging (\%)} = \frac{B-A}{B}\times 100$$

Where, A is the zone difference ($Rec^- - Rec^+$) by the mutagens (4NQO, AF-2) DNA-damaging presence in the saliva and B is the zone difference ($Rec^- - Rec^+$) by the mutagens DNA-damaging in the plate without saliva.

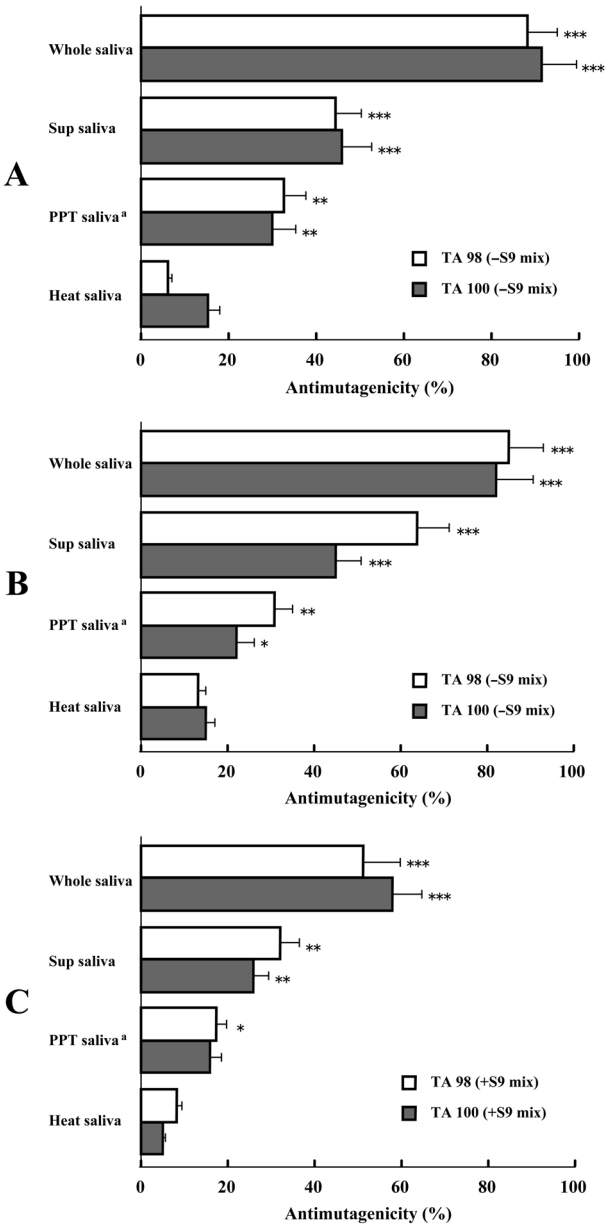


Fig. 3A–C The antimutagenic activities of the saliva four fractions against the 4NQO (0.5μg/plate)-induced (A), AF-2 (0.05μg/plate)-induced (B) without the S9 mix and B[a]P (5.0μg/plate)-induced (C) with the S9 mix activation mutagenicity in TA98 and TA100 strains

The 4NQO (0.5μg/plate) produced 422 ± 40, $1{,}245\pm162$ His^+ revertant colonies in TA98 and TA100 strains, respectively. The AF-2 (0.05μg/plate) produced 220 ± 15, $1{,}851\pm261$ His^+ revertant colonies in TA98 and TA100 strains, respectively. The B[a]P (5.0μg/plate) produced 225 ± 23, $1{,}180\pm122$ His^+ revertant colonies in TA98 and TA100 strains, respectively. [a]: 0.5 mg/plate freeze-dried cells precipitate of the oral bacteria in the saliva. The spontaneous His^+ revertant colonies were 21 ± 4, 101 ± 22 for TA98 and TA100 strains, respectively. The data are expressed as mean ± SD of five trials. The percentages of the antimutagenicity were obtained in comparison with the TA98, TA100 revertants/0.5μg 4NQO, 0.05μg AF-2, and 5.0μg B[a]P. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$

Statistical analysis

The data are expressed as mean ± SD. The data were analyzed by ANOVA followed by Newman-Keul's test. Statistical significance was set at $P<0.05$.

Results

Dose-response curves for the positive mutagens mutagenicity

In this study, the known positive mutagens, 4NQO, AF-2 and B[a]P were used as mutagens for Ames test on the His^+ revertant colonies of TA98 and TA100 strains, with or without the S9 mix. The dose-response curves for 4NQO (− S9 mix), AF-2 (− S9 mix) and B[a]P (+ S9 mix)-induced mutagenicities were obtained (data not shown). The peak mutagenic doses of 0.5μg/plate for 4NQO, 0.05μg/plate for AF-2 and 5.0μg/plate for B[a]P were chosen in order to obtain the number of His^+ revertant colonies/plate of 482 ± 65, 1,250 ± 198 for 4NQO, 260 ± 59, 1,888 ± 226 for AF-2, and 266 ± 33, 1,300 ± 155 for B[a]P, in the test strains TA98 and TA100, respectively. The spontaneous revertant colonies were 21 ± 4 and 101 ± 22 for TA98 and TA100, respectively.

In Rec-assay, the dose-response curves for 4NQO and AF-2 were also obtained. The difference zone ($Rec^- - Rec^+$ mm), at the peak mutagenic dosage were 23.7 ± 3.0 for 4NQO (0.5μg/plate) and 25.6 ± 2.8 for AF-2 (0.2μg/plate). MMC (0.1μg/plate) of 10.5 ± 1.8 was used as a positive control.

Antimutagenicity of the saliva

The antimutagenic activities of the human saliva four fractions were evaluated in TA98 and TA100 mutagenicity assay and Rec-assay. The saliva four fractions were neither mutagenic nor toxic to all of the bacteria. Figure 3A–C show the antimutagenic activity of the undiluted saliva four fractions against 4NQO (0.5μg/plate), AF-2 (0.05μg/plate) and B[a]P (5.0μg/plate)-induced mutagenicities in TA98 and TA100, respectively. When the various dilutions of the saliva four fractions were added, His^+ revertant colonies induced by each of the mutagens were reduced in a dose-dependent manner (data not shown).

The saliva inhibited 4NQO-induced mutagenicity, and their antimutagenic potency was in the order: whole saliva>Sup>PPT>Heat for both TA98 and TA100 (Fig. 3A). The mutagenic activity of 4NQO in both of TA98 and TA100 were nearly eradicated in the presence of the whole saliva. Among the saliva four fractions, the whole saliva exhibited the greatest antimutagenic activity against AF-2 (Fig. 3B). Inhibitions of the mutagenicities of 4NQO and AF-2 by the whole saliva were more than 80% in both of TA98 and TA100. The antimutagenic activity against AF-2 was also similar to those observed for 4NQO. The result of the antimutagenic activities by 5μg/plate B[a]P (with S9 mix) is illustrated in Fig. 3C. The antimutagenic activities using TA98 were 51.1%, 32.0%, 17.3% and 8.4% for the whole saliva, Sup, PPT and Heat, respectively. In addition, the saliva four fractions appeared to inhibit the mutagenicity of the indirect-acting mutagens of B[a]P, which were less effective than those of the direct acting 4NQO and AF-2. The antimutagenic activities of the saliva four fractions against the mutagenicity of 4NQO, AF-2 and B[a]P in both of TA98 and TA100 exhibited a decreasing pattern and were in the following order: whole saliva>Sup>PPT>Heat.

Inhibition of the four fractions of the undiluted saliva's DNA-damaging by Rec-assay using the cold-incubation method is shown in Fig. 4. For the

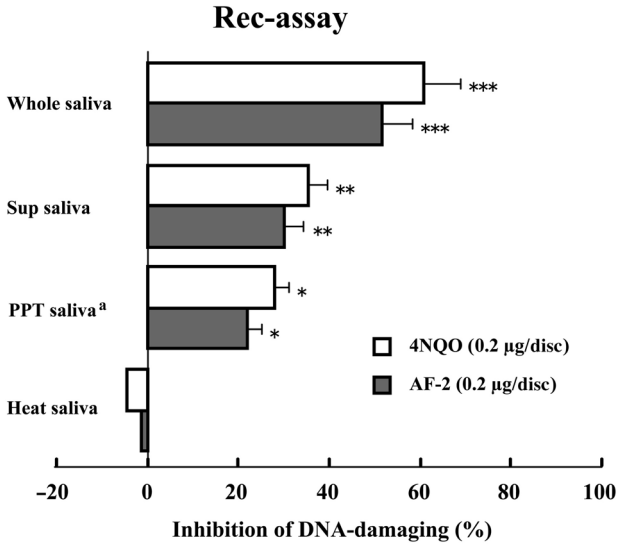


Fig. 4 The inhibition of the saliva four fractions against 4NQO (0.2μg/disc) and AF-2 (0.2μg/disc)-induced DNA-damaging by Rec-assay

[a]: 0.5 mg/plate freeze-dried cells precipitate of the oral bacteria in the saliva. 4NQO (0.2μg/disc) = 23.7 ± 3.0 mm ($Rec^- - Rec^+$). AF-2 (0.2μg/disc) = 25.6 ± 2.8 mm ($Rec^- - Rec^+$). The data are expressed as mean ± SD of five trials. The values differed significantly from the 0.2μg/disc 4NQO and AF-2. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$

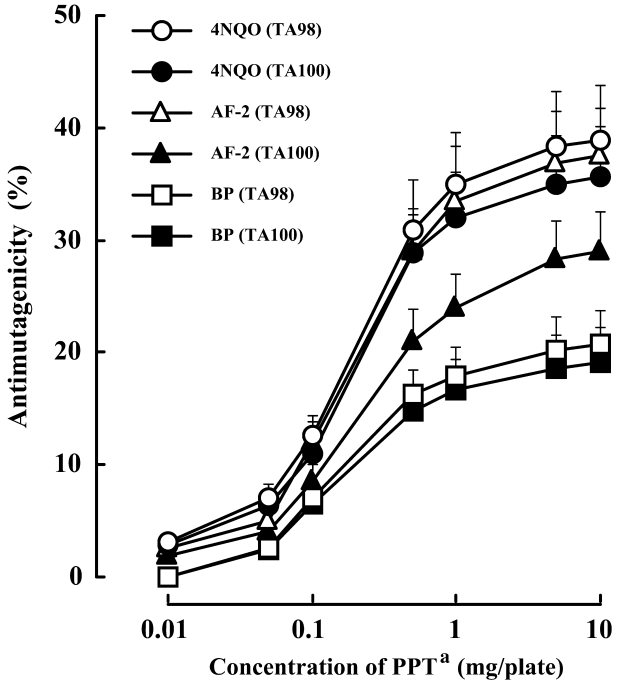


Fig. 5 The increase of the antimutagenic activity of the PPT against the 4NQO (0.5μg/plate), AF-2 (0.05μg/plate) and B[a]P (5.0μg/plate)-induced mutagenicity in TA98 and TA100 strains

[a]: freeze-dried cells precipitate of the oral bacteria in the saliva. The data are expressed as mean ± SD of five separate trials.

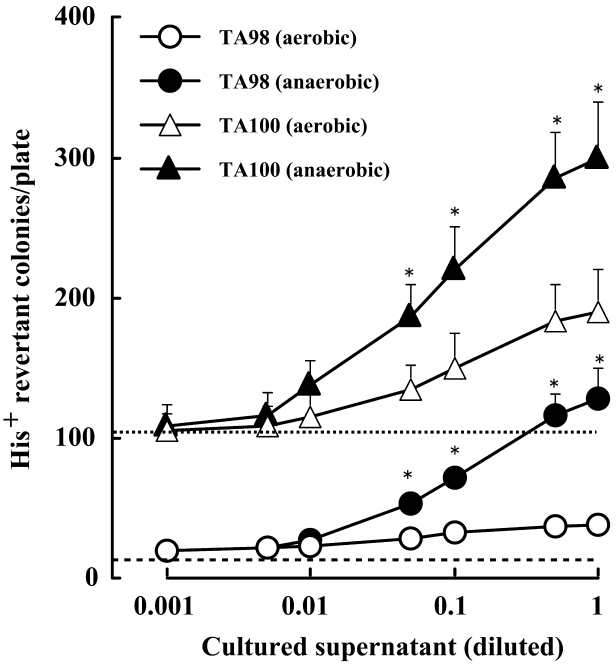


Fig. 6 The dose-response curve for the mutagens from the cultured supernatants of the anaerobic and aerobic oral bacteria

The dotted line (TA100) and dashed line (TA98) indicates the spontaneous counts for the DMSO in the absence of the cultured supernatants. *: 2-fold over the spontaneous mutation. The spontaneous His^+ revertant colonies were 25 ± 6 for TA98 strain. The spontaneous His^+ revertant colonies were 113 ± 26 for TA100 strain. The data are expressed as mean ± SD of five separate trials.

antimutagenicity studies, 0.2μg/plate 4NQO and AF-2, were chosen. With the exception of the Heat, the remaining three fractions of the saliva inhibited 4NQO and AF-2 DNA-damaging in a dose-dependent manner in Rec-assay. The whole saliva exhibited an inhibitory activity of 60.8% and 51.6% for 4NQO and AF-2, respectively. The Heat enhanced the DNA-damaging that was induced by 4NQO and AF-2. The Sup exhibited a greater inhibition on the DNA-damaging than that for the PPT. 4NQO was more sensitive to all of the saliva four fractions than that of AF-2.

In the PPT, 0.5 mg/plate exhibited an inhibitory activity in TA98 and TA100 of 30.9% and 28.8% in 4NQO, respectively (Fig. 5). At the highest dosage for the PPT (10.0 mg/plate), the mutagenicity of 4NQO in TA98 and TA100 were inhibited 38.9% and 35.6%, respectively. The PPT exhibited a dose-dependent antimutagenicity against 4NQO, AF-2 and B[a]P in both of TA98 and TA100.

Mutagens and antimutagenicity of the cultured supernatants and cell wall skeletons of the oral anaerobic and aerobic bacteria

The undiluted cultured supernatant of the anaerobic bacteria exhibited only a weak mutagenicity in the absence of the S9 mix. It showed a dose-related increase in the revertant colonies of up to two times of the background in TA98 and TA100 (Fig. 6). No antimutagenicity against all of the mutagens in both of TA98 and TA100 was found in the cultured supernatants of the anaerobic and aerobic bacteria. In TA98, the cell wall skeletons for both the anaerobic and aerobic bacteria inhibited mutagenicity of 4NQO (0.5μg/plate), AF-2 (0.05μg/plate) and B[a]P (5μg/plate) in a dose-dependent manner (Fig. 7A). The cell wall skeletons for the anaerobic and aerobic bacteria inhibited the mutagenicity of all three mutagens in the order: 4NQO>AF-2>B[a]P. In TA100, the cell wall skeletons for both anaerobic and aerobic bacteria inhibited all of the mutagens-

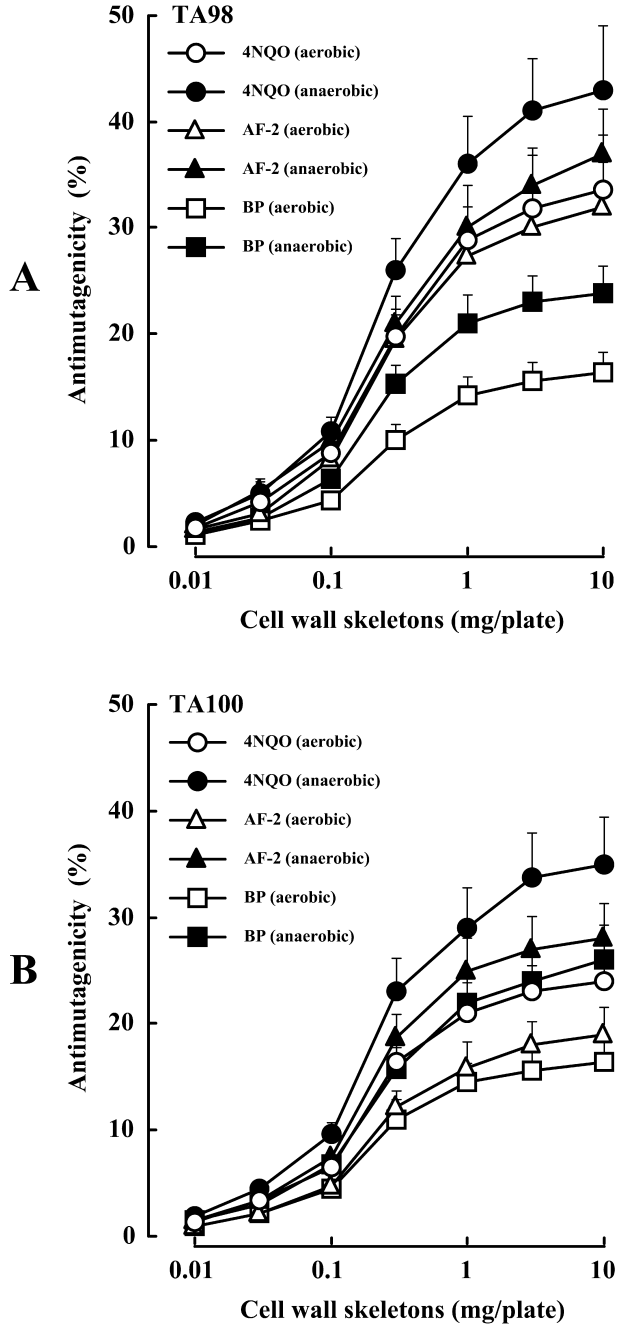


Fig. 7A–B The antimutagenic activity for the freeze-dried cell wall skeletons of the anaerobic and aerobic oral bacteria against 4NQO (0.5μg/plate), AF-2 (0.05μg/plate) and B[a]P (5.0μg/plate)-induced mutagenicities in TA98 (A) and TA100 (B)

mutagenicities in a dose-dependent manner (Fig. 7B). The cell wall skeletons of the anaerobic bacteria exhibited greater antimutagenic activity against all of the mutagens than those of the aerobic bacteria in both TA98 and TA100.

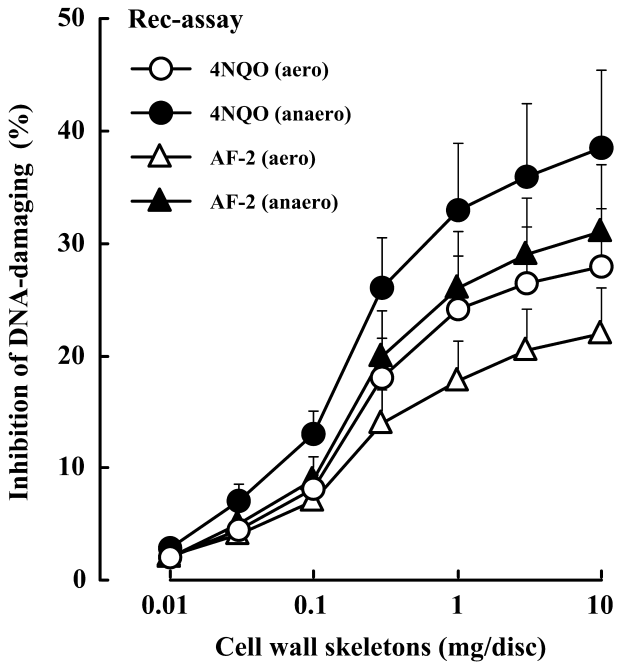


Fig. 8 The inhibited freeze-dried cell wall skeletons of the anaerobic and aerobic oral bacteria against 4NQO (0.2μg/disc) and AF-2 (0.2μg/disc)-induced DNA-damaging by Rec-assay

4NQO (0.2μg/plate) = 23.7 ± 3.0 mm ($Rec^- - Rec^+$). AF-2 (0.2μg/plate) = 25.6 ± 2.8 mm ($Rec^- - Rec^+$). The data are expressed as mean ± SD of five separate trials.

In Rec-assay, the cell wall skeletons for both anaerobic and aerobic bacteria inhibited 4NQO (0.2μg/plate) and AF-2 (0.2μg/plate)-induced DNA-damaging in a dose-dependent manner (Fig. 8). The cell wall skeletons for the anaerobic bacteria inhibited a greater DNA-damaging against these mutagens than those of the aerobic.

Discussion

Table 1, antimutagenic activities, DNA-damaging of varying potency and mutagenic activities were found in four fractions of saliva and cultured supernatants and cell wall skeletons of the oral anaerobic and aerobic bacteria. With the exception of the Heat, we observed that the three fractions of the saliva inhibited all of the mutagens in a dose-dependent manner in *Salmonella* reversion assay using TA98 and TA100 and DNA-damaging in Rec-assay. The saliva four fractions inhibited the 4NQO-induced mutagenicity. The antimutagenic potency was in the following order: whole saliva>Sup>PPT>Heat for both of TA98 and TA100 (Fig. 3A). The whole saliva

Table 1 The antimutagenic effects of saliva, and cell wall skeletons of the anaerobic and aerobic oral bacteria on mutagenicity induced by 4NQO, AF-2 and B[a]P in Ames test and Rec-assay

sample	Antimutagenic potency[a]			DNA-damaging inhibition[a]	
	4NQO	AF-2	B[a]P	4NQO	AF-2
whole saliva	+++	+++	++	++	++
Sup	+	+	+	+	+
PPT	+	+	±	+	+
Heat	±	±	±	−	−
PPT	+	+	+		
Cell Sk. Acrobic[b]	+	+	±	+	+
Cell Sk. Anaerobic[c]	+	+	+	+	+

sample	Mutagenic potency
Sup. Aerobic[d]	−
Sup. Anaerobic[e]	+

[a]: +++, strong inhibition (more than 80%); ++, inhibition (50–80%); +, weak inhibition (20–50%); ±, (less than 20%); −, no inhibition
[b]: cell wall skeletons of the aerobic oral bacteria
[c]: cell wall skeletons of the anaerobic oral bacteria
[d]: cultured supernatants of the aerobic oral bacteria
[e]: cultured supernatants of the anaerobic oral bacteria

inhibited the mutagenicities of 4NQO and AF-2 by more than 80% in both of TA98 and TA100. The inhibition of the antimutagenicity by the centrifuged and heated saliva fractions suggested that the saliva might contain antimutagen. Under similar conditions, Nishioka *et al.*[1], using Ames test, reported that the whole saliva inhibited the mutagenicity of 4NQO and AF-2 by over 80% in TA98 and TA100. The mechanism of this antimutagenicity is ascribed mainly as the decomposition of the mutagens induced by the peroxidase and by vitamin C within the saliva. The saliva four fractions that were observed also appeared to effectively inhibit the mutagenicity of the indirect-acting mutagens of B[a]P less than with the direct acting 4NQO and AF-2. The antimutagenic activities of the saliva four fractions against the mutagenicity of all of the mutagens in both of TA98 and TA100 were as follows: whole saliva>Sup>PPT>Heat.

The antimutagenic activities for the whole saliva roughly agreed quantitatively with the results for the Sup plus the PPT in the saliva. The saliva is a complex mixture of several components[21]. The antimicrobial components[22,23] in the saliva include immunoglobulins, lysozymes, lactoferrins, salivary peroxidases and anionic proteins. Further, the organic components in the saliva, especially the mucous glycoproteins, function as nutrients for many of the oral bacteria[23]. In a comparative study on the antimutagenic activity of the whole saliva against mutagens, we found that the mutagenic activity of 4NQO was eliminated by 0.5 m*l* of saliva per plate, and that the antimutagenic potency of the saliva was comparable higher than that of the β-carotene and vitamin C[7,8]. Therefore, the saliva might be a more effective antimutagen than several known antimutagens, such as, tea, vitamins and related compounds. Also, endogenous oral microflora and many of its harmful metabolic products are inhibited or neutralized by the salivary component[23]. Thus, it might be concluded that the saliva plays an important role in the maintenance of the health.

PPT showed antimutagenicity against 4NQO, AF-2 and B[a]P-induced mutagenicities in both TA98 and TA100 dose-dependently. At the highest dosage of the PPT (10.0 mg/plate), the mutagenicity of AF-2 in TA98 and TA100 were inhibited 37.5% and 29.0%, respectively (Fig. 5). The bacterial content

in the saliva is estimated to be approximately 1×10^9 bacteria per m*l* (approx. 1 mg precipitant/m*l* saliva). Therefore, our results suggested that not only the lower molecular weight substances, such as the enzymes and vitamins in the Sup, but also the bacteria and higher molecular weight substances in the PPT are responsible for the inhibition of the mutagenicity.

In the bacteria from the human whole saliva, we indicated that only the cultured supernatants of the oral anaerobic bacteria exhibited weak mutagens in both of TA98 and TA100 (Fig. 6). The mutagens may be the main metabolic products in the cultured supernatants of the anaerobic bacteria. Under this condition, the periodontopathogenic bacteria such as *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans* of the Gram-negative anaerobic bacterium were increased under the present cultured conditions[24,25].

In the human mouth, many oral bacteria can influence the nitration reactions by converting nitrate to nitrite, a reaction that can take place in foods. Bacteria from the human saliva contain nitrate- and nitrite-reducing species, and can catalyze the formation of the N-nitroso compound from a series of drugs[26]. Slots[27] also reported that the oral nitrate-reducing bacteria, such as *A. actinomycetemcomitans* produced nitrite by nitrate reduction. Nitroso compounds are a direct acting carcinogen and will induce tumors, which could be formed by an interaction of the amides with the bacterially produced nitrite[28,29]. The use of an antibacterial mouthwash containing chlorhexidine could result in the inhibition of carcinogenic nitrosamine formation[30]. Recently, Zhang *et al.*[31] reported that the fractions of the bacteria bound effectively with 3-amino-1, 4-dimethyl-5 H-pyrido[4,3-b]indole (Trp-P-1), mutagenic pyrolysate. The cell wall skeletons of the gram-negative bacteria could bind to the Trp-P-1 more effectively than those of the gram-positive bacteria. The gram-negative anaerobic bacteria cell wall is considerably more complex than its gram-positive counterpart in terms of structural heterogeneity, chemical composition, and functional activities[32]. Perhaps, the cell wall skeletons of the anaerobic bacteria may have a high adherence to be an active site for the mutagens.

In conclusion, the human whole saliva exhibited strong antimutagenic potency against the mutagens. Antimutagenic potency in centrifuged and heated saliva fractions was weak. These results indicate that saliva possess not only a significant antimutagenic activity but a inhibitory action against carcinogen-induced DNA damages. We suggest that saliva can function as a health-promoting biotherapy. The cultured supernatant of the anaerobic bacteria exhibited weak mutagens. Therefore, oral health care, especially good oral hygiene habits, is of significant importance.

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