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Peptostreptococcus sp.와 人體 棲息菌種과의 片利共生에 관한 硏究

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Studies on the Commensalism of *Peptostreptococcus* sp. with the Human Bacterial Flora

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ABSTRACT

The strain of *Peptostreptococcus* sp. S99 used in this study was isolated from the serous discharge of omphalitis of a 6 days old icteric male infant at the Clinical Laboratory of Microbiology, Seoul National University Hospital on June 9, 1973. The purpose of this study is to clarify the commensalism between *Peptostreptococcus* sp. and the human bacterial flora isolated from clinical specimens with special references to *p*H.

The results obtained were summarized as follows:

1. *Peptostreptococcus* sp. S99, an anaerobic streptococcus, could grow commensally in aerobic condition with 15 species of the human bacterial flora. Therfore, it was found to grow as satellite colonies around the colonies of the bacterial flora on the surface of agar medium. In the nutrient broth enriched with the used cultural filtrated of the growth of *Peptostreptococcus* sp. was also observed.

In these results, it is assumed that the synergism in producing pathogenic processes might have been induced by the commensal growth of non or weak pathogenic organisms in the bacterial flora. The commensal growth of anaerobic bacteria in aerobic condition may be due to the material of the electron transport system, such as vitamin K, in the case of *Bacteroides*, produced from the ost organisms.

2. The optimum *p*H of commensal growth of *Peptostreptococcus* sp. on the surface of agar medium was found o be 7.0 to 7.5. It is suggested that at this *p*H the commensal factor(s), w en grown on the surface, may be more active or more actively produced by the host organisms.

3. In the broth culture, the optimum *p*H for the growth of *Peptostreptococcus* sp. was found to be 8.0. At the lower and higher *p*H than 8.0, the lag phase was prolonged and the growth rate was also reduced.

In this result it is assumed that the commensal factor(s) is more active n vicinity of *p*H 8.0 in broth culture.

4. In the commensal growth among *Peptostreptococcus* sp., *Staphylococcus aureus* and *Corynebacterium* sp. were recognized. The commensal growth between *Peptostreptococcus* sp. and *Corynebacterium* sp. or *Staphylococcus aureus*, and also between *Staphylococcus aureus* and *Corynebacterium* sp. was observed. Furthermore the commensal growth was promoted within three species than each pairs of species.

5. *Pseudomonas aeruginosa*, one of the bacterial flora, showed an inhibition of

the commensal growth when it was grown 1 cm from the host on blood agar. When the two bacteria were grown farther apart, the *p*H range for the commensal growth between *Peptostreptococcus* sp. and the host was found broader. When the cultural filtrated of *Pseudomonas aeruginosa* was added more than 10%, the commensal growth of *Peptostreptococcus* sp. in the enriched broth was completely inhibited.

INTRODUCTION

The importance of interactions between species in the mixed microbic population in nature is recognized, but the subject is poorly understood. The need for studies in this field has been noted by Appleton (1940), Woods (1947), and Lucas(1949). Some of the manifest difficulties that beset an adequate approach to it are mentioned by Topley and Fielden(1922).

Most studies on the investigation of interactive phenomena among indigenous microflora have been concerned with inhibitory effects and have dealt mainly with the search for possible therapeutic agents or for a particular disease, e.g. diphtheria. Here are included (a) a group of reports on antibiotic substances produced by lactic streptococci and acting upon other streptococci, pneumococci, staphylococci, the *Corynebacterium diphtheriae*, and other organisms(Oxford, 1944; Hirsch and Wheater, 1954), (b) the so-called "colicins" produced by strains of *Escherichia coli* and acting upon other strains of the same species or upon other enteric bacteria (Gratia, 1925; Blackford *et al.*, 1952), and (c) both inhibitory and stimulatory effects produced by certain micrococci or staphylococci, enteric pathogens, and other bacteria(Halbert *et al.*, 1953).

Among examples of cooperative or probiotic microbial associations may be mentioned the satellitism of hemophilus influenzae by *Staphylococcus aureus*(Grassberger, 1897) and various fermentation effects described as "synergism"(Holman and Meekison, 1926).

Kadavy and Dack (1951) have shown that in bread which has a water content near the limit for growth of *Clostridium botulinum*, the growth of *Bacillus mesentericus* can raise the water content sufficiently so that the former organism can grow. One organism may alter the physiological environment and make it suitable for the growth of a second organism. Elliker(1949) have shown that *Geotrichum candidum* can grow easily by the lactic acid-producing *Streptococcus lactis*: the *p*H is thus kept high and the growth of *Streptococcus lactis* is not inhibited. On the other hand, the lactic acid may reduce the *p*H of a medium and thus make it favourable for the growth of yeasts. The growth of aerobic organisms may lead to a reduction in the redox potential permitting the growth of anaerobes(Mossel and Ingram, 1956).

Medical microbiologists have extensively inquired into the changes brought about in mixtures of organisms because of the significance of heterogeneous communities in infection and the enhancement of virulence by nonpathogens. For example, the virulence of *Proteus vulgaris* is markedly promoted by *Staphylococcus aureus*(Arndt and Ritts, 1951) and the abundance of *Borrelia vincentii* in the human mouth increased by components of a total of 15 strains, including members of the genera

Fusobacterium, *Escherichia*, *Streptococcus*, *Candida*, *Actinomyces* and other microflora characteristic of certain inflammatory process (Nevin, Hampp, and Duey, 1960). Also Robert(1967) has shown that the pathogenic synergy of *Fusiformis necrophorus* and *Corynebacterium pyogenes*.

There is a synergy of *Fusiformis necrophorus* and *Corynebacterium pyogenes*, which causes more rapid growth of each organism and greater inflammation and necrosis of the tissues than in single infections with either organism alone. *Fusiformis necrophorus* appears to be the primary invader and a necrotizing agent with *Corynebacterium pyogenes* participating less directly in the pathogenic process (Roberts *et al.*, 1968).

Peptostreptococcus is a genus of anaerobic streptococci, first described by Prevot (1925) which has been associated with human infection: yet, this genus has received little study because the isolation, cultivation, and identification of the anaerobic streptococci are technically difficult. Some species are found as members of the normal flora in the respiratory and gastrointestinal tracts, genito-urinary system, oral cavity, and the skin (Dack, 1940; Resebury, 1962).

Most recently, *Peptostreptococcus* sp. was isolated from subacute bacterial endocarditis (Nakamura *et al.*, 1970).

A strain of *Peptostreptococcus* sp. was found to produce visible colonies around the colonies of bacterial flora on the blood agar surface cultured aerobically.

This finding suggested commensalism between the bacterial flora and this strain of *Peptostreptococcus* sp. However, there was no report on the commensalism of *Peptostreptococcus* sp. with the bacterial flora of human clinical specimens.

The present study was carried out in order to clarify the cooperative and probiotic microbial association and inhibition activity between *Peptostreptococcus* sp. and the bacterial flora.

MATERIALS AND METHODS

1. Organisms and media

1) Organisms

The strain of *Peptostreptococcus* S99 was isolated from serous discharge of omphalitis of a 6 days old icteric male infant at Clinical Laboratory of Microbiology, Seoul National University Hospital on June 9, 1973.

Sixteen species of the bacterial flora were isolated from the clinical specimens at the same laboratory (Table 1).

All the species were identified according to Bergey's Manual of Determinative Bacteriology(Breed *et al.*, 1957).

Table 1. The bacterial normal flora isolated from various sources of human clinical specimen

Organism	Gram reaction	Remarks
Staphylococcus aureus	+	coccus
Staphylococcus epidermidis	+	coccus
Micrococcus tetragenes	+	coccus
Neissseria sicca	−	coccus
Neisseria perflava	−	coccus
Corynebacterium sp.	+	bacillus
Klebsiella aerogenes	−	bacillus
Paracolon bacilli	−	bacillus
Escherichia coli	−	bacillus
Proteus rettgeri	−	bacillus
Proteus morganii	−	bacillus
Alcaligenes faecalis	−	bacillus
Flavobacterium sp.	−	bacillus
Citrobacter freundii	−	bacillus
Pseudomonas aeruginosa	−	bacillus
Candida albicans	+	fungus

2) Agar media

Nutrient agar medium was prepared by adding 1.6% of Difco "Bacto Agar" to the nutrient broth medium as described in Difco Manual.

To prepare blood agar medium 0.5% of sodium chloride and 5% of rabbit whole blood were added to the nutrient agar medium at 45°C.

3) Broth media

The nutrient broth medium was prepared as described in Difco Manual.

CN-enriched broth medium: *Corynebacterium* sp. was incubated for 48 hours at 37°C in the nuturient broth (*p*H 6.8) and then the broth culture was sterilized by millipore filtration. The extract was added to the nutrient broth to make the final concentration of 5%.

SN-enriched broth medium: *Staphylococcus aureus* under the same condition and procedures described above and then the culture filtrated was added to the nutrient broth to make the final concentration of 5%.

CNS-enriched broth medium: *Corynebacterium* sp. was cultured under the same condition described above in the SN-enriched broth and then filtrated and the cultured filtrate was added to nutrient broth to make 5%.

AN-enriched broth medium: *Alcaligenes faecalis* under the same condition and procedures described above and then the culture filtrate was added to the nutrient broth to make the final concentration of 5%.

*p*H of the media were adjusted with 1 normal sodium hydroxide or hydrochloric acid.

Sterilizing procedure for broth culture by millipore filtration was as follows: Immediately after incubation for 48 hours the broth culture was centrifuged at 2°C, 3,000 r.p.m. for 15 minutes and the supernatant fluid was passed through sterile millipore filters of 0.22μ mean pore diameter.

2. The phenomenon of satellite growth

Peptostreptococcus sp. was inoculated on the center of nutrient agar surface on the straight line and then strains of the bacterial flora were cross-streaked on these plates and incubated aerobically at 37°C.

The satellite growth was investigated by measuring distance between satellite colony and the host colony using micrometer.

3. Growth inhibitory effect

After inoculation of *Peptostreptococcus* sp. on the blood agar plate the host species, *Alcaligenes faecalis*, was cross-streaked on this plate and then *Pseudomonas aeruginosa* was streaked in parallel with the distance of 1, 2, 3, 4, and 5cm from the host.

The effects of *p*H and the inoculation distance on the commensal growth inhibition of *Pseudomonas aeruginosa* were observed by measuring the satellite colony distance from *Alcaligenes faecalis*.

The broth used in the study on the growth inhibitory effects of *Pseudomonas aeruginosa* was prepared as follows: *Pseudomonas aeruginosa* was incubated in the nutrient broth medium (*p*H 6.8) for 48 hours at 37°C. The broth culture of *Pseudomonas aeruginosa* was sterilized by millipore filtration and then added to SN-enriched broth to make the final concentration of 2%, 4%, 6%, 8%, or 10%.

The effects of different *p*H on the commensal growth inhibition of *Pseudom-*

onas aeruginosa were observed by measuring turbidometrically.

4. Colony counts

Colony counts for *Peptostreptococcus* sp. were performed by the following methods: Serial dilution of *Peptostreptococcus* sp. culture in the CN-enriched broth or CSN-enriched broth was made with 0.85% sodium chloride solution containing 0.05% thioglycollic acid. 0.05M*l* of each dilution were inoculated and spread out with L-shaped glass rod thoroughly on the blood agar surface. After evaporating excessive moisture, the plate were incubated for 30 minutes at 37°C.

After 48 hours incubation, the number of colonies were counted.

Colony counts for the bacterial flora were performed by the following methods: Serial dilution were made with 0.85% sodium chloride solution. 1M*l* of each dilution were transfered to sterile Petri dish. Immediately afterward, 20m*l* of nutrient agar, previously melted and cooled to 45°C, were poured into the Petri dishes and thoroughly mixed. Number of colonies was counted after 24 hours incubation.

RESULTS

Peptostreptococcus sp., a strict anaerobe, could be commensally grown with bacterial flora on the nutrient agar under the aerobic condition. In the vicinity of the bacterial flora satellite colonies of *Peptostreptococcus* sp. were appeared (Fig. 1).

Most of the bacterial flora could induce the commensal growth of *Peptostreptococcus* sp. on the agar surface with different distance from the commenals in accordance with different *p*H. While *Pseudomonas aeruginosa* among the bacterial flora did not induced the commensal growth.

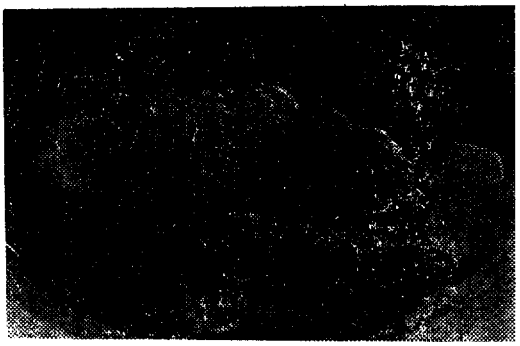

Fig. 1. Blood agar culture of *Peptostreptococcus* sp. showing satellite colonies around *Bacillus subtilis*

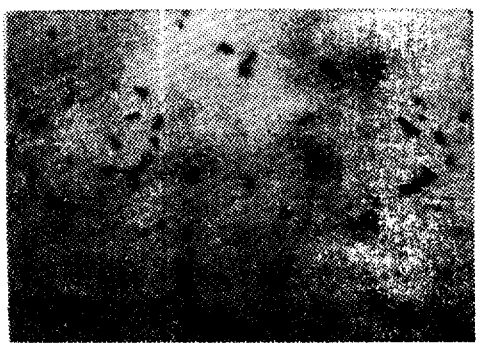

Fig. 2. *Peptostreptococcous* sp. S99, Gram stain, ×1,000.

The bacterial flora might be classified by their commensal effects on the growth of *Peptostreptococcus* sp.

Group A, at the *p*H from 6.0 to 10.0, which includes *Micrococcus tetragenes*, *Corynebacterium* sp., *Klebsiella aerogenes*, *Paracolon bacilli*, *Escherichia coli*, *Proteus morganii*, *Alcaligenes faecalis*, *Flavobacterium* sp., and *Citrobacter freundii*, and group B, which includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Proteus rettgeri* in the range of *p*H 6.5 to 10.0 induced the growth of *Peptostreptococcus* sp. In the range of *p*H 7.0 to 10.0, the commensal growth of *Peptostreptococcus* sp. was induced by the organisms of group C which includes of

Table 2. Commensal growth of *Peptostreptococcus* sp. with the bacterial flora

Group	Organism	The extent of satellite colonies appearance(mm)*	Commensal growth observed in the range of
A	*Micrococcus tetragenes*	3.5	*p*H 6.0—10.0
	Corynebacterium sp.	2.9	〃
	Klebsiella aerogenes	3.6	〃
	Paracolon bacilli	4.2	〃
	Escherichia coli	3.6	〃
	Proteus morganii	7.3	〃
	Alcaligenes faecalis	10.8	〃
	Flavobacterium sp.	6.8	〃
	Citrobacter freundii	4.7	〃
B	*Staphylococcus aureus*	9.9	*p*H 6.5—10.0
	Staphylococcus epidermidis	5.2	〃
	Proteus rettgeri	4.6	〃
C	*Neiseria sicca*	2.0	*p*H 7.0—10.0
	Neisseria perflava	6.1	〃
	Candida albicans	1.4	〃
D	*Pseudomonas aeruginosa*	—	None

* At *p*H 7.5

Neisseria sicca, *Neisseria perflava* and *Candida albicans*. Group D included *Pseudomonas aeruginosa* which could not induce the commensal growth of *Peptostreptococcus* sp.

The differences of distance between the satellite colony and the host organism on the agar surface in accordance with different *p*H are shown in the Fig. 3, 4, and 5. In the case of the group A, the maximum distance between satellite and host colony was observed at *p*H 7.5 and became shorter at lower or higher *p*H than 7.5.

At *p*H 7.5 the distances between the satellites from the host organisms were *Alcaligenes faecalis* 10.8mm, *Staphylococcus aureus* 9.9mm, *Proteus morganii* 7.3mm, *Klebsiella aerogenes* 3.6mm, *Flavobacterium* sp. 6.8mm, *Paracolon bacilli* 4.2mm, *Citrobacter freundii* 4.7 mm, *Escherichia coli* 3.6mm, *Micrococcus tetragenes* 3.5mm, and *Corynebacterium* sp. 2.9mm (Fig.3).

At *p*H 7.5 except no observable growth

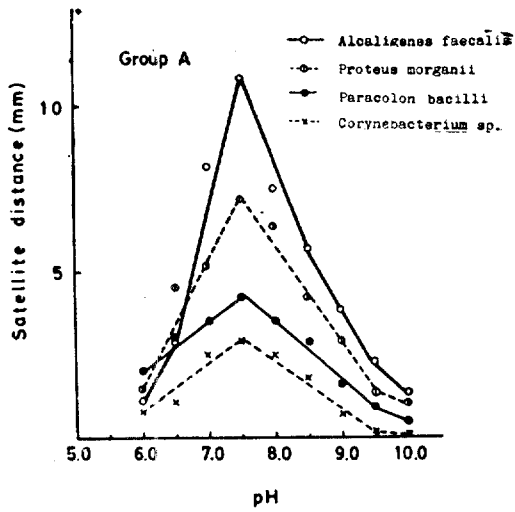

Fig. 3. Distance of satellite colony from the host grown on agar surface at various *p*H.

below *p*H 6.5 the commensal growth pattern of group B was similar to Group A. The distances between satellite colony from the host organisms were *Staphylococcus aureus* 9.9mm, *Staphylococcus epidermidis* 5.2mm, and *Proteus rettgeri* 4.6mm (Fig. 4).

In the case of group C, the commensal

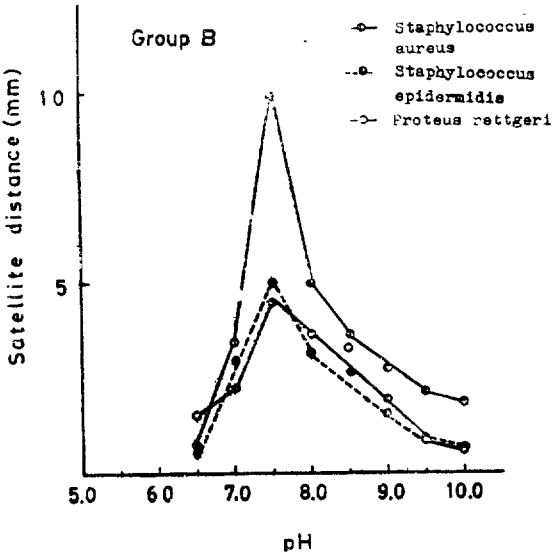

Fig. 4. Distance of satellite colony from the host grown on agar surface at various *p*H.

growth pattern was very different from those of groups A and B. The figure shows that *Peptostreptococcus* sp. did not aerobically grow on agar surface below *p*H 7.0, and the distance from the host organism was maximum at *p*H 7.0 and became to shorter in according with increasing *p*H (Fig.5).

Organism of group D, *Pseudomonas aeruginosa* could not induce the commensal growth of *Peptostreptococcus* sp. but rather inhibited the commensal growth

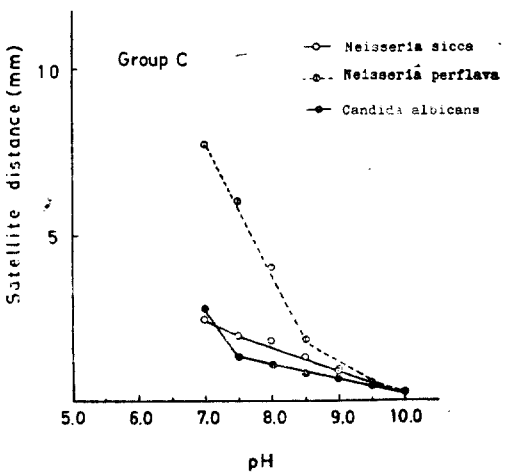

Fig. 5. Distance of satellite colony from the host grown on agar surface at various *p*H.

Table 3. Growth inhibition of *Peptostreptococcus* sp. by *Pseudomonas aeruginosa* at different *p*H(incubated for 48 hours)

*p*H	Distance(cm)*				
	1	2	3	4	5
5.5	0	0	0	0	0
6.0	0	0	0.3	0.7	1.0
6.5	0	0.2**	1.8	2.0	2.1
7.0	0	1.9	4.8	6.8	8.0
7.5	0	2.4	5.6	7.8	10.9
8.0	0	1.8	3.0	5.7	7.8
8.5	0	1.2	2.9	4.7	5.8
9.0	0	0	1.8	2.9	3.7
9.5	0	0	1.1	1.7	2.4
10.0	0	0	0	0.8	1.3

* Distance between parallel inoculation of host organism and *Pseudomonas aeruginosa*
** Distance between *Peptostreptococcus* sp. and the host

Table 4. Inhibitory effect of *Pseudomonas aeruginosa* on the commensal growth of *Peptostreptococcus* sp. at different *p*H

*p*H	Control	*Pseudomonas* content(%)				
		2	4	6	8	10
5.5	−	−	−	−	−	−
6.0	±	±	−	−	−	−
6.5	+	±	±	−	−	−
7.0	+	±	±	−	−	−
7.5	++	+	+	±	±	−
8.0	+++	++	+	+	±	−
8.5	+++	++	+	+	±	−
9.0	++	+	±	±	±	−
9.5	++	−	+	±	−	−
10.0	+	±	±	−	−	−
10.5	±	±	−	−	−	−
11.0	−	−	−	−	−	−

+++, very heavy growth
++, moderately heavy growth
+, moderate growth
±, slight growth
−, no growth
* *Pseudomonas aeruginosa* filtrate added in AN-enriched broth

of *Peptostreptococcus* sp. with other organisms.

The effects of *p*H and the inoculation distance of *Pseudomonas aeruginosa* from *Alcaligenes faecalis* on blood agar were studied (Table 3). When *Pseudomonas aeruginosa* was inoculated within 1cm from *Alcaligenes faecalis*, there was no commensal growth of *Peptostreptococcus* sp. grown in the range of *p*H 6.5 and 8.5. However, the distance was very short. The farther inoculation distance between *Pseudomonas aeruginosa* and *Alcaligenes faecalis* was, the broader *p*H range at which *Peptostreptococcus* sp. grew commensally. And also the longer distance was observed. When *Pseudomonas aeruginosa* was inoculated at 5cm from the host, there was almost no effect on the commensal growth of *Peptostreptococcus* sp.

Although there was no growth in the nutrient broth, *Peptostreptococcus* sp. grew well if the nutrient broth had been enriched with the culture filtrate of some bacterial flora (Fig. 6).

This shows that the optimum *p*H for the growth of this organism in AN-enriched broth is 8.0. At higher or lower *p*H than the optimum, the longer the lag phase became and the less the population increased. At *p*H 9.0, the lag phase took 3hours, at *p*H 7.0 and 10.0 6hours, at *p*H 6.0 9hours, and at *p*H 11.0 12hours. At *p*H 8.0 the microbial population was 8.2×10^5, at *p*H 9.0 1.0×10^5, at *p*H 7.0 8.3×10^4, and at *p*H 11.0 7.0×10^3 cells per m*l* after 24 hours (Fig. 7).

In SN-enriched broth the growth rate of *Corynebacterium* sp. was not only enhanced, but also the lag phase were shortened. The microbial population in both nutrient broth and SN-enriched broth after 24 hours incubation, at *p*H 6.0 were 6.0×10^6 and 8.0×10^3, at *p*H 7.0 1.5×10^7 and 9.3×10^5, at *p*H 8.0 7.5×10^6 and 6.0×10^5, and at *p*H 9.0 6.7×10^6 and 8.0×10^3 cells per m*l*. The data shows that *Corynebacterium* sp. could not grow in

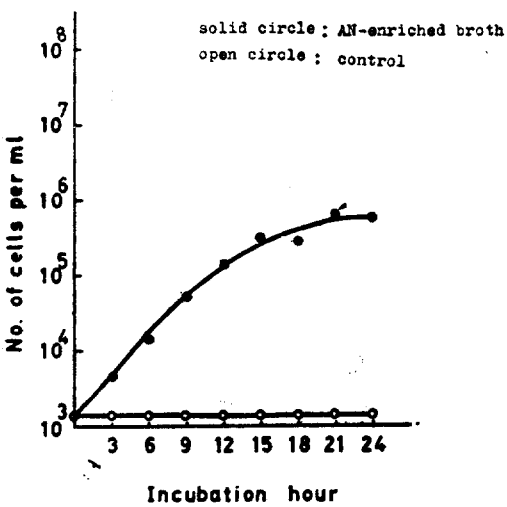

Fig. 6. Growth curve of *Peptostreptococcus* sp. S99 in AN-enriched broth at *p*H 8.0

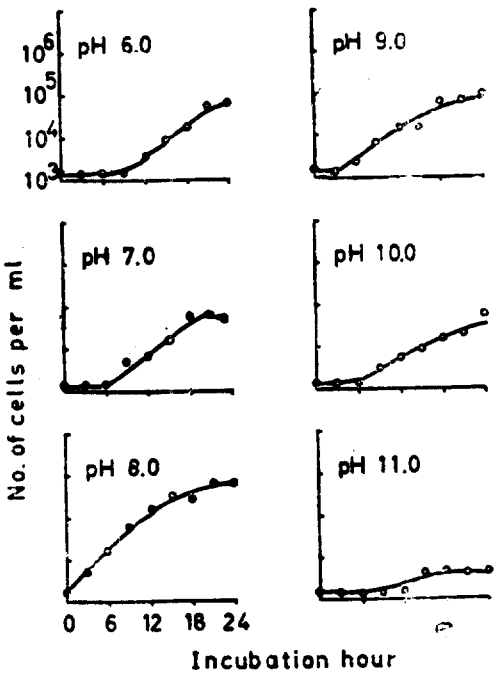

Fig 7. Growth curve of *Peptostreptococcus* sp. S99 in AN-enriched broth at different *p*H

the nutrient broth adjusted to *p*H 6.0 or *p*H 9.0. In the SN-enriched broth the lag phase was shortened from 10 to 2 hours at *p*H 7.0. These results indicate that there are commensal interaction between these two organisms (Fig. 8 and 9).

Peptostreptococcus sp. showed more or less rapid growth in the CSN-enriched broth than in the CN-enriched broth and the population of *Peptostreptococcus* sp. was also increased more greatly in the CN- and the CSN-enriched broth at *p*H from 7.0 to 9.0.

After 24 hours incubation in the CSN-enriched broth and the CN-enriched broth at *p*H 8.0 were 5.5×10^7/m*l* and 1.5×16^6/m*l* respectively. The lag phase was shortest at *p*H 8.0. However, there was no growth in the both media at *p*H 6.0. The

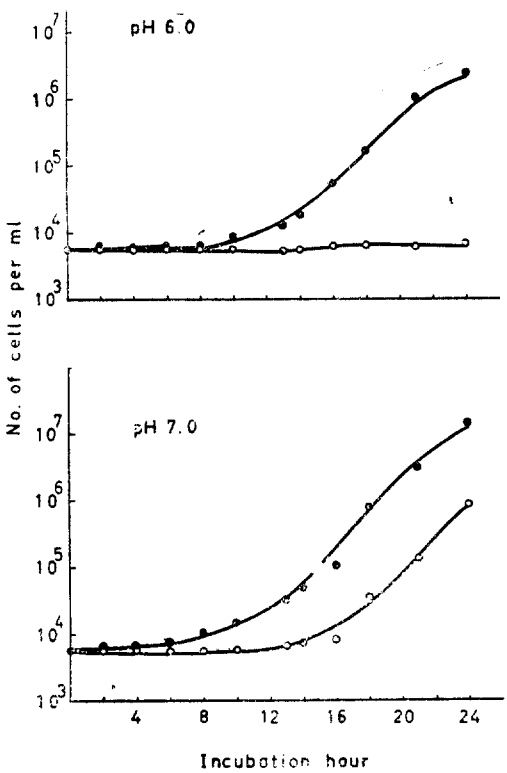

Fig. 8. Growth curve of *Corynebacterium* sp. in the nutrient broth (open circle) and the SN-enriched broth (solid circle) at different *p*H

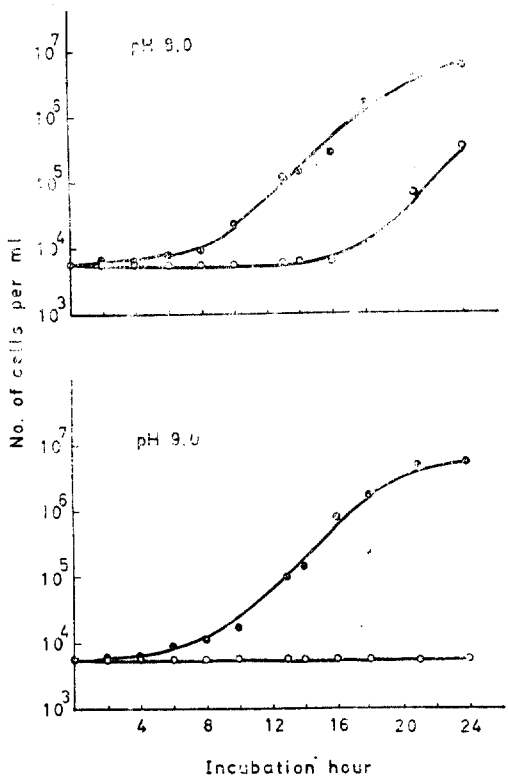

Fig. 9. Growth curve of *Corynebacterium* sp. in the nutrient broth (open circle) and the SN-enriched broth (solid circle) at different *p*H

similar growth phase of *Peptostreptococcus* sp. was shown in the CSN-enriched broth and the CN-enriched broth at *p*H 9.0 and each population was 0.1×10^6 and 8.3×10^5 cells per m*l* (Fig. 10 and 11).

These results indicate that there are commensal interactions among three species, *Peptostreptococcus* sp., *Corynebacterium* sp., and *Staphylococcus aureus*, which are indigeneous microflora in human.

The effect of the culture filtrates of *Pseudomonas aeruginosa* on the commensal growth of *Peptostreptococcus* sp. were studied by adding various amounts of the culture filtrate into the AN-enriched broth.

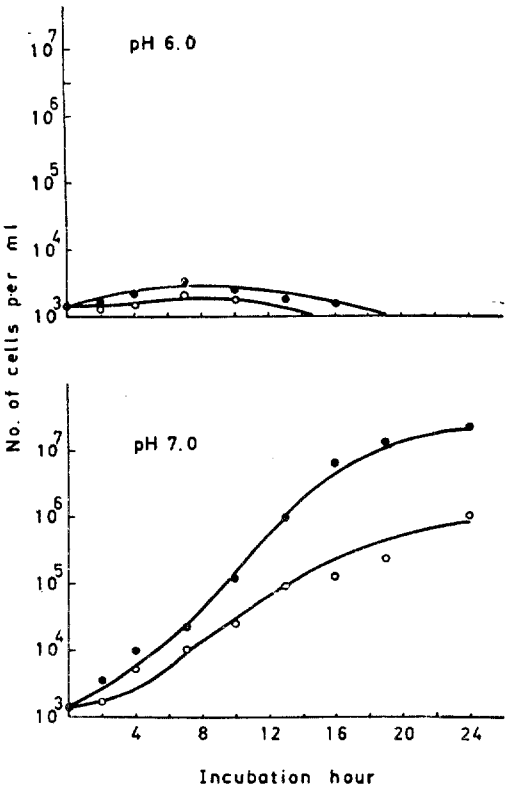

Fig. 10. Growth curve of *Peptostreptococcus* sp. S99 in the CN-enriched broth (open circle) and the CSN-enriched broth (solid circle) at different *p*H

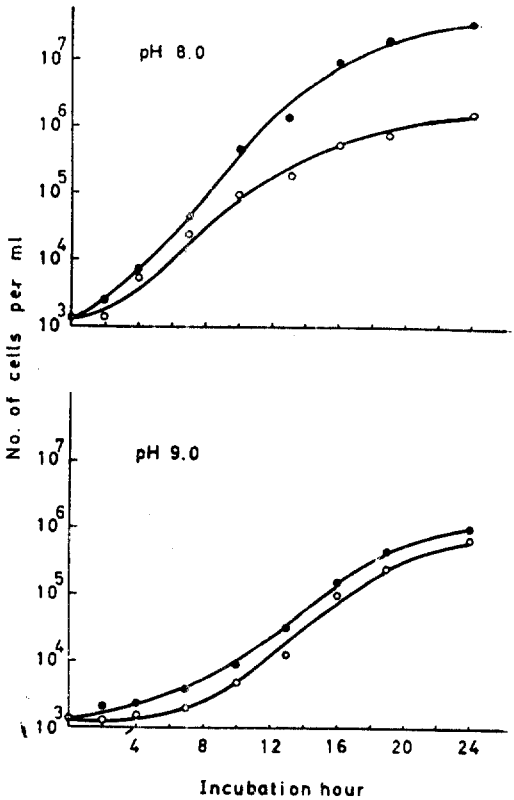

Fig. 11. Growth curve of *Peptostreptococcus* sp. S99 in the CN-enriched broth (open circle) and the CSN-enriched broth (solid circle) at different *p*H

In the AN-enriched broth containing more than 2% of the culture filtrate, the growth of *Peptostreptococcus* sp. was more or less inhibited at *p*H 6.0 to 10.0. When the AN-broth contained 10% of the filtrated the growth of *Peptostreptococcus* sp. was not observed upto 48 hours.

DISCUSSION

Since Prevot(1925) reported *Peptostreptococcus* which associated with human infection, the organism has been found as a member of the bacterial flora in the respiratory and gastro-intestinal tracts, genito-urinary system, oral cavity, and the skin (Dack, 1940; Hare, 1952; Gutierrez *et al.*, 1958; Rosebury, 1962).

As the genus has to be grown in anaerobic condition, the isolation, cultivation, and identification of this genus are technically difficult. For the reason, this genus has received little attention up-to-date.

Just there are no paper about the commensal growth of the genus *Peptostreptococcus* with the bacterial flora and(or) the synergy infection with them.

In this experiment, the commensal growth of *Peptostreptococcus* sp. with some bacterial species from the normal flora such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus tetragenes*, *Neisseria sicca*, *Neisseria perflava*, *Corynebacterium* sp., *Klebsiella aeroge-*

nes, *Paracolon bacilli*, *Escherichia coli*, *Proteus morganii*, *Proteus rettgeri*, *Alcaligenes faecalis*, *Flavobacterium* sp., *Citrobacter freundii*, and *Candida albicans* was observed. And *Peptostreptococcus sp.*, the strict anaerobe, with these organism was grown under the aerobic condition, also the commensal growth of *Corynebacterium* sp. with *Staphylococcus aureus* was found.

In commensal relationship one organism may alter the physiological environment and make it suitable for the growth of a second organism. The representative results showing the kinds of compounds serving as the basis for commensalism are as follow: *Borrelia vincentii* can grow commmensally consuming acetyl phosphate produced by *Corynebacterium* sp. (Nevin *et al.*, 1960), *Proteus vulgaris*(Shindala *et al.*, 1965), and *Lactobacillus* sp. (Challinor *et al.*, 1954), nicotinic acid or purine produced by *Saccharomyces cerevisiae* and *Tertrahymena* sp. (Stillwell, 1967), RNA produced by *Colpidium compylum* and *Phytophthora cryptogea* (Erwin *et al.*, 1961), thiamine produced by *Bacterium* sp. and *Bacterioides melaninogenicus* (Gibbons *et al.*, 1960), vitamin K produced by *Staphylococcus aureus*. In this experiment, however, it is assumed that the commensal growth of *Peptostreptococcus* sp. in aerobic condition is due to a coenzyme(s) of electron transport system rather than the acetyl phosphate or vitamin K produced by the *Corynebacterium* sp. or *Staphylococcus arueus*.

Recently, medical microbiologists have extensively studied the changes brought about in mixtures of organisms because of the significance of the possible enhancement of virulence by non or weak pathogens in indigenous human microflora (Arndt, 1961; Roberst, 1967).

The mixed infections with two or more organisms are found most often at the internal and external surfaces of the body that have an indigenous microflora (Suck *et al*, 1967; Chang *et al.*, 1973). Wilson *et al.*(1964) and Rosebury(1965) reported that harmless organisms that resided on the skin or mucous membrane are often essential to the infective process. Therefore, it is assumed that *Peptostreptococcus* sp. seemingly harmless in pure culture may reveal their pathogenicity when the organism grows together with some of the human bacterial flora.

The optimum *p*H of the commensal growth of *Peptostreptococcus* sp. with the bacterial flora on agar surface was 7.5 for group A and B, and 7.0 for group C which were lower than the optimum *p*H 8.0 in the broth culture. This result suggests that the differences of optimum *p*H may be related to production of the metabolic products by the host.

If the *p*H of the AN-enriched broth were higher or lower than the optimum *p*H 8.0, the longer the lag phase prolongs, the less the population increases. The result shows that the paticipation of the commensal factor(s) to the growth of *Peptostreptococcus* sp. are dependent on *p*H of the media.

There is the commensal growth among three species, which include *Peptostreptococcus* sp., *Cornyebacterium* sp., and *Staphylococcus aureus*. However, there is no paper about the commensal growth among three species.

The inhibitory effect on the commensal growth of *Peptostreptococcus* sp. by the

Pseudomonas aeruginosa in both broth and on agar surface was observed. This well coincides with the fact that the ability of *Pseudomonas aeruginosa* to produce pyocianin and other various antimicrobial substances *in vitro* were demonstrated by Liu (1966), Davis *et al.* (1970), and Jawetz (1970) among others.

Many species which produce various antibiotic substances have been proved quite common in the intestinal tract (Halbert, 1949; Halbert *et al.*, 1949; Gordner *et al.*, 1950), eye (Halbert *et al.*, 1952), skin (Evans *et al.*, 1949), nasopharynx(Thibault *et al.*, 1949), and oral cavity (Scrivener *et al.*, 1951).

The potential importance of the probiotic or antibiotic activities of the microflora of human tissues in recovery from or in resistance to some infectious diseases appeares to warrant more extensive investigations in this field.

摘 要

1973年 6月 9日 서울大學校 醫科大學 附屬病院 微生物 檢査室에서 黃色 葡萄球菌으로 인한 敗血症과 膿炎으로 入院한 生後 6日된 男兒의 배꼽에서 *Peptostreptococcus* sp. S99를 分離 同定하여 人體 棲息菌과의 *p*H에 따른 片利共生을 觀察하여 다음과 같은 結果를 얻었다.

1) 偏性 嫌氣性 細菌인 *Peptostreptococcus* sp.가 人體에서 分離한 15種의 人體 棲息菌과 共生할 때 好氣性으로 培養할 수 있었다. 即 普通 寒天培地上에서 *Peptostreptococcus* sp.는 人體 棲息菌의 集落 주위에 衛星集落(satellite colony)을 形成하였고 普通 液體培地에서는 人體 棲息菌의 培養液을 滅菌 濾過하여 5% 添加한 培地에서만 生長할 수 있었다.

本 實驗의 結果는 棲息菌中 非病原性菌이 片利共生을 함으로써 協同해서 感染을 일으킬 수 있을 것으로 생각되며 偏性 嫌氣性菌이 好氣性菌으로 전환되는 것은 vitamin K와 같은 전자전달계를 구성하는 어떤 물질이 宿主菌에서 生成함에 그 原因이 있지 않나 사료되는 바이다.

2) *Peptostreptococcus* sp.가 普通 寒天培地上에서 人體 棲息菌과의 片利共生에 依하여 生長할 수 있는 最適의 *p*H는 7.0~7.5였다. 이것은 *Peptostreptococcus* sp.의最適 *p*H인 8.0보다 낮은 것이다. 이와 같은 最適 *p*H의 差異는 宿主菌의 最適 *p*H와 片利共生을 위한 어떤 代謝物質을 最大로 生産할 수 있는 *p*H와의 사이에는 서로 連關關係가 있는 것으로 思料된다.

3) *Peptostreptococcus* sp. 生長의 最適 *p*H인 8.0을 中心으로 *p*H가 낮거나 높을수록 生長曲線의 lag phase가 길어지는 傾向을 보였으며 生長率도 낮았다.

이러한 현상은 commensal factor가 *p*H 8.0에서 훨씬 활성화되는 것으로 판단된다.

4) 三者間의 片利共生이 *Staphylococcus aureus*, *Corynebacterium* sp. 및 *Peptostreptococcus* sp. 사이에서 일어나는 것을 관찰하였다. 即 *Peptostreptococcus* sp.는 *Corynebacterium* sp.와 *Staphylococcus aureus*의 各菌株와 사이에서 片利共生을 할 수 있었고 또한 *Corynebacterium* sp.와 *Staphylococcus aureus*간에도 片利共生을 볼 수 있었다. 그러나 *Corynebacterium* sp.는 *Peptostreptococcus* sp.를 더욱 旺盛하게 生長시킴을 보았다.

5) 人體 棲息菌中에서 *Pseudomonas aeruginosa*만은 *Peptostreptococcus* sp.와 他菌種과의 片利共生을 방해하였다. 即 血液寒天 培地上에서 生長될 때 宿主菌과의 거리가 1cm以內에서는 *p*H에 關係없이 *Pseudomonas aeruginosa*는 *Peptostreptococcus*.sp.의 生長을 현저하게 저해했으며 2cm에서 5cm에 이르기 까지 거리가 멀어 질수록 片利共生을 시킬수 있는 *p*H의 範圍와 satellitic colony distance가 길어짐을 보았다. SN-enriched broth에서도 *Pseudomonas aeruginosa*의 培養濾過液을 2% 添加하면 *p*H에 거의 關係없이 *Peptostreptococcus* sp.가 生長할 수 있었으나 10% 添加해 주면 *p*H에 關係없이 全혀 生長할 수가 없었다.

REFERENCES

1. Appleton, J.L.T., 1940. The problems of oral microbiology as problems in ecology. *Proc. Dental Centenary Celebration*, 282—293.
2. Arndt, W.F., and Ritts, R.E., 1961. Synergism between staphylococci and proteus in mixed infection. *Proc. Soc. Exptl. Biol. Med.*, **108**, 166—169.
3. Blackford, V.L., Robbins, M.L., Roberts H. Jr., and Cook, M.K., 1952. An investigation of the antagonistic activity of "colicins". *Bact. Proc.*, 109.
4. Breed, R.S., *et al.*, 1957. Bergey's manual of determinative bacteriology, 7th ed. p. 1094, Willams & Wilkins Co., Baltimore,
5. Challinor, S.W., and Rose, A.H., 1954. Interrelationships between a yeast and a bacterium when growing together in defined medium. *Nature*, **174**, 877—878.
6. Chang, W.H., Suk, C.S., Kwon, B.S., Cinn, Y.W., and Lee, S.H., 1973. Studies on the distribution, infectious patterns, and susceptibility to various antibiotics of bacteria isolated from purulent materials. *The Seoul Journal of Medicine*, **14**(1), 41—48.
7. Dack, G.M., 1940. Non-sporeforming anaerobic bacteria of medical importance. *Bacteriol. Rev.*, **4**, 227—259.
8. Davis, B.D., *et al.*, 1970. Microbiology, 6th ed. p.774, Harper and Row, Publishers, N.Y.
9. Difco Laboratory, 1958. Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures, 9th ed. p.350, Difco Laboratory, Detroit.
10. Elliker, P.R., 1949. Practical diarly bacteria, 1st ed. p.391, McGraw-Hill, N.Y.
11. Erwin, D.C., and Katznelson, H., 1961. Studies on the nutrition of *Phytophthora crytogea*. *Can. J. Microbiol.*, **7**, 15—25.
12. Gardner, J.F., 1950. Some antibiotics formed by *Bacterium coli*. *Brit. J. Exper. Path.*, **31**, 102—111.
13. Gibbons, R.J., and MacDonald, J.B., 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. *J. Bacteriol*, **80**, 164—170.
14. Grassberger, R., 1897. Verhalten von Influenza in Mischkulturen. *Wien. klin. Wochschr.*, **10**, 485—486.
15. Gratia, A., 1952. Sur un remarquable example d'antagonisme entre deux souches de colibacille. *Compt. rend. soc. biol.*, **93**, 1040—1041.
16. Gutierrez, J., Davis, R.E., Lindahl, I.L., and Warwick, E.J., 1959. Bacterial changes in the rumen during the onset of feet-lot bloat of cattle and characteristics of *Peptostreptococcus elsdenii* n. sp. *Appl. Microbiol.*, **7**, 16—22.
17. Halbert, S.P., 1949. Relation of *Shigella* type specificity and susceptibility to antibiotic-producing strain of *Escherichia coli*. *J. Immunol.*, **61**, 271—282.
18. Halbert, S.P., and Gravatt, M., 1949. Prevalence of antibiotic-producing coliform organisms: further studies. *Pub. Health Rep.*, **64**, 313—318.
19. Halbert, S.P., and Swick, L.S., 1952. Antibiotic producing bacteria of ocular flora. *Am. J. Ophth.*, **35**, 73—81.
20. Halbert, S.P., Swick, L.S., and Sonn, C., 1953. Characteristics of antibiotic-producing strains of the ocular bacterial flora. *J.Immunol.*, **70**, 400—410
21. Hare, R., Widy, P., Billette, F.S., and Twort, D.N., 1952. The anaerobic cocci; gas formation, fermentation reactions, sensitivity to antibiotics and sulphonamides. Classification. *J. Hyg.*, **50**, 295—319.
22. Hirsch, A., and Wheater, D.M., 1951. "Streptozyme", a lytic enzyme from lactic-streptococci. *Nature*, **168**, 607.
23. Holman, W.L., and Meekison, D.M., 1926. Gas production by bacterial synergism. *J. Infectious Diseases*, **39**, 145—172.
24. Jawetz, E., Melnick, J.L., and Adelberg, E.A., 1970. Review of medical microbiology, 9th ed. p.197, Lange Medical Pub., N.Y.
25. Kadavy, J.L., and Dack, G.M., 1951. The effect of experimentally inoculating canned bread with spores of *Clostridium botulinum* and *Bacillus mesentericus*. *Food Res.*, **16**, 328—337.
26. Liu, P.V., 1966. The roles of various fractions of *Pseudomonas aeruginosa* in its pathogenesis, Ⅲ. Identity of the lethal toxin produced *in vitro* and *in vivo*. *J. Infectious Diseases*, **116**, 481—489.
27. Lucas, C.E., 1949. Selective toxicity and antibiotics. *Symposia Soc. Exptl. Biol.*, **3**, 336—356.

28. Mossel, D.A.A., and Ingram, M., 1956. The physiology of the microbial spoilage of food. *J. Appl. Bacteriol.*, **18**, 232—268.
29. Nakamura, I., *et al.*, 1970. Subacute bacterial endocarditis due to *Peptostreptococcus. Saishin Igaku*, **25**, 1996—2000.
30. Nevin, T.A., Hampp, E.G., and Duey, B.V., 1960. Interaction between *Borrelia vincentii* and an oral diphtheroid. *J. Bact.*, **80**, 783—786.
31. Oxford, A.E., 1944. Diplococcin, an antibacterial protein elaborated by certain milk streptococci. *Biochem. J.*, **38**, 178—182.
32. Prevot, A.R., 1625. Les streptocoques anaérobies. *Ann. Inst. Pasteur*, **39**, 417—447.
33. Roberts, D.S., 1967. The pathogenic synergy of *Fusiformis necrophorus* and *Corynebacterium pyogens*, Ⅰ. Influence of the leucocidal exotoxin of *F. necrophorus*. *Brit. J. Expl. Path.*, **48**, 665—673.
34. Roberts, D.S., 1967. The pathogenic synergy of *Fusiformis necrophorus* and *Corynebacterium pyogenes*, Ⅱ. The response of *F. necrophorus* to a filtrable product of *C. pyogenes*. *Brit. J. Expl. Path.*, **48**, 674—679.
35. Roberts, D.S., 1969. Synergic mechanisms in certain mixed infections. *J. Infectious Diseases*, **120**, 720—723.
36. Roberts, D.S., Graham, N.P.H., Egerton, J.R., and Parsonson, I.M., 1968. Infective bulbar necrosis (heel-abscess) of sheep, a mixed infection with *Fusiformis necrophorus* and *Corynebacterium pyogenes*. *J.Comp. Path. Ther.*, **78**, 1—8.
37. Rosebury, T., 1962. Microorganism indigenous to man, pp. 31—36, The Blakiston Division, McGraw-Hill Book Co., Inc., N.Y.
38. Rosebury, T., 1965. Bacteria indigenous to man. pp. 326—355. In Dubos, R.J., and Hirsch J.G. (ed.), Bacterial and mycotic infections of man, 4th ed., Lippincott, Philadelphia.
39. Scrivener, C.A., Myers, H.I., Moore, N.A., and Warner, B.W., 1951. Some antagonistic activity of bacteria from human oral cavity. *J. Dent. Res.*, **29**, 784—790.
40. Shindala, A., Bungay, H.R. Ⅲ, Krieg, N.R., and Culbert, K., 1965. Mixed-culture interactions, Ⅰ. Commensalism of *Proteus vulgaris* with *Saccharomyces cerevisiae* in continuous culture. *J. Bacteriol.*, 89, 693—696.
41. Stillwell, R.H., 1967. Colpidium-produced RNA as a growth stimulant for *Tetrahymena*. *J. protozool.*, **14**, 19—22.
42. Suk, C.S., Park, Y.C., and Kim, S.I., 1967. Clinico-bacteriological observation on the mixed infections. *The Korean Central Journal of Medicine*, **12**, 97—101.
43. Thibault, J., and Welsch, M., 1949. Production d'hydrogene sulfure par les corynebacteries et action negative de la penicilline sur leur sulfhydrase. *Compt. rend. soc. biol.*, **143**. 724—726.
44. Topley, W.W.C., and Fielden, H.A., 1922. The succession of dominant species in a mixed bacterial culture in a fluid medium. *Lancet*, **2**, 1164—1165.
45. Wilson, G.S., and Miles, A.A., 1964. Relapsing fever, avian spirochaetosis, and Vincent's angina, pp.2160—2169. In Topley and Wilson's Principles of Bacteriology and Immunity, 5th ed. Arnold, London.
46. Woods, D.D., 1947. Bacterial metabolism. *Ann. Rev. Biochem.*, **16**, 605—630.