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Symbiotic relationship between termite, *Reticulitermes Speratus kyushuenesis* Morimoto, and its intestinal microorganisms

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흰개미 (*Reticulitermes Speratus kyushuenesis* Morimoto)와 장내 미생물과의 공생관계

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ABSTRACT

The importance of the gut microorganisms in the termite, *Reticulitermes Speratus kyushuenesis* Morimoto, was estimated by feeding with several antibiotics. Antibiotics which killed the bacterium, but not the fungi (Ampicillin, Kanamycin, Erythromycin), or killed both the microorganisms (Cephaloridine, Tetracycline) reduced the life span of the termite from 245 days to about 15 days. On the other hand, some antibiotics, penicillin and pimaricin, did not greatly reduce the life span of the termite.

Key words : gut microorganism, antibiotic, termites, *Reticulitermes kyushuenesis*

INTRODUCTION

The gut microorganisms of termites consist of protozoa (Honigberg 1970), bacteria (Lee & Wood 1971; Breznak 1973, 1984; Kuhnigk et al. 1995; Sch fer *et al.* 1996), and fungi (Cleveland 1925, Hungate 1936, O'Brien & Slaytor 1982; Breznak & Brune 1994; Varma *et al.* 1994). The role of these microorganisms in termite nutrition is still largely a matter of conjecture.

Protozoa have been found in the hindgut of representatives of all families of termites, but the unique genera and species of oxymonad, trichomonad and hypermastrgote flagellates, capable of ingesting wood particles, are restricted to the five families of the lower termites. These protozoa appear to be indispensable for the survival of their host (Honigberg 1970). In the higher termites bacteria have been implicated in cellulose digestion, but the evidence is conflicting with some workers claiming the isolation of cellulose-degrading bacteria (Mannesmann 1972; Thayer 1976). Fungi which appear to be incidental occupants of the gut, do not seem to

be involved in cellulose degradation (Lee & Wood 1971).

Some of the gut bacteria seem to be responsible for methane formation (Breznak 1975), and also for dinitrogen fixations (Benemann 1973; Breznak *et al.* 1973; French *et al.* 1976). Nitrogen-fixing bacteria have been isolated from the gut of several species of termites (French et al. 1976; Potrikus & Breznak 1977). The role of spirochaetes, a group of bacteria which are conspicuous when the gut is viewed microscopically because of their size and shape, is completely unknown. Cleveland (1928) claimed that removing them from the gut of a termite with acid fuchsin did not affect the termite.

This study was carried out to find the effects of microorganisms on the survival of the termite, *Reticulitermes Speratus kyushuenesis* Morimoto.

MATERIALS AND METHODS

Termites

Reticulitermes Speratus kyushuenesis Morimoto was collected at the southern part of Korea, Kyungnam, and was maintained in their own nest material in plastic bags. The termites were fed filter paper, and the nest was kept humid by spraying with water.

Feeding of antibiotics and fungicide

Micronests containing 90 workers and 10 soldiers were prepared as previously described (Eutick *et al.* 1976). Filter papers sterilized to kill any fungi or other microorganisms were soaked in a solution of 20㎎ of antibiotic dissolved in, either water, or water:methanol (1:1). After air drying, sterile filter papers were placed on the surface of the micronests, which were then incubated at 30 ℃ in the dark.

Counting of gut bacteria

Workers were removed from the micronests at intervals of usually 2 days to determine the effect of the ingested antibiotic, or dye, on the gut microorganisms. The number of bacteria in the gut was determined by dissecting out the gut of workers after surface sterilization, and homogenizing them in 2.5㎖ of sterile Krebs-Ringer buffer (pH 7.4). The suspension was then serially diluted with the same buffer and spread on freshly-prepared reinforced clostridial medium (RCM) agar plates (Oxoid Ltd, London). The plates were incubated aerobically at 30℃ for 48hrs and the bacterial colonies were counted.

Sensitivity of gut bacteria to antibiotics

The sensitivity of the isolated gut bacteria of the termites to antibiotics was tested by spreading each bacterium on a RCM agar plate. Antibiotic multidisks (Oxoid Ltd, London) were placed on the plate and after incubation of the plates at 30℃ for 48hrs, they were examined for zones of inhibition around each disk.

RESULTS

A major bacterial species, *Bacillus amyloliquefaciens*, was isolated from the hindgut of *R. Speratus kyushuenesis* (Park & Bae, in preparation). The bacterium was isolated from termites not previously subjected to antibiotic treatment and was tested for its sensitivity with a range of antibiotics as described in Materials and Methods.

The effects of the different antibiotics on the gut microorganisms and on the life span of *R. Speratus kyushuenesis* are shown in Table 1. The usual practice of determining the time taken by the antibiotics to kill 50% of the termites was not in this experiment. Instead, the duration of the whole micronests to die was determined. This approach was adopted from Eutick *et al.* (1978). Control micronests, without the antibiotics, were

also maintained under the same conditions as the test micronests to determine the life span of untreated termites.

Table 1. Effect of antibiotics and pimaricin on the gut microorganisms and life span of *R. Speratus kyushuenesis*.

Antibiotic	Microorganism surviving*	Life span (days)
Control	B. F	245
Penicillin	B. F	〉200
Streptomycin	B. F	100
Ampicillin	F	20
Metronidazole	B. F	17
Kanamycin	F	24
Erythromycin	F	20
Cephaloridine	None	17
Tetracycline	None	15
Pimaricin	B	255

* B: bacterium, F: fungi

Antibiotics which killed the bacterium, but not the fungi (Ampicillin, Kanamycin, Erythromycin), or killed both the microorganisms (Cephaloridine, Tetracycline) caused the death of all termites over a period of 15~20 days. The termites in the control nest lived for 245 days. Several of the antibiotics (Penicillin, Streptomycin, Pimaricin) did not appear to affect the gut microorganisms of *R. Speratus kyushuenesis*, as shown in the result that the termites lived for 100 days to in excess of 200 days. Removal of the gut fungi by feeding fungicide pimaricin did not affect the life span of R. kyushuenesis.

The aforementioned experiments discussed only an indication of the dependence of the termite on its different gut microorganisms. Using the results in Table 1 as a guide, it was estimated the relationship between the life span of the termite and the number of its gut microorganisms. The effect of some of the antibiotics on the termite was determined by preparing a series of micronests. At regular intervals one of the nests of each series was deliberately broken up to count the surviving termites and to measure the number of bacterium surviving in the gut. A series of control nests, without antibiotics, was also examined. Over a period of 25 days, there was no decline in the number of termites in the control nests. The number of bacterium present in the gut of the control termites oscillated between about 10^5 and 2.5 x 10^5 per gut (Fig. 1).

The effect of penicillin on the termite is shown in Figure 1. The antibiotic did not cause the death of any of the termites over 15 days, but did have an effect on the gut bacterial population causing it to decline rapidly. After this bacterial population recovered quickly reaching the normal concentration by day 10. These results were in agreement with those shown in Table 1, and with the experiments using antibiotic sensitivity discs, which showed that penicillin was only weakly active against the gut bacterium of *R. Speratus kyushuenesis*.

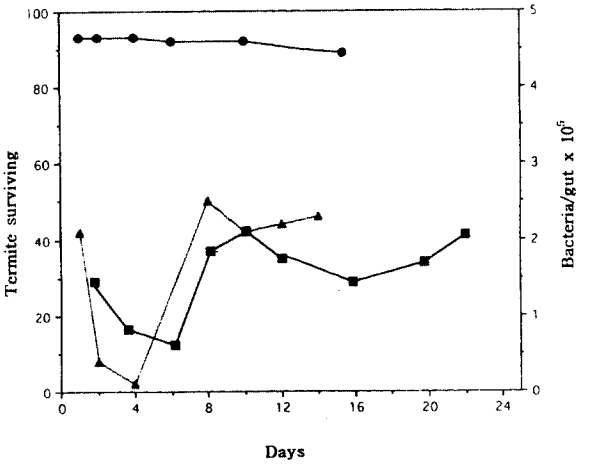

Fig. 1. Oscillation of the gut bacterial population of *R. Speratus kyushuenesis* in control nests and effect of penicillin on life span of *R. Speratus kyushuensis* and its gut bacterial population. : termites fed penicillin, : gut bacterium, *Bacillus amyloliquefaciens*, of penicillin fed termites, : gut bacterium of control termites.

The effects of ampicillin and tetracycline on the termite are shown in Figure 2. The death rate of the termite was the same in both cases with 50% mortality by day 12 and 100% mortality at day 20 and at day 15, respectively. Both antibiotics caused a rapid decline in the bacterial population in the gut within 2~4 days to about one-tenth of their normal concentration.

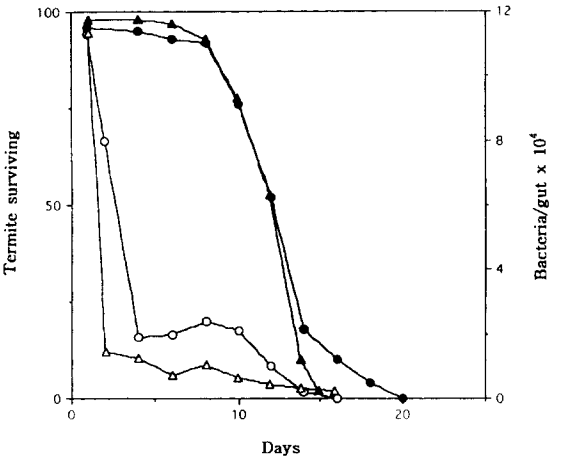

Fig. 2. Effects of ampicillin and tetracycline on life span of *R. Speratus kyushenesis* and its gut bacterial population. ● : termites fed ampicillin, ▲: termites fed tetracycline, ○: gut bacterium of ampicillin-fed termites, △: gut bacterium of tetracycline-fed termites.

Figure 3 shows the results of feeding metronidazole to the termite. The death rate was similar to that shown in Figure 2. However, the bacterial population was only slightly affected.

DISCUSSION

In any study on the effect of antibiotics on the flora of insects, it is necessary to determine, if possible, whether the effects observed can be attributed to the effect of the antibiotic on the flora, or on the insect itself. True control experiments to determine this in the case of

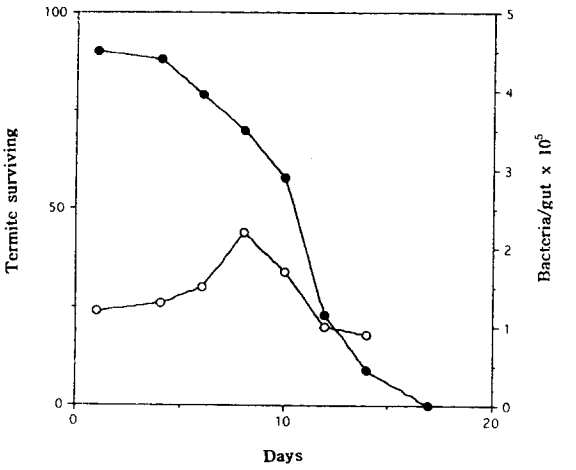

Fig. 3. Effect of metronidazole on life span of *R.. Speratus kyushenesis* and its gut bacterial population. ● : termites fed metronidazole, ○ : gut bacterium of metronidazole-fed termites.

termites are virtually impossible since termites are dependent on their gut microorganisms. The perfect control would require a germ-free termite, a state of affairs not yet achieved. However, the results of this study can be attributed to the antibiotic primarily affecting the gut microorganisms. Therefore, there was a correlation between the death of the termite and the death of one, or more, components of its gut microorganisms.

In the case of antibiotics which had no effect on the gut microorganisms of *R. Speratus kyushuensis*, the termites lived for a much longer period than those in which the microorganisms were killed. The somewhat shortened life span caused by feeding streptomycin may have been due to some deleterious effect of the antibiotic on the termite itself over the long time span of the experiments. The results obtained with penicillin and ampicillin, all of which are chemically related (Franklin & Snow 1971), also argue for the antibiotics not affecting the termite. Thus, penicillin did not affect the life span of the termite, nor did they affect the gut

microorganisms. Ampicillin, on the other hand, killed the bacterium of the termite, and also reduced its life span.

One of the major problems in this study is to separate the effects of the antibiotics on the bacterium and on the termite itself. The most direct way of showing this would be to reinfect the termite with bacterium after they had excreted all of the ingested antibiotic and observed whether its life span returned to normal.

적 요

흰개미 장내 공생 미생물의 중요성을 파악하기 위해 여러 항생제를 투여하여 실험한 결과, Ampicillin, Kanamycin, Erythromycin의 경우 장내 세균, 혹은 장내 세균과 곰팡이를 모두 살균하여 흰개미의 수명을 245일에서 15일 정도로 단축시켰다. 반면, penicillin 이나 pimaricin의 경우에는 흰개미의 수명에 큰 영향을 미치지 않았다.

검색어 : 장내미생물, 항생제, 흰개미, *Reticulitermes Speratus kyushuenesis*

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