Appl. Ent. Zool. **15** (2) : 129—134 (1980)

Properties of Intracellular Symbiotes of the Smaller Brown Planthopper *Laodelphax striatellus* FALLÉN (Hemiptera : Delphacidae)

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(Received December 20, 1979)

Sensitivity of isolated intracellular symbiotic microorganisms of *Laodelphax striatellus* FALLÉN, Ls-1 and Ls-2, to high temperature and antibiotics was studied. Ls-1 completely lost its viability when stored at 37°C, while Ls-2 was able to survive. Ls-2 was killed by the addition of 2.4 μg/ml of cycloheximide at 37°C.

INTRODUCTION

Symbiotic microorganisms have been observed within and outside the cell of various insects as reviewed by BUCHNER (1965). Roles played by intracellular symbiotes in insects have attracted the interest of many investigators (BUCHNER, 1965; KOCH, 1967). The isolation of the intracellular symbiotes has been reported in some cases, though very few of them were identified with the microorganisms living in their host cells.

In the smaller brown planthopper, *Laodelphax striatellus* FALLÉN, yeast-like symbiotes were found in the mycetocytes of eggs and fat bodies of abdomen (NASU, 1963). The symbiotes were transmitted vertically from a mother to her progeny, which suggested the establishment of a very intimate symbiotic relationship between the yeast-like microorganisms and their host. A yeast-like organism was isolated from the medium of the cultured cells of this insect by MITSUHASHI (1975), who failed to keep the isolated yeast-like organism alive. Recently, isolation methods for intracellular symbiotic microorganisms from various plant- and leaf-hoppers have been developed in our laboratories. According to the method, two very close but different yeast-like microorganisms were isolated from *L. striatellus*. These two microorganisms were identified with their insect counterpart through immunological methods (KUSUMI et al., 1979).

In this paper, the effect of elevated temperature and antibiotics upon their viabilities will be described.

MATERIALS AND METHODS

Biological materials. Two yeast-like microorganisms isolated from *L. striatellus*,

Ls-1 and Ls-2 (KUSUMI et al., 1979), were used. Both microorganisms were subcultured in 50 ml of YM broth (Difco Laboratories, Detroit, Michigan) at 25°C. The cell growth was monitored either by measuring the increase of turbidity of the medium at 660 nm or with a Klett-Summerson Photoelectric Colorimeter.

Effect of various treatment. Microorganisms were placed in a Temperature Gradient Incubator Model TN-3 (Toyo Kagaku Co. Ltd., Tokyo) under constant oscillation (24 oscillations/min.). The effect of temperature on their growth was examined by measuring the turbidity of the culture medium after 16 hr incubation.

The temperature-viability relationship was studied by incubating the microorganisms at a given temperature up to 12 days, followed by the incubation at 25°C either in fresh YM broth or YM agar plates.

Effect of antibiotics on the growth of test organisms was examined at 25°C and 37°C.

Chemicals. Cycloheximide (Nakarai Chemicals Ltd., Kyoto), amphotericin B (P. L. Biochemicals Inc., Milwaukee, Wisconsin), tetracycline (Lederle Japan Ltd., Tokyo) and chloramphenicol (Takeda Pharmaceutical Industries Ltd., Osaka) were purchased from Nakarai Chemicals Ltd. All other reagents used were of the analytical grade.

RESULTS

Effect of temperature on the growth of symbiotes of L. striatellus in vitro

The effect of temperature on the growth of two yeast-like intracellular symbiotes isolated from *L. striatellus*, Ls-1 and Ls-2, was studied at temperatures ranging from 15°C to 40°C. As shown in Fig. 1, Ls-1 could not grow either below 22°C or over 33°C, but grew well between 25°C and 31°C. Ls-2 failed to grow below 22°C and over 40°C. It grew well between 25°C and 35°C. The optimum temperature for growth of both strains was 30°C, though Ls-2 was able to grow at higher temperature than Ls-1.

The differences observed in the growth of these two symbiotes, Ls-1 and Ls-2, became more distinct when the viability of both microorganisms at 25°C was compared after storage at 37°C. As shown in Fig. 2a, if the cells of Ls-1 were kept at 37°C for 6 days or longer, a complete loss of viability was observed. In contrast, the growth of Ls-2 was merely hindered at 37°C and immediately returned to normal at 25°C even after storage at 37°C for 9 days (Fig. 2b).

The above results may indicate that the treatment at 37°C exerts a lethal effect on Ls-1 but only a static effect on Ls-2.

Effect of antibiotics on Ls-1 and Ls-2

Treatment of Ls-1 with antiobiotics was carried out at 25°C for 7 days. As shown in Table 1, amphotericin B and cycloheximide inhibited the growth after application of 30 μg and 2.5 mg per m*l*, respectively. The loss of viability of the test organisms occurred only in the presence of cycloheximide. When both antibiotics were added to the cell suspension, the concentration of the drug needed to kill the microorganisms was reduced to one third of the amount needed to inhibit the growth when added separately. Chloramphenicol and tetracycline had no effect on the growth of Ls-1.

In contrast, Ls-2 was more sensitive to cycloheximide and to the combined ad-

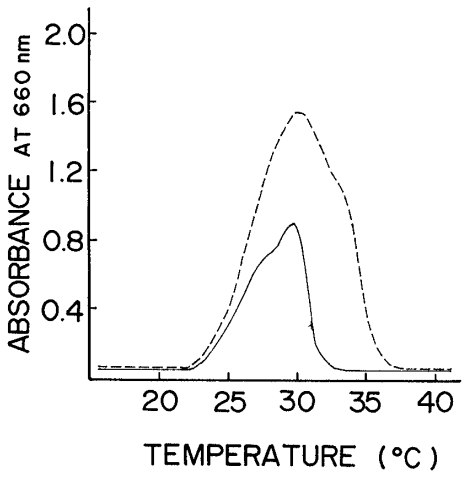

Fig. 1. Effect of temperature on the growth of isolated intracellular symbiotes of *L. striatellus*, Ls-1 and Ls-2. Growth was monitored by measuring the increase in turbidity of the medium at 660 nm after incubation at a given temperature for 16 hr. ——, Ls-1: ----, Ls-2.

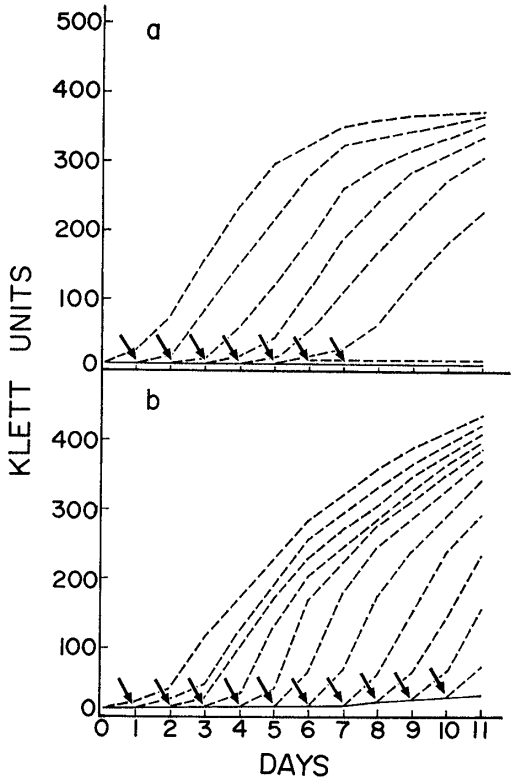

Fig. 2. Effect of treatment at 37 °C upon viability of Ls-1 and Ls-2. a) Ls-1 (7.0×10^3 cells/m*l*) was incubated at 37 °C up to 8 days. Cells were transferred to 25°C at the time indicated by arrows. b) Ls-2 (7.0×10^3 cells/m*l*) was incubated at 37°C up to 8 days. Viability was examined at 25°C as in the case of Ls-1.

Table 1. Minimum Concentration of Antibiotics Required for the Complete Inhibition of Isolated Symbiotic Microorganisms

Antibiotics	Ls-1[a] 25°C	Ls-2 25°C	Ls-2 37°C
Amphotericin B	30 μg	no effect	30 μg
Cycloheximide	2.5mg	30 μg	2.4 μg
Amphotericin B +Cycloheximide	9 μg+0.8mg	30 μg+3 μg	40 ng+0.8 μg

[a] Ls-1 (7×10^3 cells/m*l*) and Ls-2 (7×10^3 cells/m*l*) were treated with antibiotics either at 25°C or 37°C for 7 days.

dition of cycloheximide and amphotericin B. Results are summarized in Table 1. At 25°C, Ls-2 could not grow in the presence of 30 μg/m*l* of cycloheximide. Although amphotericin B itself had no effect on the growth of Ls-2, in the presence of 30 μg/m*l* of amphotericin B, the concentration of cycloheximide needed to kill the test organisms could be reduced to 2.4 μg/m*l*.

The effect of antibiotics on cell death became more distinct at 37°C as only 2.4 μg/m*l* of cycloheximide were able to kill Ls-2. The presence of 40 ng/m*l* of amphotericin B could reduce the amount of cycloheximide to 0.8 μg/m*l*. Chloramphenicol and tetracycline had no effect on the growth of Ls-2.

By comparing the sensitivity to antibiotics between Ls-1 and Ls-2, it was found

that Ls-2 was readily killed by the addition of cycloheximide.

DISCUSSION

As described in our preceding paper (KUSUMI et al., 1979), two yeast-like symbiotes were isolated in our laboratory from the smaller brown planthopper, *L. striatellus*, and were identified with the microorganisms living in the mycetocytes of the insect using immunological techniques.

The major purpose of studying intracellular symbiotes of insects is to elucidate the role played by the symbiotes in the host insects. Therefore, knowledge of the sensitivity of the isolated symbiotes to temperature and drugs was found to be essential for preparing symbiote-free insects in the least harmful way.

Of the two symbiotes, Ls-1 was killed completely following storage at 37°C for 7 days, while Ls-2 could survive and merely experienced arrested growth. On the other hand, Ls-2 was very sensitive to cycloheximide and 30 μg/m*l* of cycloheximide were able to kill it without exerting any deleterious effect on Ls-1.

These observations may suggest that the rearing of *L. striatellus* at 37°C on artificial diet containing cycloheximide make it possible to free the insect from the symbiotes.

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