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Symbiotes of Planthoppers : II. Isolation of Intracellular Symbiotic Microorganisms from the Brown Planthopper, *Nilaparvata lugens* STÅL, and Immunological Comparison of the Symbiotes Associated with Rice Planthoppers (Hemiptera : Delphacidae)

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Two morphologically different yeast-like symbiotes were isolated from eggs of the brown planthopper, *Nilaparvata lugens* STÅL. They were identified immunologically with the intracellular symbiotes found in eggs and in the adult insects. The two isolated microorganisms from the egg have common antigenicity with those isolated from the smaller brown planthopper, *Laodelphax striatellus* FALLÉN, and from the white backed planthopper, *Sogatella furcifera* HORVÀTH.

INTRODUCTION

The existence of a symbiotic association between host insects and microorganisms has been reported in various insects (BUCHNER, 1965). The effect exerted by the symbiotic microorganisms on their host insects has not been clarified due to the difficulty in isolating them, especially those living within cells.

NASU (1963) demonstrated that intracellular yeast-like symbiotes are distributed among rice planthoppers. The isolation of a yeast-like symbiote from the culture fluid of cells derived from the smaller brown planthopper was achieved by MITSUHASHI (1975), who failed, however, to identify the symbiotes with those present in the host insect. Two yeast-like intracellular symbiotes were isolated from the smaller brown planthopper, *L. striatellus*, in our laboratory (KUSUMI et al., 1979). The method of isolation can be applied to several other insects which carry intracellular microorganisms such as the brown planthopper, the white backed planthopper and the green rice leafhopper, etc.

This reports describes the isolation of the intracellular yeast-like symbiotes from the mycetocytes of eggs and fat bodies of the abdomen of the brown planthopper, *N. lugens* and the immunological identification of the isolated microorganisms with symbiotes present in the eggs. Immunological comparison of the intracellular symbiotes of three species of rice planthoppers, *N. lugens*, *L. striatellus* and *S. furcifera*, will also

be described.

MATERIALS AND METHODS

Biological Materials. The brown planthopper, *N. lugens*, was used for the isolation of intracellular symbiotic microorganisms. The smaller brown planthopper, *L. striatellus*, and the white backed planthopper, *S. furcifera*, were used for immunological comparison of the symbiotes. These three planthoppers were reared on rice seedlings at 25°C.

Isolation of yeast-like microorganisms from *N. lugens* was performed on agar plate containing GRACE TC medium (1962), supplemented with penicillin and streptomycin, but free immobilized hemolymph according to the method of KUSUMI et al. (1979). The isolated microorganisms were maintained in Bacto YM broth (Difco Laboratories, Detroit, Michigan).

Antisera. Antisera against the isolated microorganisms were prepared by immunization in guinea pigs. Immunogloblin G was partially purified by precipitation with ammonium sulfate as described by KUSUMI et al. (1979). FITC-conjugated rabbit antiserum against guinea pig IgG (Miles laboratories Inc., Kankakee, Illinois) was used for the indirect fluorescent antibody staining of symbiotic microorganisms.

Indirect Fluorescent Antibody Staining of Symbiotes. Indirect fluorescent antibody staining was carried out by the modified method of WELLER et al. (1954) under conditions described by KUSUMI et al. (1979).

RESULTS

Intracellular Symbiotic Microorganisms of Brown Planthopper

In the brown planthopper, *N. lugens*, two morphologically different yeast-like symbiotes were identified by microscopic examination of mycetocytes of both egg and fat bodies from the abdomen of nymph and adult insect. Namely, oval-shaped cells (8–10 μm in length) and sheath-like cells (15–20 μm) were found in both egg and fat body (Fig. 1A and 1B). The latter form was the predominant species in the insects. These two yeat-like microorganisms were isolated from sterilized eggs of brown planthopper according to the method described by KUSUMI et al. (1979). They were designated as Nl-1 (Fig. 2A), and Nl-2 (Fig. 2B) which correspond to the sheath-like cells and oval-shaped cells (Fig. 1A and 1B), respectively.

Immunological Identification of Symbiotes

To determine whether the two microorganisms were derived from symbiotes of *N. lugens*, identification of the intracellular microorganisms was achieved by the indirect fluorescent staining method. The results are summarized in Table 1. Guinea pig antibodies against Nl-1 or Nl-2 were specific for the microorganisms used for immunization, though there was some indication that they might have certain antigen(s) in common. As shown in Fig. 3 and Fig. 4, the mycetocytes treated with the specific antibodies against Nl-1 and Nl-2 (Fig. 3A and Fig. 4A) and the indirect staining of the smear with FITC-cojugated rabbit anti-guinea pig IgG antibodies showed the existence of the stained symbiotes (Fig. 3B and Fig. 4B). However, when the anti-guinea pig Nl-1 antibodies adsorbed by Nl-2 were applied to the smear,

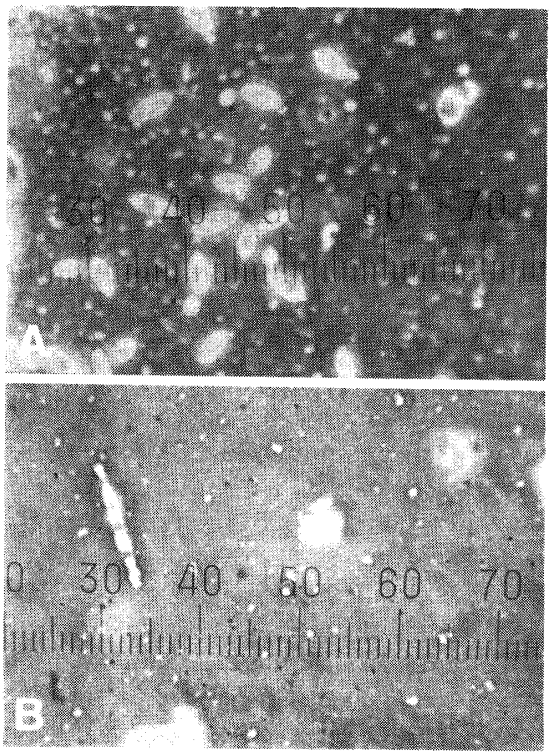

Fig. 1. Yeast-like symbiotes in the mycetocytes of *N. lugens*. Oval-shaped cells(A) and sheath-like cells(B), light-microscopy, ×400.

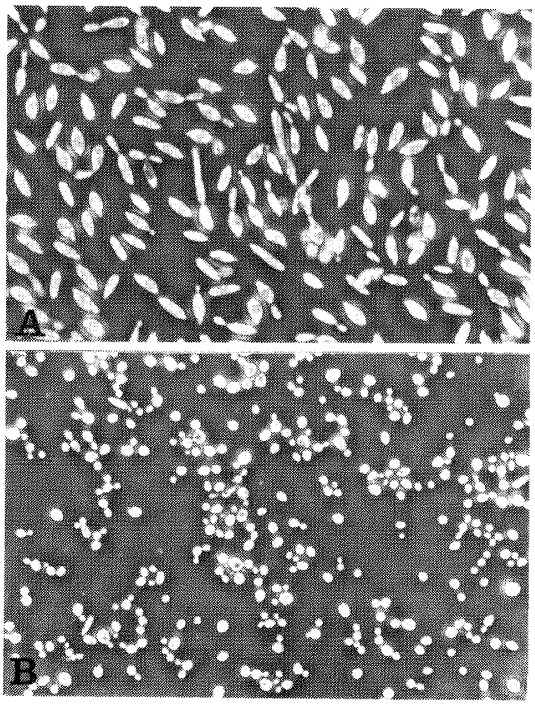

Fig. 2. Yeast-like symbiotes from *N. lugens* were cultured on YM-agar medium. Nl-1 cells(A) and Nl-2 cells(B), light-microscopy, ×400.

Table 1. Specificity of Antibodies against N1-1 and N1-2 Cells as Revealed by Indirect Immunofluorescent Method

Antibody	Antigen	Reaction
anti N1-1	N1-1	+
anti N1-1	N1-2	±
anti N1-1	symbiote	+
anti N1-1 adsorbed with N1-1	symbiote	−
anti N1-1 adsorbed with N1-2	symbiote	±
anti N1-2	N1-1	±
anti N1-2	N1-2	+
anti N1-2	symbiote	+
anti N1-2 adsorbed with N1-1	symbiote	+
anti N1-2 adsorbed with N1-2	symbiote	−

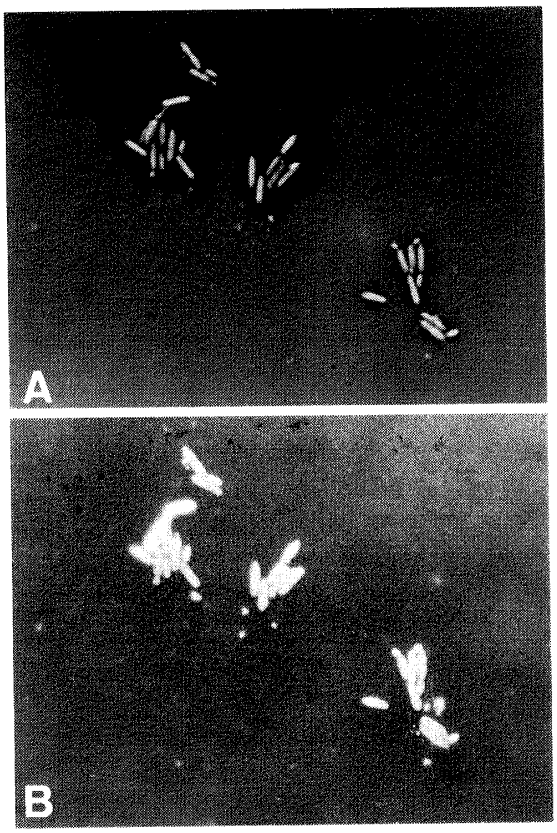

Fig. 3. Stained symbiotes in mycetocytes of *N. lugens*. After staining by the indirect immunofluorescent method using specific anti Nl-1 IgG, microphotographs were taken under ordinary(A) and UV-light(B).

the brilliancy of the staining markedly decreased. Along with the data shown in Fig. 1 and Table 1, the results suggested that the two microorganisms, isolated from *N. lugens*, originated from the two yeast-like intracellular organisms and shared certain common surface antigens.

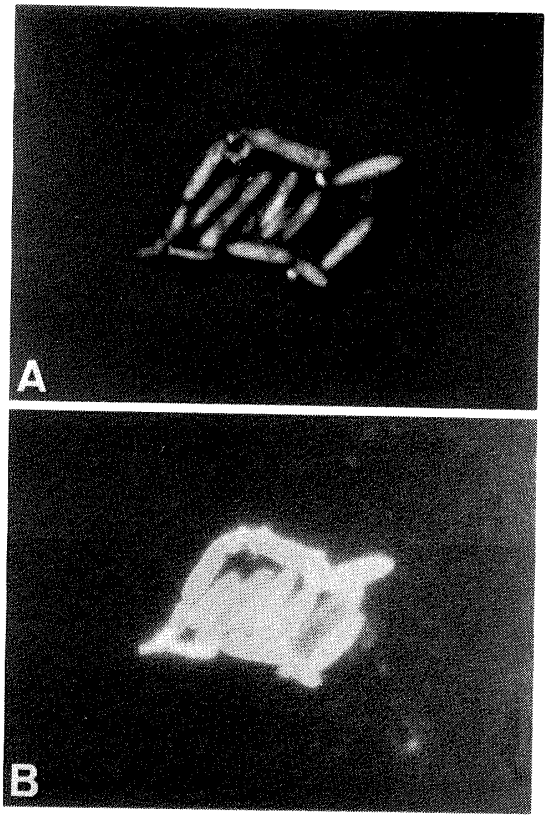

Fig. 4. Stained symbiotes in mycetocytes of *N. lugens*. After staining by the indirect immunofluorescent method using specific anti Nl-2 IgG, microphotographs were taken under ordinary(A) and UV-light(B).

Immunological Identification of Yeast-like Symbiotes Found in Rice Planthoppers

Various rice planthoppers harbour yeast-like symbiotes in the mycetocytes of their embryonic cells and in fat bodies of the abdomen of both nymph and adult insects. The four antibodies prepared against the symbiotes isolated from the brown planthopper, *N. lugens*, and the smaller brown planthopper, *L. striatellus*, were applied to smears of mycetocytes of the brown planthopper, smaller brown planthopper and the white backed planthopper, *S. furcifera*, followed by staining with FITC-conjugated anti-guinea pig IgG antibodies. As summarized in Table 2, the intracellular symbiotes of these insects are divided into two groups according to the number of stained cells in smeared mycetocytes : Those reacting to anti-Nl-1 and Ls-2, and those stain-

Table 2. IMMUNOLOGICAL COMPARISON OF INTRACELLULAR SYMBIOTES OF THREE SPECIES OF RICE PLANTHOPPERS

Tested symbiotes in planthoppers	Antibody			
	anti-Ls-1	anti-Ls-2	anti-Nl-1	anti-Nl-2
smaller brown planthopper	+	++	++	+
brown planthopper	++	++	++	++
white backed planthopper	+	++	++	+

++ : Majority of cells were stained, + : some cells were found to be stained.

ed by Nl-2 and Ls-1. Shapes and sizes of these yeast-like microorganisms vary according to the culture conditions employed. These results suggest that the two serologically identical yeast-like intracellular symbiotes are harboured in cells of the rice planthoppers.

DISCUSSION

Among the rice planthoppers, the brown planthopper, *N. lugens*, carries two yeast-like intracellular symbiotes, which can be distinguished morphologically by microscopic examination of mycetocytes of eggs and fat bodies of the abdomen. The two microorganisms, Nl-1 and Nl-2, which have been isolated from eggs, share the identical surface antigens. The two yeast-like intracellular symbiotes, Ls-1 and Ls-2, have already been isolated previously from *L. striatellus* (KUSUMI et al., 1979). Immunological specificity of the intracellular symbiotes of rice planthoppers has been studied using four antisera prepared against two symbiotes isolated from *N. lugens* and two microorganisms from *L. striatellus*. The intracellular symbiotic microorganisms associated with the three species of rice planthoppers are classified in either one of two types : one which is stained by the antibodies against Nl-1 and Ls-2, and the other which is stained by the antisera against Nl-2 and Ls-1. These findings raise the question whether or not all species of rice planthoppers carry the two immunologically distinct microorganisms in common. To clarify this problem, immunological comparison of the symbiotes in various species of rice planthoppers has been undertaken and the results will be published in the near future.

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