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Isolation and identification of culturable gut microbiota in the larval stage of lesser mealworm (*Alphitobius diaperinus*)

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INTRODUCTION

The highly prevalent pest *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) causes significant structural damage in poultry farms. Despite previous investigations on its carriage of pathogenic microorganisms, our understanding of its microbiome remains limited. This study aimed to analyze the diversity of culturable gut microbiota in *A. diaperinus* obtained from laboratory breeding.

MATERIALS AND METHODS

Fifteen seventh instar larvae underwent a 24-hour starvation period, followed by surface disinfection. Dissected midguts were homogenized and plated on nutrient agar (NA), brain heart infusion agar (BHI), and Bacillus cereus agar (BC). The cultured isolates were subjected to gram staining, phylogenetic analysis, biochemical property evaluation, and metabolic activity assessment.

RESULTS AND DISCUSSION

-Higher bacterial counts in BHI ($2.51x10^5$ CFU/gut) compared to NA ($2.25x10^5$ CFU/gut), possibly due to nutrient richness.

-NA exhibited a dominant colony morphology of gram-negative bacilli, while BHI displayed additional distinct colonies of gram-positive cocci. Surprisingly, yeast-like colonies were observed on BC plates (Figure 1).

-Based on 16S rRNA gene sequences, eight bacterial isolates were identified as *Enterobacter* sp., and two as *Staphylococcus* sp. Using RNA gene ITS region sequences, two yeast isolates were identified as *Debaryomyces* sp. and *Hyphopichia* sp.

-The resulting 16S rRNA gene sequences have been deposited in the GenBank database (Table 1). Based on rRNA gene ITS region sequencing, we classified the two isolated yeasts. These sequences have also been deposited in the GenBank database (Table 1).

-A preliminary species-level identification of bacteria (*Enterobacter cloacae*, *Staphylococcus gallinarum*, and *Staphylococcus succinus*) was achieved using API systems and complementary biochemical tests.

-Discrepancies between phylogenetic analysis (Figures 2 and 3) and phenotypic data suggest the potential existence of new species or subspecies. Further comprehensive studies are required to confirm this hypothesis.

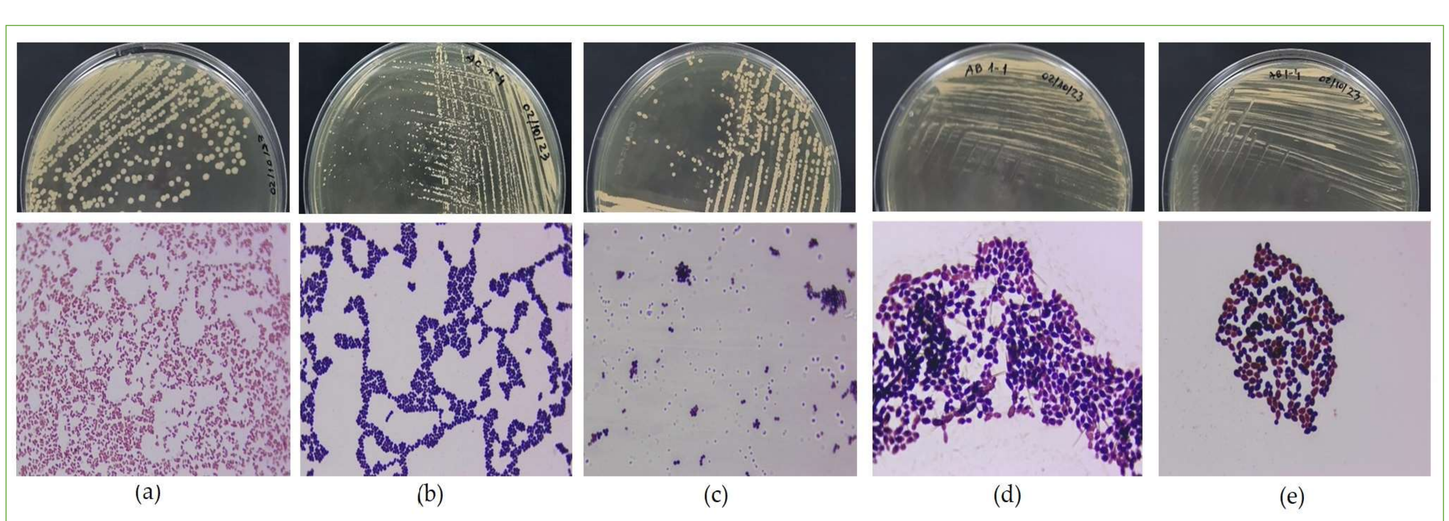

Figure 1. Pure cultures of the isolates were grown in NA medium and incubated for 24 hours at 29°C. Gram staining was performed and observed under a 1000x magnification immersion lens, revealing the following isolates: INTA AN1-1 (a), INTA AC1-4 (b), INTA AC1-8 (c), INTA AB1-1 (d), and INTA AB1-4 (e). It's important to note that only one representative gram-negative isolate is included in the figure for clarity.

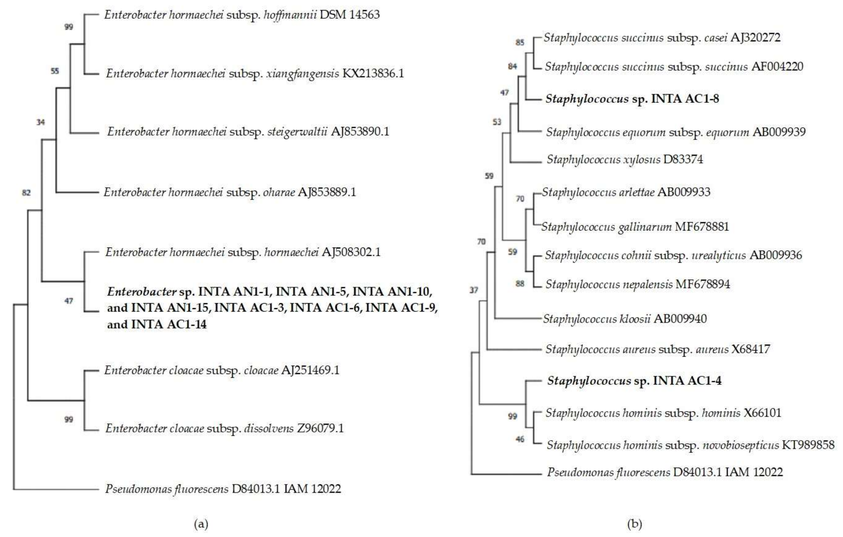

Figure 2. The phylogenetic tree of 16S rRNA sequences illustrates the relationships among the gram-negative isolates and type strains of *Enterobacter* species (a), as well as among the two gram-positive isolates and type strains of *Staphylococcus* species (b). The tree was constructed using the neighbor-joining method. The numbers displayed at specific nodes indicate consensus bootstrap values based on 1,000 replications.

Table 1. Nucleotide lengths and GenBank accession numbers of sequences obtained from selected gut isolates of *Alphitobius diaperinus*.

Isolate	*Sequenced gene/genes*	*Nucleotide length (bp)*	*GenBank accession number*
INTA AN 1-1	*16S rRNA*	*1401*	*OP339834.1*
INTA AN 1-5	*16S rRNA*	*1369*	*OP346784.1*
INTA AN 1-10	*16S rRNA*	*1396*	*OP346981.1*
INTA AN 1-15	*16S rRNA*	*1397*	*OP347118.1*
INTA AC 1-3	*16S rRNA*	*1395*	*OP348220.1*
INTA AC 1-6	*16S rRNA*	*1396*	*OP348874.1*
INTA AC 1-9	*16S rRNA*	*1399*	*OP348886.1*
INTA AC 1-14	*16S rRNA*	*1369*	*OP351273.1*
INTA AC 1-4	*16S rRNA*	*1417*	*OP348929.1*
INTA AC 1-8	*16S rRNA*	*967*	*OP348932.1*
INTA AB 1-1	*rRNA genes ITS region*	*446*	*OP348991.1*
INTA AB 1-4	*rRNA genes ITS region*	*612*	*OP348992.1*

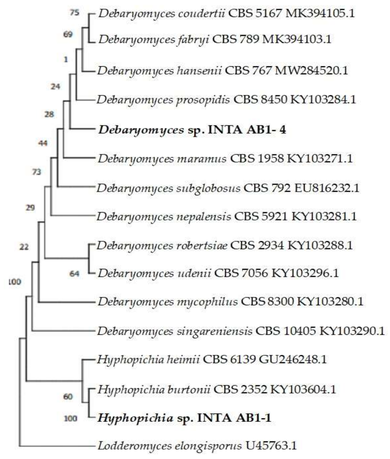

Figure 3. The phylogenetic tree of rRNA gene ITS region sequences illustrates the relationships among the yeast isolates and the type strains of *Debaryomyces* and *Hyphopichia* species. The tree was constructed using the neighbor-joining method, and the numbers shown at specific nodes represent consensus bootstrap values derived from 1,000 replications.