Preliminary Evidence of Antibiotic-Producing Gram-Negative Bacteria from Hemolymph of Cerambycidae Beetles

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Title

Preliminary Evidence of Antibiotic-Producing Gram-Negative Bacteria from Hemolymph of Cerambycidae Beetles

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Abstract

Fifty-two Gram-negative strains were isolated from Passalid (approximately 85 % of the samples) and Cerambycidae beetles in Amazonian forests near Itacoatiara, Brazil. Samples included hemolymph, larval surface washes, adult abdominal washes, gallery swabs, and live adult intestinal contents. Screening against *Bacillus cereus* ATCC 11778 and *Escherichia coli* ATCC 25922 identified three most active isolates—4 (H 11.1.3), 43 (LV 4.1.1), and 50 (H 11.1.1). Isolates 4 and 50 originated from Cerambycidae larval hemolymph; isolate 43 from a Cerambycidae larval surface wash. All three produced inhibition zones > 15 mm against both testers. Crude 48 h LB fermentations of these isolates also inhibited *E. coli*, *B. cereus*, and *Staphylococcus aureus* (halos 14–18 mm). Colony morphology: isolate 4 formed translucent light-caramel, creamy, mucoid colonies; isolate 43 formed translucent caramel-orange colonies; isolate 50 formed translucent orange-caramel colonies. Although further work was curtailed by funding constraints, these findings suggest Cerambycidae larval niches (hemolymph and surface) harbor antibiotic-producing Gram-negative symbionts.

Keywords:

Cerambycidae · insect hemolymph · Gram-negative bacteria · antibiotic discovery · Amazonian beetles

Introduction

Insect hemolymph is typically sterile owing to potent innate defenses (hemocytes, antimicrobial peptides) [1]. Nevertheless, some insects maintain defensive symbioses with antibiotic-producing bacteria. For example, rove beetles (*Paederus*

spp.) harbor *Pseudomonas* in their hemolymph that produces pederin [2]; *Lagria villosa* eggs are protected by *Burkholderia gladioli* secreting lagriamide [3]; and entomopathogenic nematodes rely on *Xenorhabdus* and *Photorhabdus* to kill hosts and suppress competitors [4, 5]. Cerambycidae (longhorn) larvae develop in microbe-rich wood but remain unstudied for hemocoelic symbionts. Surveys of *Oberea linearis* larvae isolated Gram-negative taxa (e.g., *Serratia*) considered pathogenic [6]. To our knowledge, no antibiotic-producing Gram-negative bacteria have been reported from Cerambycidae larval hemolymph or associated washes. We thus screened Gram-negative isolates from Amazonian Cerambycidae larval samples for antimicrobial activity.

Materials and Methods

Sample Collection

Cerambycidae larvae (n = 4) and adults (Cerambycidae and Passalidae) were collected from decaying logs near Itacoatiara, Amazonas, Brazil. Larvae were surface-sterilized (70 % ethanol, 5 min; 2.5 % sodium hypochlorite, 5 min; sterile water, 10 min). Hemolymph was drawn via a small abdominal incision. Larval surface washes (pre-disinfection rinse), adult abdominal washes, gallery swabs (vortexed in 1 mL 0.9 % saline), and live adult intestinal contents (aseptic dissection, vortexed in saline) were also collected. All specimens were transported to the UFAM Itacoatiara Mycology Laboratory at ambient temperature and processed within 24 h.

Isolation of Gram-Negative Bacteria

Collected fluids (hemolymph, larval washes, adult washes, gallery swabs, intestinal contents) were serially diluted (≥ 10^{-3}) and plated on MacConkey agar, then incubated at 24–26 °C for up to 72 h. Distinct colonies were purified by repeated streaking. In total, 52 nonidentical Gram-negative isolates were obtained: ~ 85 % from Passalidae sources; the remainder from Cerambycidae larval hemolymph or washes.

Primary Antimicrobial Screening

Colony Plug Assay (First-Test Activity):

- After purification on MacConkey agar (48 h incubation at 24–26 °C), 5 mm plugs were aseptically cut from actively growing colonies of each isolate.
- A sterile plug cut from an uninoculated MacConkey plate served as a negative control.

- Mueller–Hinton agar plates were prepared and seeded with either *B. cereus* ATCC 11778 or *E. coli* ATCC 25922 (swabbed to achieve a confluent lawn at ~ 10^8 CFU/mL).
- Each 5 mm colony plug was inverted (agar side down) and gently pressed onto the surface of the preinoculated Mueller–Hinton agar. Controls included: (i) negative control plug (uninoculated MacConkey), and (ii) a 30 µg chloramphenicol disk as positive control.
- Plates were incubated at 24–26 °C for 24 h. Zones of inhibition around each plug were measured (mm). Isolates yielding zones > 10 mm against either *B. cereus* or *E. coli* were advanced to follow-up characterization.

Secondary Fermentation and Crude Extract Assays

- Each active isolate (4, 43, 50) was cultured in 10 mL LB (loop inoculum, 24 h static, 24–26 °C), then transferred to 100 mL LB (inoculated with pre-inoculum, 48 h agitation at 100 rpm, 24–26 °C).
- Cultures were centrifuged (6 000×g, 10 min), and cell-free supernatants collected.
- Ten µL of each crude supernatant was spotted onto Mueller–Hinton agar plates seeded with either *B. cereus* ATCC 11778, *E. coli* ATCC 25922, or *S. aureus* (laboratory strain).
- Following incubation (24 h at 24–26 °C), inhibition halos (14–18 mm) were recorded.

Morphological Characterization

Gram stain and 1000× light microscopy assessed cell morphology. Colony appearance on MacConkey after 48 h at 24–26 °C:

- **Isolate 4 (H 11.1.3):** Diplobacilli; colonies translucent light-caramel, creamy, mucoid, ~ 2 mm diameter.
- **Isolate 43 (LV 4.1.1):** Bacilli; colonies translucent caramel-orange, ~ 2 mm diameter.
- **Isolate 50 (H 11.1.1):** Short diplobacilli; colonies translucent orange-caramel, ~ 1.5 mm diameter.

No molecular (16S rRNA) identification was performed due to resource constraints.

Results

Origin of Active Isolates: Two most active isolates (4, 50) derived from Cerambycidae larval hemolymph; isolate 43 derived from a Cerambycidae larval surface wash. Passalidae-derived isolates showed weak or no activity (< 10 mm).

Primary Screening (Colony Plug Assay):

- Isolates 4, 43, 50 produced inhibition zones > 15 mm against both *B. cereus* and *E. coli*.
- No other isolate yielded reproducible halos > 10 mm.

Secondary Fermentation and Crude Extract Activity:

- Crude 48 h LB supernatants of isolates 4, 43, 50 inhibited *E. coli*, *B. cereus*, and *S. aureus*, with halos of 14–18 mm.

Morphology:

- **Isolate 4:** Diplobacilli; translucent light-caramel, creamy, mucoid colonies (~ 2 mm).
- **Isolate 43:** Bacilli; translucent caramel-orange colonies (~ 2 mm).
- **Isolate 50:** Short diplobacilli; translucent orange-caramel colonies (~ 1.5 mm).

Discussion

Cerambycidae larval niches—hemolymph and surface washes—harbor Gram-negative bacteria capable of producing extracellular antibiotics. Isolates 4 and 50 (hemolymph) and 43 (surface wash) inhibited both Gram-positive (*B. cereus*, *S. aureus*) and Gram-negative (*E. coli*) bacteria. Colony and cell morphologies suggest genera such as *Pseudomonas*, *Serratia*, or *Enterobacter*, though taxonomic assignment requires 16S rRNA sequencing [5, 6].

As far as we know, no previous study reported antibiotic-producing Gram-negative symbionts from Cerambycidae larvae. Scieuzo *et al.* (2023) identified antimicrobial peptides from *Hermetia illucens* hemolymph but not Gram-negative isolates from Cerambycidae [4]. Technical–financial constraints prevented molecular identification and compound purification here. Nonetheless, these preliminary results justify follow-up: (1) 16S rRNA sequencing to resolve taxonomy; (2) solvent extraction and chromatographic fractionation of active compounds; (3) minimum inhibitory concentration (MIC) determinations; (4) screening against multidrug-resistant clinical isolates [5, 6].

Conclusion

This short communication reveals that Cerambycidae larval hemolymph and surface washes contain antibiotic-producing Gram-negative bacteria. The three most active isolates (4, 43, 50) consistently inhibited *B. cereus*, *E. coli*, and *S. aureus*. Despite

resource limitations, these findings justify comprehensive taxonomic and chemical investigations to evaluate their novelty and therapeutic potential.

Acknowledgments

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Conflict of Interest

The author declares no conflict of interest.

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