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Antibiotic susceptibility and functional enzyme profiling of gut bacteria isolated from fall armyworm (*Spodoptera frugiperda*) feeding on maize

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Abstract

The fall armyworm (*Spodoptera frugiperda*) is a highly destructive invasive pest of maize with remarkable adaptability to diverse agroecosystems. Increasing evidence suggests that gut-associated bacteria play a crucial role in insect nutrition, digestion, and tolerance to environmental stressors, including antimicrobial compounds. The present study aimed to isolate cultivable gut bacteria from fall armyworm larvae feeding on maize and to characterize them based on antibiotic susceptibility patterns and functional enzymatic activities. Seventeen bacterial isolates were recovered from larval gut homogenates and subjected to antibiotic susceptibility testing against erythromycin, colistin, polymyxin, nadifloxacin, methicillin, and rifampicin using the disc diffusion method. Functional characterization included screening for protease and urease activities. The isolates showed high susceptibility to nadifloxacin (88.2%), followed by colistin and polymyxin (64.7%), whereas resistance was frequently observed against methicillin and erythromycin. One isolate exhibited resistance to all tested antibiotics. Protease activity was detected in six isolates, with the highest activity recorded in FWG-14 (22.66), while urease activity was observed in six isolates (35.3%). The coexistence of antibiotic resistance and functional enzyme production highlights the ecological and physiological significance of gut bacteria in fall armyworm nutrition and adaptability. These findings provide insights into insect-microbe interactions and may contribute to the development of microbiome-based pest management strategies.

Keywords: Fall armyworm, gut microbiota, antibiotic susceptibility, protease, urease, maize

1. Introduction

The fall armyworm (*Spodoptera frugiperda* J.E. Smith) is a polyphagous lepidopteran pest that has emerged as a major threat to maize production worldwide. Native to the Americas, the pest has rapidly invaded Africa and Asia, causing severe economic losses due to its high reproductive capacity, migratory behavior, and ability to develop resistance to insecticides (Goergen *et al.*, 2016; Day *et al.*, 2017) [7, 4]. Maize is among its preferred hosts, and heavy infestations can lead to substantial yield reductions.

Insects harbor complex microbial communities in their gastrointestinal tract, which play essential roles in host physiology, including digestion, nutrient assimilation, immune modulation, and detoxification of xenobiotics (Moran *et al.*, 2019; Douglas, 2015) [10, 6]. In herbivorous insects, gut bacteria are particularly important for the breakdown of plant-derived macromolecules and the mitigation of plant defensive compounds (Dillon and Dillon, 2004; Visóto *et al.*, 2009) [5, 12].

Antibiotic resistance among environmental and insect-associated bacteria has become an increasing concern. Agricultural ecosystems are subjected to continuous selective pressure due to the widespread use of antibiotics, pesticides, and other agrochemicals, which can promote the emergence and persistence of resistant microbial populations (Martínez, 2009; Allen *et al.*, 2010) [9]. Insect gut bacteria may act as reservoirs of antibiotic resistance genes, potentially enhancing microbial survival and host adaptability (Xia *et al.*, 2018) [13].

In addition to resistance traits, functional enzymes produced by gut bacteria are critical for insect nutrition. Proteases play a key role in the hydrolysis of dietary proteins into absorbable amino acids, while urease facilitates nitrogen recycling by converting urea into ammonia, an important process in nitrogen-limited herbivorous diets (Zhang *et al.*, 2022; Salvadori *et al.*,

2012)^[8, 11]. Despite their importance, limited information is available on the combined antibiotic susceptibility and functional enzyme profiling of gut bacteria associated with fall armyworm feeding on maize.

Therefore, the present study aimed to isolate cultivable gut bacteria from *S. frugiperda* larvae and to characterize them based on antibiotic susceptibility patterns and protease and urease activities.

2. Materials and Methods

2.1 Collection of Fall Armyworm Larvae

Larvae of *Spodoptera frugiperda* were collected from maize fields exhibiting characteristic fall armyworm damage. The larvae were transported to the laboratory in sterile containers and processed within 24 hours of collection.

2.2 Surface Sterilization and Gut Dissection

Larvae were surface sterilized using 70% ethanol for 1-2 minutes and rinsed three times with sterile distilled water to remove external contaminants (Li *et al.*, 2022)^[8]. Under aseptic conditions, the gut was dissected and transferred into sterile phosphate-buffered saline.

2.3 Isolation and Purification of Gut Bacteria

The dissected guts were homogenized in sterile phosphate-buffered saline and serially diluted. Appropriate dilutions were spread-plated on nutrient agar and incubated at 37 °C for 24-48 hours. Morphologically distinct colonies were purified through repeated streaking and designated as FWG-1 to FWG-17.

2.4 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Nutrient agar (Bauer *et al.*, 1966)^[2]. The antibiotics tested included erythromycin, colistin, polymyxin, nadifloxacin, methicillin, and rifampicin. Plates were incubated at 37 °C for 24 hours, and isolates were categorized as sensitive or resistant based on zone of inhibition.

Calculation of Percentage Susceptibility

The percentage susceptibility for each antibiotic was calculated using the formula:

$$\text{Percentage susceptibility} = \left(\frac{\text{Number of susceptible isolates}}{\text{Total number of isolates}} \right) \times 100$$

2.5 Protease Activity Assay

Protease activity was assessed using skim milk agar medium. Isolates were spot-inoculated and incubated at 37 °C for 24-48 hours. Clear zones around colonies indicated protease production, and enzyme activity was quantified based on the diameter of the hydrolysis zone (Zhang *et al.*, 2022)^[8].

2.6 Urease Activity Assay

Urease activity was determined using Christensen's urea agar medium. A color change from yellow to pink after incubation indicated a positive urease reaction (Christensen, 1946)^[3].

2.7 Data Analysis

Antibiotic susceptibility results were expressed as percentages, while protease activity values were recorded quantitatively. Urease activity was recorded as positive or negative. Data were analyzed descriptively.

3. Results

3.1 Antibiotic Susceptibility of Gut Bacterial Isolates

The antibiotic susceptibility profiles of the seventeen gut bacterial isolates are summarized in Table 1. Nadifloxacin was the most effective antibiotic, with 88.2% of isolates showing sensitivity. Colistin and polymyxin exhibited moderate effectiveness, with 64.7% susceptibility. In contrast, methicillin showed the lowest effectiveness (29.4%), followed by erythromycin (47.1%). Rifampicin displayed moderate activity, with 41.2% of isolates being sensitive. One isolate (FWG-12) demonstrated resistance to all tested antibiotics, indicating a multidrug-resistant phenotype.

3.2 Protease and Urease Activities

Protease activity was detected in six of the seventeen isolates, indicating functional diversity within the gut microbiota. The highest protease activity was observed in FWG-14 (22.66), followed by FWG-6 (18.66) and FWG-1 (13.66). Urease activity was recorded in six isolates (35.3%), distributed among both protease-positive and protease-negative groups, suggesting independence between these enzymatic traits.

4. Discussion

The present study demonstrates that the gut of fall armyworm larvae feeding on maize harbors cultivable bacteria with diverse antibiotic susceptibility patterns and functional enzyme activities. High susceptibility to nadifloxacin suggests its broad-spectrum activity against gut-associated bacteria, whereas frequent resistance to methicillin and erythromycin may reflect prolonged exposure to antimicrobial agents in agricultural environments (Martínez, 2009; Allen *et al.*, 2010)^[9, 11].

The detection of multidrug-resistant isolates supports earlier reports that insect guts can serve as reservoirs of resistant bacteria, potentially enhancing host survival under selective pressure (Dillon and Dillon, 2004; Xia *et al.*, 2018)^[5, 13]. Such resistance traits may indirectly contribute to the resilience of fall armyworm populations against chemical control measures.

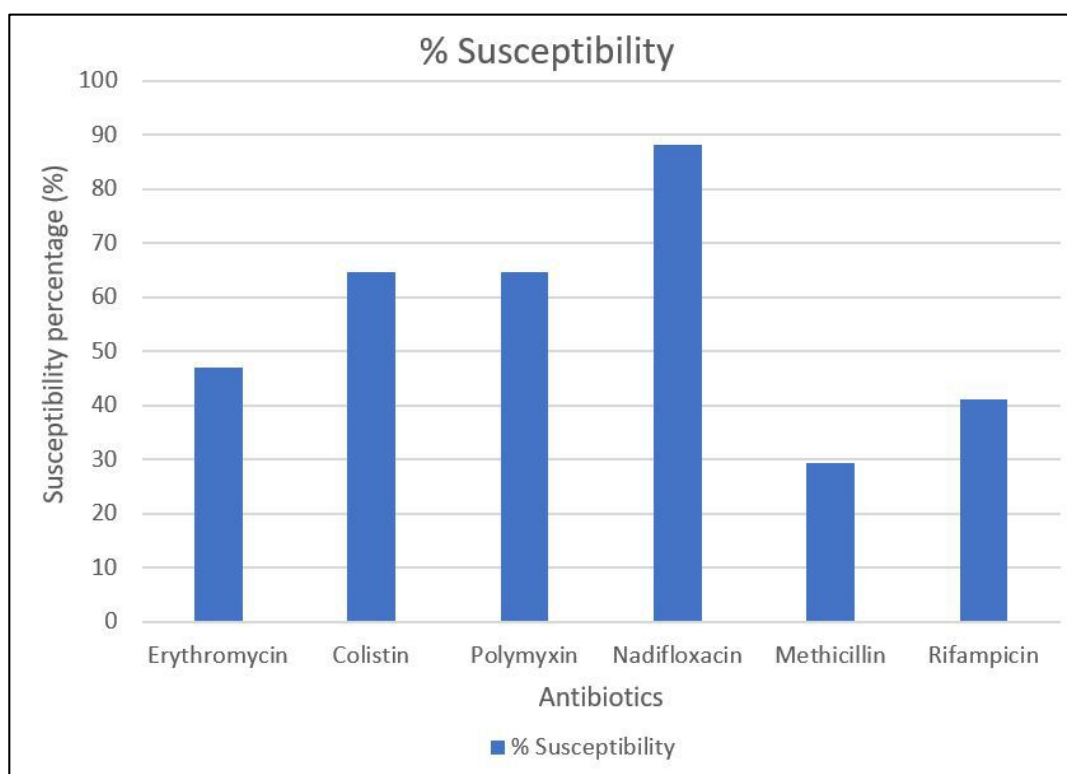
Protease-producing bacteria likely contribute to protein digestion in the fall armyworm gut by supplementing host digestive enzymes. Similar roles of gut bacterial proteases have been reported in other lepidopteran insects (Visôto *et al.*, 2009; Zhang *et al.*, 2022)^[12, 8]. Urease-producing bacteria may facilitate nitrogen recycling, which is particularly important for herbivorous insects feeding on nitrogen-limited diets (Salvadori *et al.*, 2012)^[11].

The coexistence of antibiotic resistance and functional enzyme production underscores the ecological significance of gut bacteria in enhancing host nutrition, adaptability, and survival. Understanding these interactions may provide new avenues for microbiome-targeted pest management strategies.

Table 1: Antibiotic susceptibility profile of gut bacterial isolates from fall armyworm (*Spodoptera frugiperda*) feeding on maize

Isolate Name	Antibiotic susceptibility test					
	Erythromycin	Colistin	Polymyxin	Nadifloxacin	Methicillin	Rifampicin
FWG-1	-	+	+	+	-	+
FWG-2	+	+	+	+	+	+
FWG-3	-	+	+	+	-	+
FWG-4	+	+	+	+	+	+
FWG-5	+	+	+	+	-	-
FWG-6	-	+	+	+	-	+
FWG-7	-	+	+	+	-	+
FWG-8	-	+	+	+	-	-
FWG-9	-	+	+	+	-	-
FWG-10	+	+	+	+	-	+
FWG-11	+	-	-	+	-	-
FWG-12	-	-	-	-	-	-
FWG-13	-	+	+	+	-	-
FWG-14	+	+	+	+	-	+
FWG-15	+	-	-	+	+	-
FWG-16	-	+	+	+	-	-
FWG-17	+	-	-	+	-	-

(+) indicates sensitive reaction and (-) indicates resistant reaction based on the presence or absence of a zone of inhibition in the disc diffusion assay. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Nutrient agar after incubation at 37 °C for 24 h. FWG-1 to FWG-17 represent individual gut bacterial isolates recovered from *Spodoptera frugiperda* larvae feeding on maize.



Values represent the percentage of gut bacterial isolates (n = 17) showing sensitivity to each antibiotic as determined by the Kirby-Bauer disc diffusion method. Antibiotic susceptibility testing was performed on Nutrient agar and results were recorded after incubation at 37 °C for 24 h.

Fig 1: Percentage antibiotic susceptibility of gut bacterial isolates from fall armyworm (*Spodoptera frugiperda*) feeding on maize

Table 2: Protease and urease activities of gut bacterial isolates from fall armyworm (*Spodoptera frugiperda*)

Isolate code	Protease activity (mm/index)	Urease activity
FWG-1	13.66 ± 0.15	+
FWG-2	7.00 ± 0.12	–
FWG-3	11.00 ± 0.23	–
FWG-4	0.00	+
FWG-5	0.00	–
FWG-6	18.66 ± 0.44	–
FWG-7	0.00	+
FWG-8	0.00	–
FWG-9	0.00	+
FWG-10	0.00	–
FWG-11	0.00	–
FWG-12	9.33 ± 0.08	–
FWG-13	0.00	+
FWG-14	22.66 ± 0.14	–
FWG-15	0.00	–
FWG-16	0.00	+
FWG-17	0.00	–

Values in each column are mean of three replications ($\pm$ standard error). Protease activity was determined on skim milk agar based on the diameter of the clear zone formed around bacterial colonies. Urease activity was assessed using Christensen's urea agar; (+) indicates urease positive and (–) indicates urease negative reaction.

5. Conclusion

The study reveals that fall armyworm gut bacteria associated with maize exhibit variable antibiotic susceptibility and functional enzymatic traits. The presence of protease-and urease-producing bacteria, along with multidrug-resistant isolates, highlights their potential role in host nutrition and adaptation. These findings contribute to a better understanding of fall armyworm gut microbiota and may support the development of sustainable pest management approaches.

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