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Isolation of Cellulolytic Bacterium from the Digestive Tract of the *Leptinotarsa decemlineata* (Colorado Potato Beetle)

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Abstract

The objective of this study was to isolate and identify cellulose-degrading bacteria from the adult digestive tract of the *Leptinotarsa decemlineata* (Colorado potato beetle). Soluble carboxymethyl cellulose (CMC) degradation assays were used to identify five bacterial strains and assess their cellulolytic activity. The development of a clear zone on CMC plate media and the cellulolytic index were used to calculate cellulolytic activity. The highest cellulolytic index (2.2) was found in MOC6. This strain was identified and described using morphological, biochemical, and partial 16S rDNA sequencing. New cellulose-degrading bacteria from the *Leptinotarsa decemlineata*'s gut have been identified in this investigation. The isolate had relatively high cellulolytic activity was identified as *Bacillus pumilus* strain MOC6. The potential use of this strain in the breakdown of cellulose makes its identification significant.

Key Words: Cellulolytic bacterium, Digestive tract, *Leptinotarsa Decemlineata*

Introduction

The most diverse and prevalent group of animals on the planet are insects, whose ability to survive depends mostly on their exceptional environmental adaptations and food sources. In order to accomplish this, insects have developed highly specialized and complex digestive systems that can break down a wide range of meals, primarily lignocellulose, which is the dry substance found in plant cell walls (Dar et al., 2022). The guts of insects are home to a variety of bacteria that benefit their hosts physiologically and ecologically (Ozdal et al., 2016; Jang and Kikuchi, 2020). Insect digestive systems have been known as the smallest natural bioreactors in the world because of their exceptional lignocellulose digestion (Breznak and Brune, 1994).

Among this diverse group of insects, one species with significant agricultural importance is Leptinotarsa decemlineata (Colorado potato beetle). Belonging to the order Coleoptera and the family Chrysomelidae, this species is a pest primarily feeding on plants of the Solanaceae family, causing substantial yield losses in potato fields worldwide (Muratoğlu et al. 2011). The adult individuals measure 10–12 mm in length, with a yellow-reddish coloration and a strongly convex dorsal surface. The elytra bear a total of ten longitudinal black stripes, five on each side. The mature larvae have a hunched posture, with a dark brown head and a bright orange body (Kekillioğlu and Yılmaz, 2018). *L. decemlineata* overwinters in the soil as an adult. In the spring, it leaves its overwintering sites in search of host plants, during which process it can disperse over large areas (Muratoğlu et al., 2011).

In terms of feeding habits, *L. decemlineata* primarily consumes the leaves of the plant, and then feeds on the stems and tubers. Both larvae and adults typically feed by starting from the edges of the leaves and moving inward, or by creating holes on the leaf surface and continuing to feed (Bouchard et al., 2003). The most destructive period is the late larval stage, which accounts for approximately 90% of total leaf consumption. Furthermore, *L. decemlineata* not only causes physical damage but also serves as a vector for various pathogens, including brown rot, potato ring rot, and spindle tuber viroid**.** The feeding habits and digestive processes of *L. decemlineata* result in a significant specialization of its gut microbiota. The gut of *L. decemlineata* supports a complex microbiota capable of degrading lignocellulose and other plant-derived components (Efimenko et al., 2022).

The aim of this study is to isolate cellulolytic microorganisms from the *L. decemlineata* gut. Numerous insect guts have been found to harbor significant populations of bacteria with cellulolytic activity. (Uddin et al., 2021; Han et al., 2024). Therefore, insects are considered attractive biological sources for discovering new cellulolytic enzymes with extraordinary catalytic potential.

Materials and Methods

Collection of *Leptinotarsa decemlineata* specimens

Leptinotarsa decemlineata collected from potato fields in Erzurum, Pasinler were used in the study. The collected adult specimens were placed in glass jars. These jars were covered with a fine-mesh cloth and transported to the laboratory environment where the experiments were carried out. The beetles were fed on potato plant leaves for a week.

Isolation of bacteria

The beetles were surface sterilized in 70% ethanol, rinsed with sterile distilled water and air dried for 5 minutes at room temperature (25ºC). The beetles were then dissected with a sterile scalpel and the entire gut was removed and suspended in 20 ml of 0.85% NaCl. 0.2 ml of the gut suspension was inoculated into Nutrient Agar (NA, Merck). After 24 hours, colonies that developed on the medium were isolated separately.

Morphological and molecular identification of the bacterial isolates

A colony of each isolate was then moved onto sterile glass slides after the isolates had been subcultured on nutrient agar plates for twenty-four hours. The KOH test (Powers, 1995) and Gram stain (Coico, 2006) were used. To find the gram response, the KOH test employs 3% potassium hydroxide. The cultural, morphological and biochemical properties of the bacterial species (Gram, cell shape, catalase, oxidase) were determined (Harley and Prescott, 2002; Ozdal et al., 2016). The 16S rDNA gene was utilized for molecular identification.

Screening for cellulose-degrading capacity

The isolated bacteria were inoculated individually onto Nutrient Agar (NA, Merck) medium containing 1% CMC. CMC degradation was tested by staining the plates with 1 g/L congo red dye (Sigma, USA) for 15 min and then washing with 1M NaCl. After staining with congo red dye, clear zones around bacterial colonies on CMC plates indicated cellulolytic activity. Cellulase activity index (CAI) was then calculated according to the following equation CAI=[(Diameter clear zone – Diameter bacterial colony)/Diameter bacterial colony] (Handique et al., 2017; Nelson et al., 2021).

Results and Discussion

A total of twenty bacterial strains were isolated from adult digestive tract of the *Leptinotarsa decemlineata*. Purified isolates were screened on CMC plates for cellulase activity. Based on the widths of the clear hydrolysis zones surrounding the colonies on CMC plates, five possible cellulotic isolates were chosen for the quantitative cellulase activity assay (Figure 1).

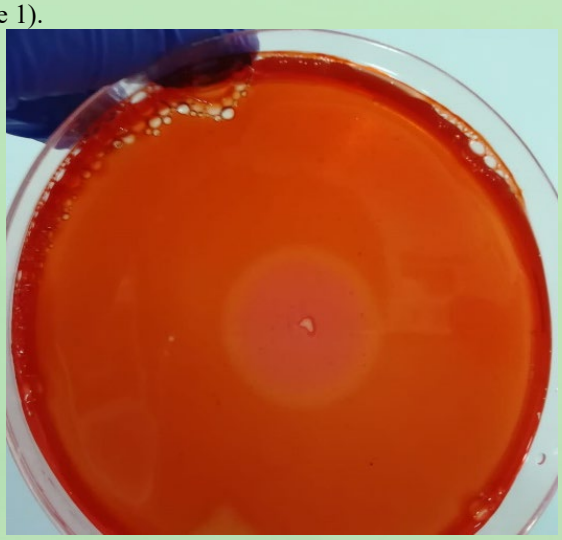

Figure 1. Cellulolytic hydrolysis zones around the streaks of isolate grown on CMC plate for 2 days at 30 °C.

In this study, three Gram-positive and two Gram-negative bacterial species that were determined to produce cellulase were isolated. Additionally, it was shown that every isolated bacterium exhibited catalase and protease activity (Table 1). A range of 0.8 to 2.2 for the cellulolytic index indicated significant cellulase production. MOC6 had the highest cellulolytic index (2.2), whereas MOC3 had the lowest (0.8) (Table 1). The sequence data based on 16 S rDNA region analysis, were compared with sequences in the NCBI database. The MOC6 isolate was identified as *Bacillus pumilus*.

Bacillus group is commonly found in many insect digestive systems due to its cellulolytic properties. The study by MsangoSoko et al. (2020) supports these results; Bacillaceae was reported as the dominant group of cellulolytic gut bacterial symbionts in Coleopteran insects. *B. licheniformis* cellulolytic bacteria have been reported isolated from the gut of white worm species (Van Dyk et al., 2009). Similar to the B. pumilus bacteria we isolated in this study, there are many studies showing that this species produces cellulase (Shankar et al., 2021; Dar et al., 2022; Yilmaz and Gurkok, 2025).

Table 1. Identification of cellulase producing isolates on the basis of morphological / biochemical tests and cellulolytic activities.

Tests	MOC3	MOC4	MOC5	MOC6	MOC7
Gram strain	+	+	-	+	-
Catalase	+	+	+	+	+
Oxidase	+	+	-	+	-
Urease	-	-	+	-	+
Protease	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod
CAI	0.8	1.3	2.0	2.2	1.6

The findings of this article were that bacteria with cellulolytic activity were isolated from the digestive system of the *Leptinotarsa decemlineata* and *B. pumilus* was determined as the most effective species. In conclusion, complex cellulose-rich organic wastes may be broken down by cellulolytic bacteria that were isolated from *L. decemlineata* guts. Therefore, more research is required to assess how well these cellulolytic bacterial strains break down organic waste and agricultural byproducts. In order to prepare strong strains of bacterial consortium that degrade organic matter and use them for the generation of biofuel at the industrial level, more research is necessary to identify and characterize cellulolytic bacteria from the gut of cellulolytic-feeding *L. decemlineata*.

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