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Original article

Cellulolytic bacteria associated with gut of yellow mealworm larvae (*Tenebrio molitor*, Coleoptera: Tenebrionidae) - isolation and molecular identification

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Abstract

The study aimed to isolate and identify cellulolytic bacteria associated with the gut of yellow mealworm larvae (*Tenebrio molitor*) and to assess their main phenotypic and enzymatic features. Bacteria capable of utilizing cellulose as the sole carbon source were cultured on CMC medium under aerobic and anaerobic conditions. Molecular identification based on *16S rRNA* gene sequencing revealed two isolates: *Mammaliicoccus lentus* (ML01) and *Pseudocitrobacter vendiensis* (PV02), both showing high sequence similarity to GenBank references (≥ 99.4%). Phylogenetic analysis confirmed their close relationship with corresponding species. Microscopy and biochemical tests indicated that ML01 is a Gram-positive, oxidase-positive coccus, whereas PV02 is a Gram-negative, oxidase-negative rod. Both strains exhibited γ-hemolysis and strong growth on TSA and LB media. API 20E profiling showed that ML01 could ferment several carbohydrates and produce β-galactosidase and arginine dihydrolase, while PV02 also expressed β-galactosidase activity. Cellulolytic activity, confirmed by Congo red and iodine staining, was observed in both isolates, with PV02 demonstrating the highest hydrolytic capacity. These findings indicate that the gut of *T. molitor* harbors cellulolytic bacteria with potential zoonotic risk, highlighting the need for their further monitoring.

Keywords: Cellulolytic bacteria, *gut microbiota*, *Mammaliicoccus lentus*, *Pseudocitrobacter vendiensis*, *Tenebrio molitor*

Introduction

The yellow mealworm (*Tenebrio molitor*, Linnaeus, 1758) is a widely distributed beetle from the family Tenebrionidae, commonly found in dark and humid environments such as under tree bark, bird nests, poultry litter, grain stores, silos, and human dwellings (Eriksson et al. 2020, Eberle et al. 2022). Mealworms are holometabolous insects, undergoing four developmental stages: egg, larva, pupa, and adult. The duration of their life cycle is highly variable (from 60 days up to 1-2 years) and depends primarily on temperature and humidity, with an optimum temperature range of 22-28°C (Li et al. 2013, Zunzunegui et al. 2024). Females lay 400-500 eggs, which hatch into larvae within 7-15 days under favorable conditions (Gkinali et al. 2022, Parsa et al. 2023). Larvae undergo multiple molts, eventually reaching a size of 2.5-3 cm and a weight of 0.2 g before pupating (Ghaly and Alkoaik 2009).

Mealworm larvae are increasingly valued as a sustainable source of protein and fat for animal feed and human consumption. Fresh larvae contain approximately 18% protein and 22% fat, while dried larvae contain almost twice these amounts. They are also rich in minerals, particularly magnesium and zinc, as well as B vitamins and vitamin E, with the oil exhibiting antioxidant properties (Siemianowska et al. 2013, Rumbos et al. 2020, Moruzzo et al. 2021, Errico et al. 2022). The European Union has authorized the marketing of *T. molitor* as a novel food in various forms, including powdered, frozen, and dried (Commission Regulation (EU) 2021/1372; Implementing Regulation (EU) 2022/169).

In addition to their nutritional value, mealworms exhibit the ability to degrade diverse organic and synthetic compounds, including cereal products, vegetable residues, and plastics such as polystyrene, polyethylene, and cellulose waste (Przemieniecki et al. 2020, Jin et al. 2023). This capacity is attributed, at least in part, to their gut microbiota, which can produce enzymes capable of cellulose and hemicellulose degradation (Urbanek et al. 2020, Ferreira et al. 2001). Studies on gut microbial communities of insects, including mealworms, termites, and wood-feeding cockroaches, indicate a key role of symbiotic bacteria in plant biomass utilization (Cruden and Markovetz 1979, Schäfer et al. 1996, Wenzel et al. 2002, Przemieniecki et al. 2020). The importance of these bacteria can be considered beyond the area of benefits for the insect, but also in biotechnological matters (importance for humans).

They can play a significant role in waste disposal (in the biodegradation processes of agricultural and organic waste), the food and feed industry, due to the fact that the enzymes produced by these bacteria can be used to improve the digestibility of products (especially in the case of animals), or in the production of biofuels as organisms that decompose lignocellulosic biomass into simple sugars, which are then fermented into bioethanol or biomethane (Fu et al. 2024).

It is worth noting, however, that previous studies on the microbiome capable of degrading cellulose or lignocellulose in *T. molitor* have focused mainly on such bacterial genera as *Selenomonas, Agromyces, Cellulomonas, Mycobacterium, Xenophilus, Stenotrophomonas* and *Pseudomonas* (Qi et al. 2011, Przemieniecki et al. 2020). To the authors' current knowledge, species such as *Mammaliicoccus lentus* and *Pseudocitrobacter vendiensis* have not been studied in this direction.

The aim of this study was to isolate cellulolytic bacterial symbionts from the gut of *T. molitor* larvae reared on a conventional diet (oat flakes and vegetables) and to identify them to the species level using molecular methods. Understanding the composition and enzymatic potential of gut microbiota contributes to both insect nutrition research and potential biotechnological applications in biodegradation and waste management.

Materials and Methods

Isolation of bacteria

The research does not require the consent of the Local Ethics Committee. Adult mealworm larvae (measuring on average 1.8-2.2 cm (n=5) (Fig. 1) came from our own breeding at the Department of Epizoology and Clinic of Infectious Diseases and were delivered to the Department of Biology and Fish Diseases, University of Life Sciences in Lublin (Poland) in a living state for dissection and further experiments. We decided that the size of our sample (n=5) is sufficient and that it is a representative research group, allowing us to conclude about our own breeding. In order to be killed, the individuals were incubated in a freezer at -20°C for 30 minutes. No anesthetic or chemical agents were used for this purpose due to the possibility of disturbing the composition of the intestinal microflora. After 30 minutes, each of the 5 larvae was surface-sterilized by immersing it three times in 70% ethyl alcohol (Stanlab, Poland) and thoroughly rinsing it with sterile distilled water. Subsequent to rinsing in H_2O, the dissection was performed under laminar flow using sterile laboratory tools (scissors, scalpels, dissecting needles). The contents of the dissected and cut intestines were scraped into an Eppendorf tube containing 1 ml of sterile 0.85% NaCl (POCH, Poland) and the sample was vortexed. Cut intestinal fragments were also placed

Fig.1. Appearance of yellow mealworm larvae (*Tenebrio molitor*) from our own culture used in research.

in the test tube. The liquid CMC culture media prepared in the flasks (200 ml) were inoculated with the entire content of the Eppendorf tube, and then the flasks were incubated for 3 weeks at 32°C. Two liquid cultures were carried out in parallel - one under aerobic conditions in a laboratory incubator, the other under anaerobic conditions in an anaerobic jar incubated at 32°C. To create an oxygen free (anaerobic) environment the Anaerocult A reagent (Merck, Germany) was used. After 3 weeks, samples were taken from the culture and inoculated onto plates with solid CMC medium. They were incubated at 32°C for 48 hours until the bacterial colonies grew. All inoculations were performed in triplicate and again carried out under both aerobic and anaerobic conditions. Pure bacterial cultures were obtained by repeated sieving of all morphologically different bacterial colonies grown on solid CMC medium. These pure bacterial cultures were stored in the Microbank system (Pro-lab Diagnostics, UK) at -80°C for further analysis. Each time, the incubation temperature was 32°C and, depending on the origin of the colony, cultures were carried out in aerobic and anaerobic conditions.

Culture media composition

For the isolation of bacteria from mealworm intestines, a liquid CMC medium was used, the composition of which (per 1 liter of volume) was as follows: carboxymethyl cellulose (CMC): 10.0 g; KH_2PO_4: 1.5 g; $Na_2HPO_4 \cdot 7H_2O$: 2.5 g; $MgSO_4 \cdot 7H_2O$: 0.3 g; NaCl: 0.5 g; $CaCl_2$: 0.1 g; $FeSO_4 \cdot H_2O$: 0.005 g; $MnSO_4$: 0.0016 g at pH 6.5 (Biswas et al. 2022).

In turn, to obtain pure cultures, solid CMC medium was used, the composition of which was as follows (per liter of distilled water): CMC: 10.0 g; peptone: 10.0 g; NaCl: 0.5 g; $MgSO_4 \cdot 7H_2O$: 0.2 g; $CaCl_2$: 0.1 g; Agar: 15.0 g at pH 6.5 (Biswas et al. 2022).

The physical parameters of the main component of the medium (solid and liquid), i.e. carboxymethyl cellulose (CMC), were as follows. Purity ≥ 99.5% Water ≤ 10.0% Degree of substitution ≥ 0.65 pH 6.0-8.5 Viscosity 1500-3000 cP. Bacterial media were sterilized in a laboratory autoclave at 126°C for 15 minutes.

Morphological and biochemical characteristics of isolates

In order to fully determine the biochemical properties of the obtained isolates, commercial API-20E kits (bioMerieux, France) were used according to the manufacturer's recommendations. *Escherichia coli* ATCC 25922 served as a quality control strain. One technical replicates was performed for the API 20E test.

A test for the ability of bacteria to produce the cytochrome oxidase enzyme was also performed using a commercial oxidase reagent (bioMerieux, France). The preference of the tested strains to utilize lactose on MacConkey medium (MacConkey LAB-AGAR™, Biomaxima, Poland) and the ability to produce hemolysins on blood-agar plates (BD Difco, USA) were also checked. Using the plate method, a swimming motility test was performed in semi-liquid LB medium (Invitrogen, Thermo Fisher Scientific, USA) containing 0.3% bacteriological lab agar (BioMaxima, Poland). The presence of motility zones was checked after 24 hours of incubation at 30°C.

The morphological evaluation of the tested isolates was performed by staining the cell smears according to the standard Gram stain protocol (Beveridge 2000).

It was also checked whether isolated bacteria grow

on traditional, universal media such as tryptic soy agar (TSA, Oxoid, UK) and LB agar (Invitrogen, Thermo Fisher Scientific, USA).

Cellulolytic activity assay

In order to confirm the cellulolytic activity of isolated bacteria, 48-hour cultures grown on CMC agar plates were stained using 0.1% (w/v) Congo-red solution and iodine solution used for Gram stain (Gohel et al. 2014). The prepared dyes were poured onto the plates and incubated for 15 minutes. After this time, the plates were washed with distilled water (in the case of iodine-stained plates) or with 1M NaCl (in the case of Congo red-stained plates). The presence of clear zones around the growth of bacteria indicated the ability to decompose cellulose contained in the substrate. These zones were measured.

DNA extraction and molecular identification

Genomic DNA from the obtained bacteria was isolated using the commercial Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer's recommendations.

For amplification sequence of partial *16S rRNA* gene the universal primers 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 1492R (5' TAC GGY TAC CTT GTT ACG AC TT 3') were used.). The reaction mixture (25 µl) consisted of 12.5 µl 2x PCR Master Mix (A&A Biotechnology, Poland), 1 µl (10 pmol μl^{-1}) forward and reverse primers (Genomed, Poland), 2 µl of template DNA, and 8.5 µl of nuclease-free water (A&A Biotechnology, Poland). PCR was performed in the GeneExplorer Thermal Cycler GE-96G (Bioer Technology, China) using the following amplification conditions: initial denaturation at 95°C for 5 min and then 30 cycles of denaturation at 95°C (30 s), annealing at 60°C (40 s), elongation at 72°C (60 s), and a final extension (72°C, 7 min). Amplification products were separated by electrophoresis (120 V) on a 1.5% agarose gel stained with Simply Safe (EURx, Poland). The expected product length was 1500 bp. Obtained amplicons of such size were eluted and purified using Gel-Out kit (A&A Biotechnology, Poland) and next subjected to the Sanger sequencing method (Genomed, Poland), performed with a forward and reverse primer for PCR.

Finch TV software ver. 1.4.0 was used to analyze the sequencing results.

The BLAST tool in NCBI (https://blast.ncbi.nlm.nih.gov) served as a tool to determine the similarity of the assembled nucleotide sequences of the partial *16S rRNA* gene.

Phylogenetic analysis

The obtained sequences, after being deposited as records in the GenBank database available from NCBI (https://www.ncbi.nlm.nih.gov), were used to construct phylogenetic trees by the neighbor-joining method (500 bootstraps). Both sequences and their closest matches were aligned using the ClustalW tool. The MEGA 11 program was used for cluster analysis while the evolutionary distances were computed using the Maximum Composite Likelihood algorithm.

Results

Gut-associated bacteria of yellow mealworm larvae (*Tenebrio molitor*) were able to grow on CMC medium with cellulose as the sole carbon source under both aerobic and anaerobic conditions using Anaerocult A reagent (Merck, Germany). Among the morphologically different pure cultures isolated from both types of environments, it was possible to confirm their affiliation to two species using the 16S rRNA gene fragment amplification method. One of them was *M. lentus* and the other was *P. vendiensis*. Following genetic identification and *16S rRNA* sequencing of isolated bacteria the sequences showed high level of identity to sequences deposited in GenBank. In the case of *M. lentus* it was 99.93% to NCBI Reference Sequence: NR_043418.1 and exactly the same to other records (PV104459, MN399938, MF948914, OR449164) (Table 1). The sequence obtained from *P. vendiensis* showed an identity of 99.41% with NCBI Reference Sequence: NR_180316 and similarities of 99.93% with OQ405690, OQ405696, OQ405713 and 99.70% with OQ406087 (Table 2). The phylogenetic tree based on partial 16S rRNA sequences of the studied isolates, their closest matches and other members of the genus and drawn using the neighbor-joining confirms the close relationship of the obtained sequences to their references deposited as records in GenBank. In the case of *M. lentus*, a division into two phylogenetic clades is visible, one of which is formed by representatives of the species *M. sciuri* and *M. stepanovicii*. The second group includes representatives of the species *M. fleurettii*, *M. vitulinus* and *M. lentus* together with the sequence obtained in this study and its closest matches (Fig. 2). The phylogenetic tree of *P. vendiensis* illustrates the division of the isolates sequences into two clades, although one of them contains only the sequence of the *Pseudocitrobacter* sp. sgm9.3 strain. The second clade groups all sequences closely related to the sequence of *P. vendiensis* strain PV02 obtained during the study and belonging to the same species, as well as isolates obtained from the species *P. faecalis*, *P. corydidari*, and the sequence of the strain *Pseudocitrobacter* sp. (Fig. 3)

Table 1. National Center for Biotechnology Information reference sequence and other sequences deposited in GenBank to which the partial sequence of *16S* ribosomal RNA gene obtained from *Pseudocitrobacter vendiensis* strain PV02 showed the highest degree of identity.

Accession number of nucleotide sequence	Description	Geographical location	Host/isolation source	Percent identity (%)
OQ405690	*P. vendiensis* strain H17 *16S rRNA* gene, partial sequence	China	No data	99.93
OQ405696	*P. vendiensis* strain H23 *16S rRNA* gene, partial sequence	China	No data	99.93
OQ405713	*P. vendiensis* strain H40 *16S rRNA* gene, partial sequence	China	No data	99.93
OQ406087	*P. vendiensis* strain S64 *16S rRNA* gene, partial sequence	China	No data	99.70
NCBI Reference Sequence: NR_180316	*P. vendiensis* strain CPO20170097 *16S rRNA* gene, complete sequence	Denmark	*Homo sapiens*/ perineum swab	99.41

Table 2. National Center for Biotechnology Information reference sequence and other sequences deposited in GenBank to which the partial sequence of *16S* ribosomal RNA gene obtained from *Mammaliicoccus lentus* strain ML01 showed the highest degree of identity.

Accession number of nucleotide sequence	Description	Geographical location	Host/isolation source	Percent identity (%)
PV104459	*M. lentus* strain CE8 *16S rRNA* gene, partial sequence	Turkey	Swab samples of cats with otitis	99.93
MN399938	*M. lentus* strain BFA42 *16S rRNA* gene, partial sequence	India	*Hyalomma anatolicum*/ gut	99.93
MF948914	*M. lentus* strain PSB295 *16S rRNA* gene, partial sequence	South Korea	Soil within the fairy ring of *Tricholoma matsutake*	99.93
OR449164	*M. lentus* strain V1678 *16S rRNA* gene, partial sequence	China	No data	99.93
NCBI Reference Sequence: R_043418	*M. lentus* strain MAFF 911385 *16S rRNA*, partial sequence	France	Goat udder	99.93

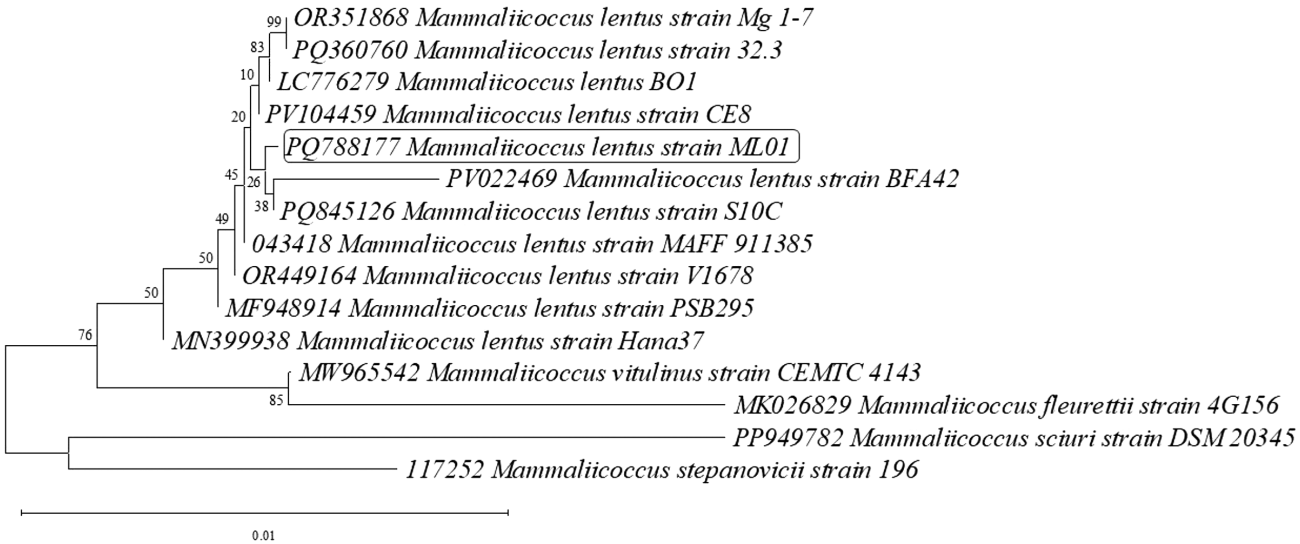

Fig. 2. Phylogenetic tree based on the partial *16S rRNA* gene sequences of *Mammaliicoccus lentus* strain ML01 isolated from gut of yellow mealworm larvae (*Tenebrio molitor*) and partial 16S rRNA gene sequences of fourteen other closely related species deposited in the GenBank database.

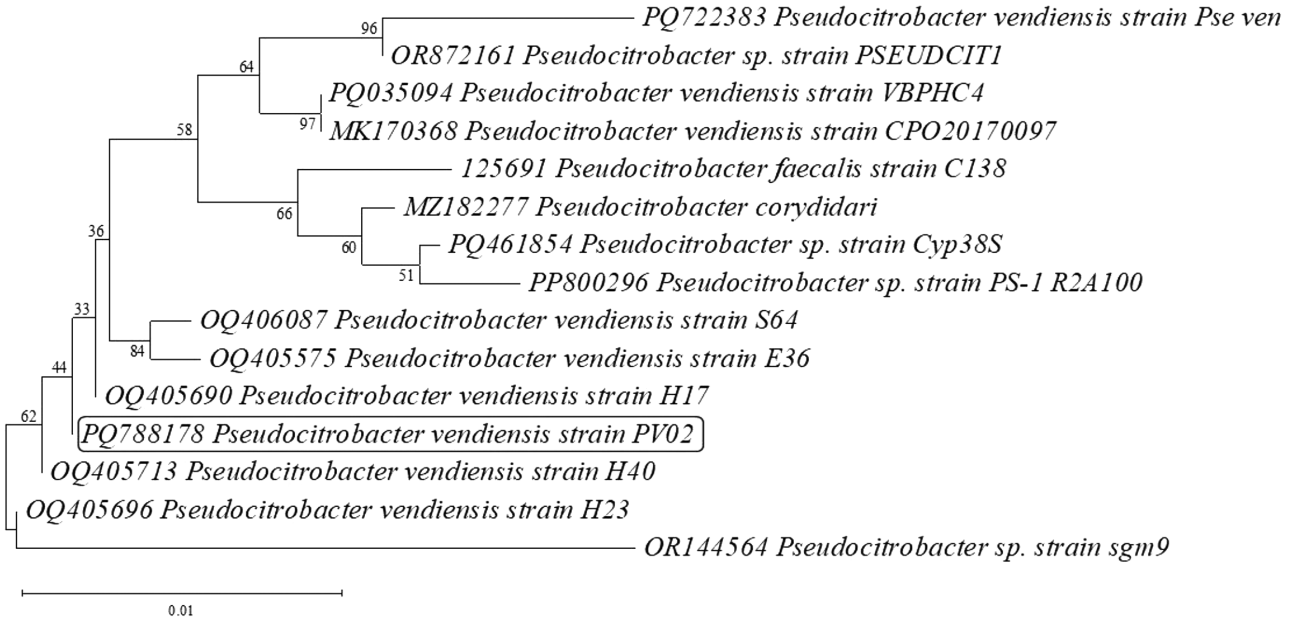

Fig. 3. Phylogenetic tree based on the partial *16S rRNA* gene sequences of *Pseudocitrobacter vendiensis* strain PV02 isolated from gut of yellow mealworm larvae (*Tenebrio molitor*) and partial 16S rRNA gene sequences of fourteen other closely related species deposited in the GenBank database.

Microscopic observations combined with Gram staining revealed that isolate ML01 is a Gram-positive coccus-shaped bacteria, whereas PV02 is a Gram-negative rod-shaped bacteria. In addition, strain ML01 was oxidase-positive while PV02 was oxidase-negative (Table 3). Both of them showed γ-hemolysis when grown on blood-agar plates and also showed abundant growth on TSA and LB solid media.

API 20E tests were used to identify metabolic features of the tested isolates. Based on their results, the *M. lentus* isolate ML01 showed a positive ONPG test (which confirmed its ability to produce β-galactosidase) and was able to produce arginine dihydrolase. Moreover, in fermentation (oxidation) tests it was able to use substrates such as glucose, mannitol, sorbitol, rhamnose, melibiose, amygdaline and arabinose (Table 3). This bacterium was negative for the remaining tests included in panel 20E. In the case of the *P. vendiensis* PV02 isolate, the results of fermentation tests on different substrates coincide with those previously reported for *M. lentus* ML01. In the remaining reactions contained on the API 20E strip, the isolate showed only the ability to produce b-galactosidase in the ONPG test (Table 3).

After 48 hours of growth of the tested isolates on CMC agar plates and staining with Congo-red solution and iodine solution, it was proven that in the case of both isolates (and cultures conducted under aerobic and anaerobic conditions) clear zones (unstained) were visible, indicating the decomposition of cellulose contained in the medium by bacteria. In the case of isolate ML01 and cultured under aerobic conditions, the zone diameter was 15 mm for Congo-red staining and 16 mm for iodine solution staining. In anaerobic conditions, it was 15 and 15 mm, respectively. In turn, for isolate PV02, the zone diameter under aerobic conditions was 19 mm for Congo-red staining and 24 mm for iodine solution staining. In oxygen-free conditions, it was 20 mm and 19 mm (Fig. 4). For comparison, cellulose-degrading bacteria from the gut of termites from India formed halo zones ranging from 4-7 mm after staining with Congo red dye and formed clear zones ranging from 6-12 mm after staining with iodine solution (Shinde et al. 2017). In turn, environmental isolates isolated from garden soil, for which cellulolytic properties were proven, formed clear zones ranging from 8.5-17 mm after staining with Congo red dye and 19-23 mm after staining with iodine solution (Gohel et al. 2014).

Discussion

Cellulolytic bacteria play a key role in the digestion of plant materials rich in cellulose, which are difficult to break down by animal hosts. The ability of cellulolytic bacteria to degrade cellulose in the insect gut not only facilitates digestion but may also contribute to the decomposition of plant materials in the environment, which is important for organic recycling and carbon cycling in ecosystems. To date, the literature has reported the isolation of cellulolytic bacteria associated with the intestines of arthropods such as *Macrotermes gilvus* (Ferbiyanto et al. 2015), *Helicoverpa armigera* (Dar et al. 2021), *Holotrichia parallela* (Huang et al. 2012),

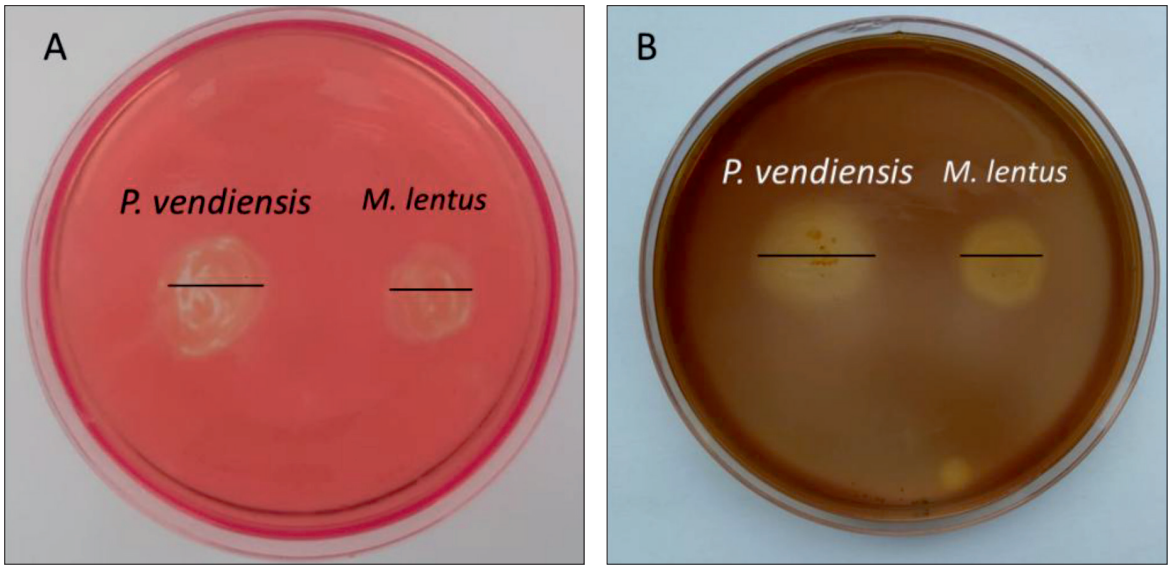

Fig. 4. Results of staining methods for bacteria growing on CMC medium. The plates were stained at room temperature (20°C±2°C), for 15 minutes. (A) Staining with Congo-red solution: *P. vendiensis*: diameter of the clear zone = 19 mm; *M. lentus*: diameter of the clear zone = 15 mm. (B) Staining with iodine solution used for Gram methods: *P. vendiensis*: diameter of the clear zone = 24 mm; *M. lentus*: diameter of the clear zone = 16 mm.

Table 3. Phenotypical and biochemical characterization of bacteria isolated from gut of yellow mealworm larvae (*Tenebrio molitor*).

Characteristics	*M. lentus*	*P. vendiensis*
Gram reaction	Gram-positive	Gram-negative
Cell shape	coccus-shaped	rod-shaped
Oxidase reaction	+	-
Hemolytic test	*γ*-hem.	*γ*-hem
Growth on MacConkey agar	inhibited (complete)	lactose-positive
Growth on TSA medium	+	+
Growth on LB medium	+	+
ONPG test (β-galactosidase)	+	+
Arginine dihydrolase	+	-
Lysine decarboxylase	-	-
Ornithine decarboxylase	-	-
Trisodium citrate utilization	-	-
H,S production	-	-
Urease production	-	-
Deamination of tryptophan	-	-
Indole production	-	-
Acetoin production (VP test)	-	-
Gelatin liquefaction	-	-
D-glucose fermentation	+	+
D-mannitol fermentation	+	+
Inositol fermentation	-	-
D-sorbitol fermentation	+	+
L-rhamnose fermentation	+	+
D-saccharose fermentation	-	-
D-melibiose fermentation	+	+
Amygdaline fermentation	+	+
L-arabinose fermentation	+	+

Heterotermes indicola (Azhar et al. 2024), *Spodoptera litura* (Dar et al. 2022) or *Prionomma bigibbosum* (Biswas et al. 2022). These studies, like those in our manuscript, focused on the use of CMC plates as a hydrolyzable substrate.

In the digestive tracts of insects, such as *Tenebrio molitor*, cellulolytic bacteria enable efficient utilization of cellulose as a source of energy and nutrients. Qi et al. (2011) reported the presence of cellulolytic bacteria closely related to *Agromyces* sp., *Mycobacterium astroafricanum*, *Xenophilus azovorans*, *Stenotrophomonas* sp., and *Pseudomonas stutzeri*. In the present study, we isolated two bacterial strains from the gut of *T. molitor* larvae: *P. vendiensis* PV02 and *M. lentus* ML01. Both strains demonstrated the ability to degrade cellulose, as evidenced by clear zones on CMC medium and activity in Congo red and iodine assays. To date, there are no literature reports on the cellulolytic activity of *P. vendiensis* or *M. lentus*. Moreover, Battisti et al. (2024) did not observe cellulolytic activity in two *M. lentus* isolates obtained from poultry manure. Przemieniecki et al. (2020), studying changes in gut microbiome structure depending on diet, reported that bacteria characteristic of a cellulose-rich diet belonged to the genus *Selenomonas*, whereas *Pseudocitrobacter* and *Mammaliicoccus* were not detected.

P. vendiensis is a species of the family Enterobacteriaceae, first described in 2020 by Kämpfer et al. (2020) based on strain CPO20170097, isolated from a human host. Additionally, *P. vendiensis* has been shown to produce the carbapenemase IMP-1 and exhibit resistance to multiple antibiotics, including cephalosporins, carbapenems, trimethoprim-sulfamethoxazole, and chloramphenicol (De Andrade et al. 2022).

M. lentus (formerly *Staphylococcus lentus*) is a Gram-positive, coagulase-negative bacterium of the family Staphylococcaceae, reclassified from the genus *Staphylococcus* based on phylogenomic analyses by Madhaiyan et al. (2020). It is currently recognized as a species of both clinical and ecological relevance. *M. lentus* is considered an opportunistic and zoonotic pathogen. Although infections are rare, cases of endocarditis, peritonitis, folliculitis, septic shock, urinary tract infections, sinusitis, and wound infections in humans have been reported (Stepanović et al. 2002, Mazal and Sieger 2010, Gorrin et al. 2014, Al-Salamy et al. 2017, Shareef et al. 2019, Hay and Sherris 2020, Ankad et al. 2023).

There is no doubt that monitoring their presence in various environments is necessary, although on the other hand their cellulolytic properties create a potential that can be used in many industries (biofuel production, paper industry, food and feed industry, waste management and bioremediation, or medicine), which leads to the need to expand research towards a thorough understanding of the capabilities of the bacteria described in this manuscript and to search for other isolates with a similar potential.

Conclusions

This study is the first to report the presence of *M. lentus* and *P. vendiensis* in the gut of *T. molitor* larvae. Both isolates exhibited cellulolytic activity, suggesting that the gut microbiota contribute to cellulose degradation and the efficient utilization of plant materials by the larvae. To date, cellulolytic activity of these species has not been reported in the literature, highlighting the novelty of these findings. At the same time, the presence of *M. lentus* and *P. vendiensis*, species that are potentially opportunistic and antibiotic-resistant, indicates the need for monitoring their occurrence in insect rearing facilities and assessing the potential risk to public health.

Author Declarations

Ethics approval

The study did not require approval from an ethics committee (according to the currently applicable law, studies involving arthropods do not require such approval).

Use of generative artificial intelligence

No generative artificial intelligence (AI) tools were used in the preparation of this manuscript.

Conflict of interest

The authors declare that they have no financial, personal, or institutional conflicts of interest related to this work

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