



Phenotypic Characterization of Intestinal Bacteria Isolated from Coconut Crabs (*Birgus latro*) on Liwo Island North Maluku

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ABSTRACT

The coconut crab (*Birgus latro*) is a terrestrial crustacean species known for its unique dietary habits and ecological significance in island ecosystems. This study aims to isolate and characterize intestinal microorganisms from coconut crabs collected from Liwo Island, North Maluku, Indonesia. A total of nine microbial isolates were obtained using traditional culture-based methods. Morphological characterization, Gram staining, and biochemical tests (catalase, oxidase, motility, indole, and sugar fermentation) were conducted for preliminary identification. All bacterial isolates were found to be Gram-positive, consistent with characteristics of lactic acid bacteria (LAB). Based on phenotypic and biochemical profiles, the dominant isolates were identified as *Lactobacillus desiosus*, *Lactobacillus coryneformis*, *Lactobacillus xylosus*, and *Candida crusei*. The predominance of LAB indicates their potential ecological role in maintaining intestinal balance and supporting digestive processes in *B. latro*. These findings provide foundational insight into the composition of the gut microbiota of coconut crabs and emphasize the ecological importance of LAB–yeast associations in their gastrointestinal ecosystem.

INTRODUCTION

The coconut crab (*Birgus latro*) is the largest terrestrial arthropod in the world, predominantly distributed across islands in the Pacific and Indian oceans. As an omnivorous scavenger, this species consumes a wide variety of plant and animal matter, with a notable preference for coconuts. Its diverse diet not only influences its nutritional intake but also plays a crucial role in shaping its ecological niche and adaptive behavior (Caro *et al.*, 2020; Khan *et al.*, 2024). *B. latro* exhibits remarkable adaptations to island ecosystems, including tree-climbing behavior to access coconuts and the use of powerful

pincers to crack hard shells, making it an important subject of ecological, physiological, and behavioral studies (Moher *et al.*, 2020).

Although extensive studies have addressed the ecology and behavior of *B. latro*, limited information is available regarding its intestinal microbial communities. Gut microbiota are increasingly recognized as vital contributors to host nutrition, digestion, metabolism, immune defense, and overall health across diverse animal taxa (Aris, 2023; Khan *et al.*, 2024). In invertebrates, particularly those inhabiting remote island ecosystems, the gut microbiota often reflect unique symbiotic relationships shaped by diet, habitat, and environmental conditions (Narayan *et al.*, 2020).

The present study aims to investigate the cultivable intestinal microbial diversity of coconut crabs collected from Liwo Island, North Maluku, Indonesia. Traditional culture-based methods were employed to isolate and characterize gut microorganisms, including observation of colony morphology, Gram staining, microscopic examination, and biochemical assays (catalase, oxidase, indole, motility, and sugar fermentation tests) to identify their phenotypic traits (Aris, 2023).

This research provides foundational insight into the intestinal microbial ecology of *Birgus latro* from Liwo Island. By characterizing lactic acid bacteria (LAB) and yeast species such as *Lactobacillus* and *Candida crusei*, this study contributes to a deeper understanding of host–microbe interactions in terrestrial crustaceans and their adaptive symbioses within island ecosystems (Li *et al.*, 2020; Aris, 2023).

MATERIALS AND METHODS

Sample collection

Coconut crabs (*Birgus latro*) were collected from Lowo Island and the sampling locations are shown on the map (Fig. 1). Three individuals were taken from each location, totaling nine samples. Each crab was placed in a sterile container, and intestinal contents were aseptically extracted immediately after humane euthanasia using sterile dissection tools. The samples were transferred into sterile tubes, kept in a cool box at approximately 4°C, and transported to the laboratory within 24h to ensure microbial viability.

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Fig. 1. Map showing the sampling location of *Birgus latro* (coconut crab). The yellow box indicates Liwo Island, located in North Patani District, Central Halmahera Regency, North Maluku Province, Indonesia

Isolation of microorganisms

Upon arrival in the laboratory, the intestinal samples were homogenized in sterile phosphate-buffered saline (PBS) and subjected to tenfold serial dilutions. A volume of 100 μ L from each dilution (10^{-2} to 10^{-6}) was spread on De Man, Rogosa and Sharpe (MRS) agar to isolate lactic acid bacteria, and on Sabouraud Dextrose Agar (SDA) to isolate yeasts. The plates were incubated aerobically at 37°C for 48h (MRS) and 30°C for 72 h (SDA). Colonies showing distinct morphological features were selected for further analysis.

Colony morphology characterization

Selected isolates were characterized based on macroscopic colony morphology including color, shape, elevation, edge, surface texture, opacity, and diameter (mm). Consistency (mucoid, dry, or creamy) was assessed by gently touching colonies with a

sterile loop. These characteristics were recorded systematically to guide further identification.

Microscopic examination and Gram staining

Isolates were cultured in nutrient broth and subjected to Gram staining using standard procedures. Stained smears were examined under a light microscope at 1000× magnification. Gram reaction (positive or negative), cell shape (coccus, bacillus, or yeast), and arrangement (single, chain, or cluster) were observed. Motility was assessed using semi-solid agar by stab inoculation and incubation for 24h.

Biochemical identification

Biochemical tests were performed to determine physiological and metabolic traits of each isolate, including catalase, oxidase, urease, nitrate reduction, hydrogen sulfide (H₂S) production, indole formation, methyl red (MR), Voges-Proskauer (VP), citrate utilization, and β-galactosidase (ONPG). Carbohydrate fermentation tests used glucose, mannitol, lactose, sucrose, xylose, and arabinose as substrates. All tests were carried out following standard microbiological methods (Cappuccino & Sherman, 2014; Bergey's Manual, 2015), and results were recorded based on color changes or gas production.

RESULTS AND DISCUSSION

Colony characterization

Microbial colonies isolated from the intestinal tract of *Birgus latro* exhibited consistent morphological characteristics across all samples (Table 1). The colonies were creamy white in color, circular in shape, with convex elevation and irregular to slightly undulate margins. Colony diameters ranged from 0.86 to 3.53mm, and all isolates showed a moist consistency.

The uniformity of these traits suggests the dominance of morphologically similar microbial groups within the coconut crab's intestinal microbiota. The creamy pigmentation and moist texture are typical of lactic acid bacteria (LAB), particularly those of the *Lactobacillus* genus, known for their carbohydrate fermentation ability. Minor variations in colony diameter may reflect strain-specific growth rates or nutrient utilization efficiency within similar intestinal ecological niches. The consistent morphology across isolates from different sampling sites indicates that environmental variations have a limited effect on the dominant cultivable microbiota of *B. latro*, suggesting the presence of functionally important symbionts involved in digestion and nutrient assimilation.

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Table 1. Colony characterization of microbial isolates from coconut crabs

Parameter	K11	K12	K13	K21	K22	K23	K31	K32	K33
Colony Color	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
Colony Diameter (mm)	1.03	0.86	3.41	1.07	0.89	3.53	1.24	0.97	3.29
Colony Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colony Margin	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Colony Elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
Colony Consistency	Moist	Moist	Moist	Moist	Moist	Moist	Moist	Moist	Moist

Gram staining and microscopic observation

Gram staining and microscopic analysis (Table 2) revealed that most bacterial isolates were Gram-positive rods or coccobacilli, with no visible motility. These are hallmark characteristics of LAB, particularly those within the genus *Lactobacillus*. Additionally, two isolates K23 and K33 (Fig. 2) demonstrated budding, oval-shaped cells typical of yeasts, supporting their identification as *Candida crusei*.

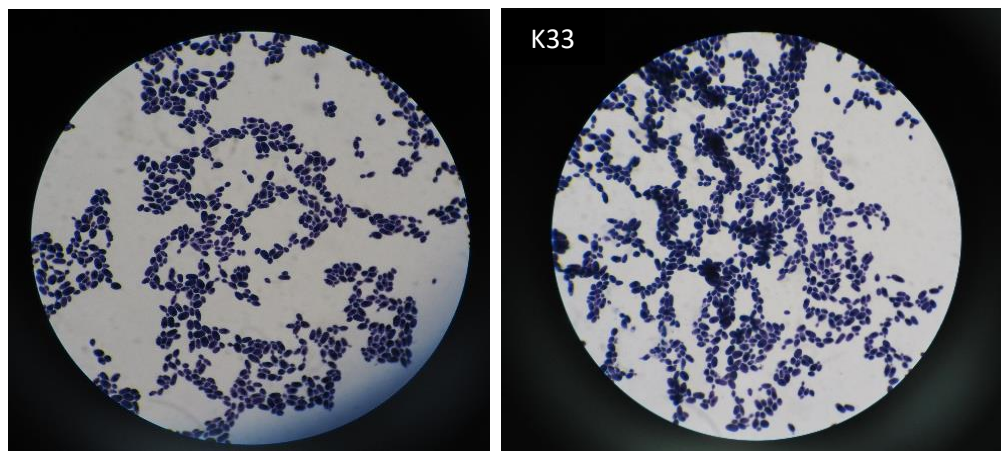


Fig. 1. Gram staining (*Candida crusei*)

Carbohydrate fermentation tests demonstrated the ability of several isolates to utilize diverse carbon sources such as glucose, mannitol, xylose, sucrose, and arabinose. This broad substrate utilization reflects the metabolic adaptability typical of *Lactobacillus* spp., supporting the degradation of plant polysaccharides and oligosaccharides within the host gut. For instance, isolates K11, K21, and K32 fermented arabinose, suggesting the ability to degrade arabinans from plant cell walls, while isolates K13 and K23 fermented mannitol and xylose efficiently, indicating adaptability to marine and plant-derived nutrients.

All isolates exhibited positive β -galactosidase (ONPG) activity, despite their inability to ferment lactose, implying the utilization of alternative β -galactoside substrates in the gut via specialized sugar transport systems. Urease activity, observed in most isolates, may assist in ammonia production and pH regulation, enhancing microbial survival under variable intestinal conditions. Variable citrate utilization patterns further reflect metabolic plasticity, as some isolates can use citrate as a sole carbon source an adaptive advantage under nutrient-limiting conditions (Vernocchi *et al.*, 2020; Aris, 2023).

The presence of *Lactobacillus* species, as confirmed by biochemical traits, supports previous findings that these bacteria are dominant in the gut microbiota of crustaceans (Ramos *et al.*, 2020; Wang *et al.*, 2022). The occurrence of *Candida crusei*, a yeast species, suggests a complementary symbiotic role in carbohydrate fermentation and vitamin production (Vernocchi *et al.*, 2020; Mukherjee *et al.*, 2024). Together, these microbes likely contribute to digestive processes and the maintenance of intestinal homeostasis in *B. latro*.

Microbial identification and ecological implications

Integrating the phenotypic, microscopic, and biochemical characteristics enabled the preliminary identification of the microbial isolates obtained from the intestinal samples of coconut crabs (*Birgus latro*) collected from Liwo Island. The identified microorganisms are presented in Figs. (3– 6), with the following classification:

- K11, K21, K32: *Lactobacillus desiosus*
- K12, K22, K31: *Lactobacillus coryneformis*
- K13: *Lactobacillus xylosus*
- K23, K33: *Candida crusei*

The predominance of lactic acid bacteria (LAB), particularly *Lactobacillus* species (Figs. 3 – 5), indicates their ecological fitness and essential role in the intestinal environment of *Birgus latro*. LAB are known to contribute to host nutrition, modulate gut pH, produce antimicrobial compounds, and enhance immune tolerance (Ramos *et al.*, 2020; Marra *et al.*, 2023). Their metabolic versatility allows them to utilize a wide range

of substrates, supporting the host's adaptation to a mixed diet of fibrous vegetation and decomposed organic matter.

Meanwhile, the occurrence of *Candida crusei* (Fig. 6) suggests a complementary role in fermentation and possible metabolic cross-feeding interactions with LAB. Yeasts are also known to contribute essential vitamins, cofactors, and enzymes that may assist in detoxifying secondary metabolites derived from plant sources (Mukherjee *et al.*, 2024). Microscopic examination confirmed that most isolates exhibited rod-shaped (*bacilli*) or coccobacilli morphologies, consistent with typical characteristics of *Lactobacillus* species (Martino *et al.*, 2022). In contrast, two isolates (K23 and K33) displayed budding cell morphology, a distinctive trait of *Candida* species, which commonly occur as part of the intestinal microbiome. The coexistence of both bacterial and yeast isolates in the intestinal samples underscores the microbial diversity inhabiting the gut of *B. latro*, reflecting a complex and dynamic microbial ecosystem (Hara, 2024).

All isolates were observed to be non-motile, which supports their classification as lactic acid bacteria and yeasts microorganisms typically adapted to stable ecological niches within the gastrointestinal tract (Palma *et al.*, 2022; Marra *et al.*, 2023). The absence of motility suggests that these microbes rely on passive dispersion or host-associated mechanisms for colonization and persistence. This adaptation enhances their ability to thrive in the gut environment, contributing to digestion, nutrient absorption, and the maintenance of intestinal homeostasis (Feng, 2023).



Fig. 2. Bakteri *Lactobacillus desiosus* (K11, K21, K32)



Fig. 3. Bakteri *Lactobacillus coryneformis* (K12, K22, K31)

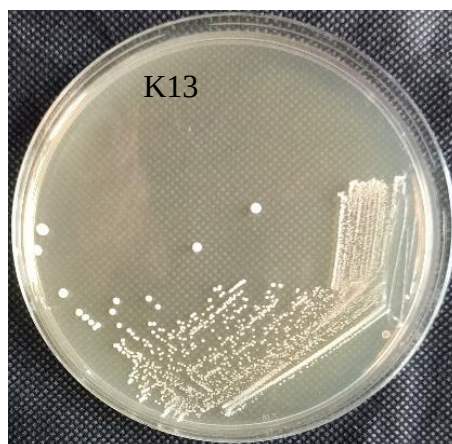


Fig. 4. Bakteri *Lactobacillus xylosus* (K13)

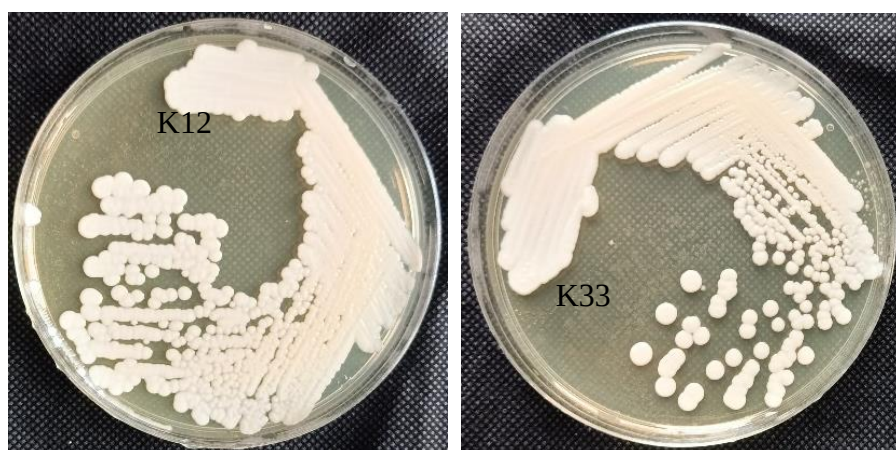


Fig. 5. Bakteri *Candida crusei* (K23, K33)

CONCLUSION

This study provides a comprehensive characterization of intestinal microorganisms isolated from the coconut crab (*Birgus latro*), revealing a predominance of lactic acid bacteria (LAB), primarily *Lactobacillus* species, alongside the yeast *Candida crusei*. The isolates exhibited consistent colony morphology, Gram-positive cell walls, non-motile behavior, and diverse biochemical activities, including carbohydrate fermentation, nitrate reduction, and β -galactosidase activity. These features indicate their potential functional roles in digestive processes, nutrient assimilation, and the maintenance of intestinal health. The coexistence of both bacterial and yeast species suggests a complex and cooperative microbial ecosystem within the gut of *B. latro*, which likely facilitates the digestion of its omnivorous diet and enhances adaptation to fluctuating nutrient availability in island environments. Collectively, this symbiotic microbial community may play a crucial role in supporting the host's digestive efficiency, ecological fitness, and overall survival.

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