Probiotic Potential of Bacterial Isolates from Chinese Water Chestnut (*Eleocharis dulcis*)

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Abstract

Chinese water chestnut (*Eleocharis dulcis*) is a major economic crop in Central Thailand. It is used in traditional Chinese medicine, and certain chemical and nutritional constituents, including antioxidants and dietary fibers, have contributed to its health benefits. Nonetheless, information about beneficial microorganisms associated with Chinese water chestnut is limited, particularly those having probiotic properties that might enhance its health benefits. This study aimed to isolate and assess the probiotic potential of microorganisms derived from Chinese water chestnut corms, specifically focusing on lactic acid bacteria (LAB). LABs were isolated from three samples obtained from different locations in Suphan Buri province. Identification of bacterial species was conducted using 16S rRNA gene sequence analysis. Several probiotic properties, including tolerance to acidic environments (pH 2.0, 2.5, 3.0, and 4.0) and bile salts (0.2%, 0.3%, and 0.4%), were tested. Antibacterial efficacy against foodborne pathogens was also assessed. As a result, 37 isolates, representing three species, *Lactococcus lactis*, *Lactococcus garvieae*, and *Weissella cibaria*, were obtained. Of these, 18 isolates exhibited high survival rates (> 80%) under acidic conditions at pH 3.0, and 10 maintained such a high survival rate in 0.3% bile salts. Moreover, some isolates exhibited inhibitory effects against *Bacillus cereus*, *Staphylococcus aureus, Salmonella enterica*, and *Escherichia coli*. The results indicate that LAB derived from Chinese water chestnut corms has probiotic potential, but additional analyses are required to confirm their probiotic properties.

Chinese Water Chestnut: Benefits

Name and taxonomy
- Common name: Chinese water chestnut
- Cyperaceae family
- Species: *Eleocharis dulcis*

Nutritional constituents
- Non-starch carbohydrates, dietary fibers and resistant starch
- Vitamins and minerals (e.g. vitamin A, vitamin C)

Microorganisms
- Ammonia-producing bacteria, Aluminum-tolerant bacteria and Nitrogen-fixing bacteria
- Studies Lactic acid bacteria (LAB) and probiotic microorganisms associated with Chinese water chestnut remains on limited, highlighting the need for investigation.

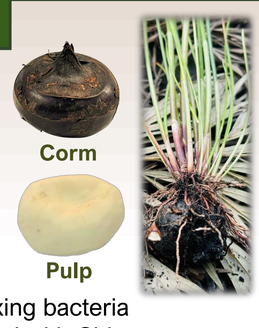


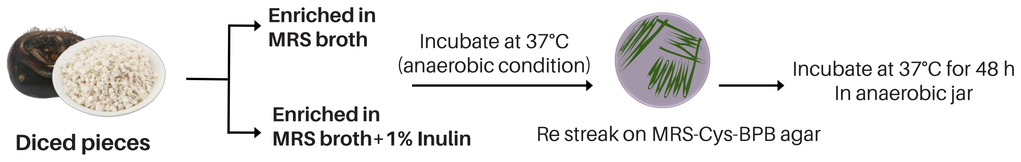
Photo reference: Department of Agricultural Extension, Thailand (2023).

Objectives

1. To isolate of Lactic acid bacteria (LAB) from Chinese water chestnut pulp
2. To identify species of selected isolates selected LAB
3. To investigate some probiotic properties of isolates

Materials and methods

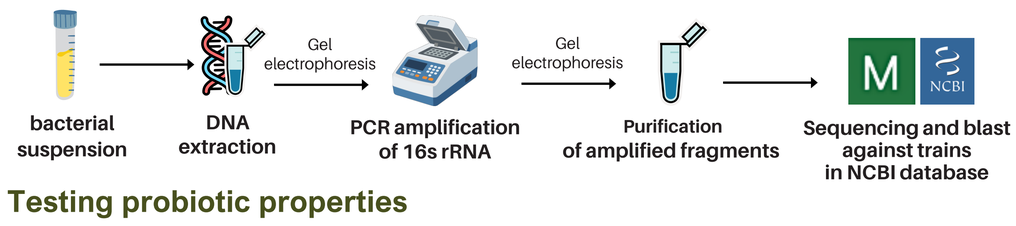
Isolation of Lactic acid bacteria bacteria



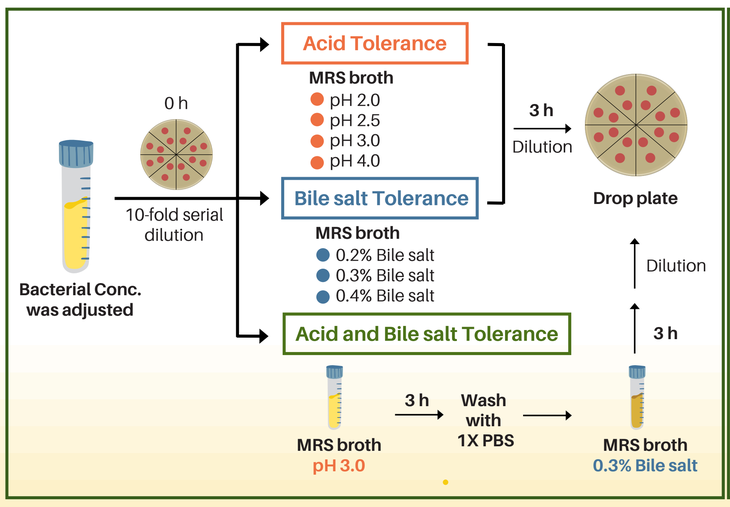
Study of morphological and biochemical characteristics

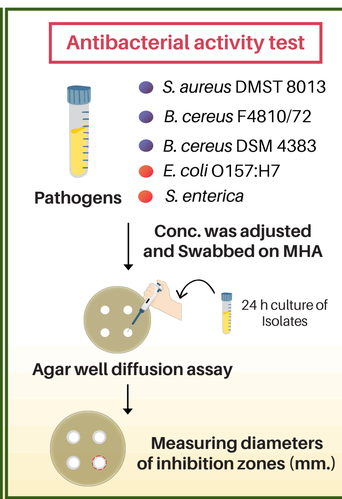


Genetic identification



Testing probiotic properties





Results

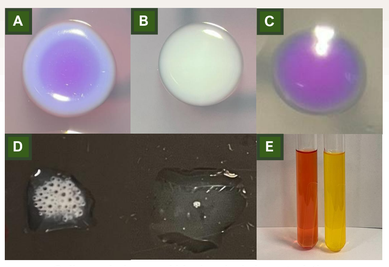
Morphological and biochemical characteristics of bacterial isolates



Chinese water chestnut pulp

LAB
- Gram-positive: 28 bacterial isolates
- Rod- and cocci-shaped
- Catalase-negative
- Lactose fermenters (4 isolates)

Other bacteria
- Gram-positive: 5 isolate (rod shaped)
- Gram-negative: 4 isolate (rod shaped)
- Catalase-positive



Cell morphology and biochemical characteristics of isolates from Chinese water chestnut:
[A] Isolate M101 (Cocci,+); [B] Isolate N102 (Cocci,+); [C] Isolate M107 (Cocci,+); [D] Catalase-positive and negative; [F] Non-lactose fermenting (left), Lactose fermenting (right)

Identification of bacteria in Chinese water chestnut

Isolate	Closest strain	Accession no.	Percent identity
• M101, M104 M107, M109 • N104	*Lactococcus garvieae* FDAARGOS_929	CP065637.1	99.93%
• M103 • N102	*Lactococcus lactis* subsp. lactis T-21	AP038894.1	100.00%
• N118	*Weissella cibaria* Y1-C-4	OK272217.1	100.00%
• N119	*Weissella cibaria* bcpca-qj-3	KX247765.1	100.00%

Lactic acid bacteria

Lactococcus lactis
- Commercial probiotics

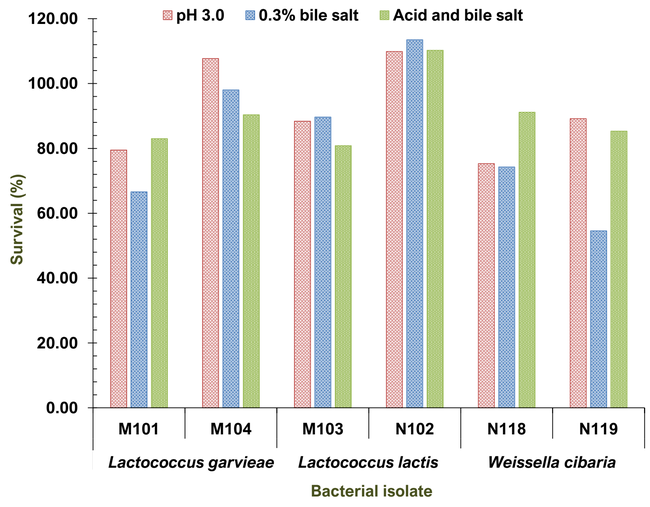
Lactococcus garvieae
- Opportunistic pathogens in humans

Weissella cibaria
- Used in oral care

Probiotic properties

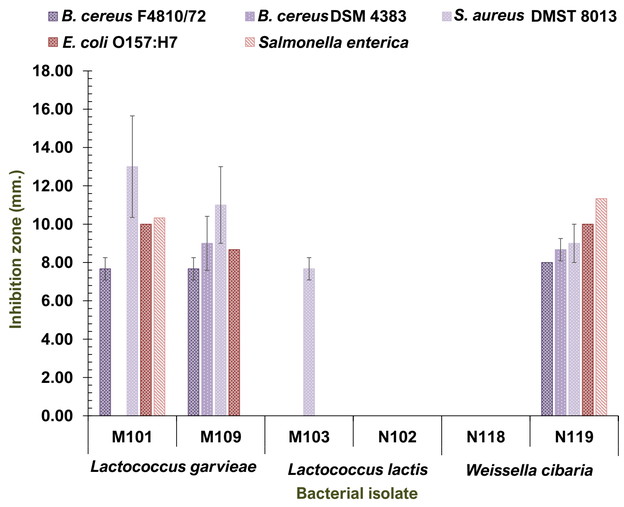
Acid and Bile salt Tolerance

Survival rate ≥ 80%
under pH 3.0 acidic and 0.3% bile salts conditions.



Antibacterial activities

Isolates were found to exhibit inhibitory activity against all five pathogenic strains



Conclusions

- A total of 37 bacterial isolates were recovered from Chinese water chestnuts obtained from three sources.
- The isolates belonged to three lactic acid bacteria (LAB) species: *Lactococcus lactis*, *Weissella cibaria,* and *Lactococcus garvieae*.
- Several isolates showed tolerance to acidic conditions (pH 3.0) and 0.3% bile salts (survival rate ≥ 80%).
- Eight isolates exhibited broad-spectrum inhibitory activities against Gram-positive and Gram-negative pathogenic bacterial strains.
- *Lactococcus lactis* N102 showed the highest tolerance to acid and bile salts, indicating its potential as probiotic candidate.

Acknowledgements

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