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Identification of *Lactobacillus reuteri* P2M1 from Swine and Evaluation of its Probiotic Function *in Vitro*

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INTRODUCTION

The post-antibiotic era, characterized by rising antimicrobial resistance, demands innovative strategies to enhance livestock health. Probiotics, particularly *lactic acid bacteria* (LAB), offer multifaceted benefits, including pathogen inhibition, immune modulation, and oxidative stress mitigation. *Limosilactobacillus reuteri* (*L. reuteri*), a gut-adapted LAB, has shown promise in improving feed efficiency and intestinal barrier function.

AIM

▲Identified the swine-derived *L. reuteri* P2M1 strain through biochemical and molecular methods.

▲Evaluated its probiotic potential, including acid/bile tolerance, antibiotic sensitivity, antioxidant activity, and safety.

METHOD

▲Strain Isolation and Characterization.

The strain isolated from healthy pig feces was biochemically identified, and molecular biological identification was performed using bacterial 16S rDNA sequence PCR amplification and sequencing techniques.

▲Functional Assays.

The growth curve and acid production curve of the strain were drawn to evaluate the tolerance of the strain to acid and bile salts, and the sensitivity of the strain to antibiotics was detected. The free radical-scavenging ability and extracellular polysaccharide production of the strain were determined

▲Safety Evaluation.

The in vitro safety test was performed to evaluate the safety of the strain to mice.

RESULTS

Table 1 Biochemical identification results of strain P2M1.

Items	Results	Items	Results
Sucrose	+	Xylose	-
Ribose	-	Lactose	+
Trehalose	-	Galactose	+
Glucose	+	Cellobiose	+
Melezitose	-	Fructose	-
Indole test	-	Gelatin of liquefaction test	-
Nitrate reduction test	-	Hydrogen peroxide test	-

+: Positive result; -: Negative result.

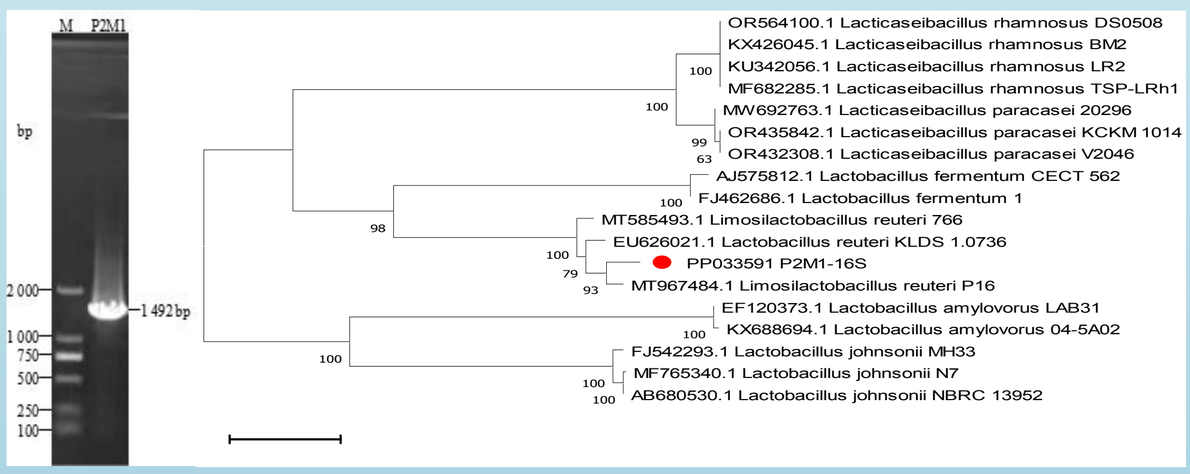

Fig.1 Identification results of strain P2M1 16S rDNA

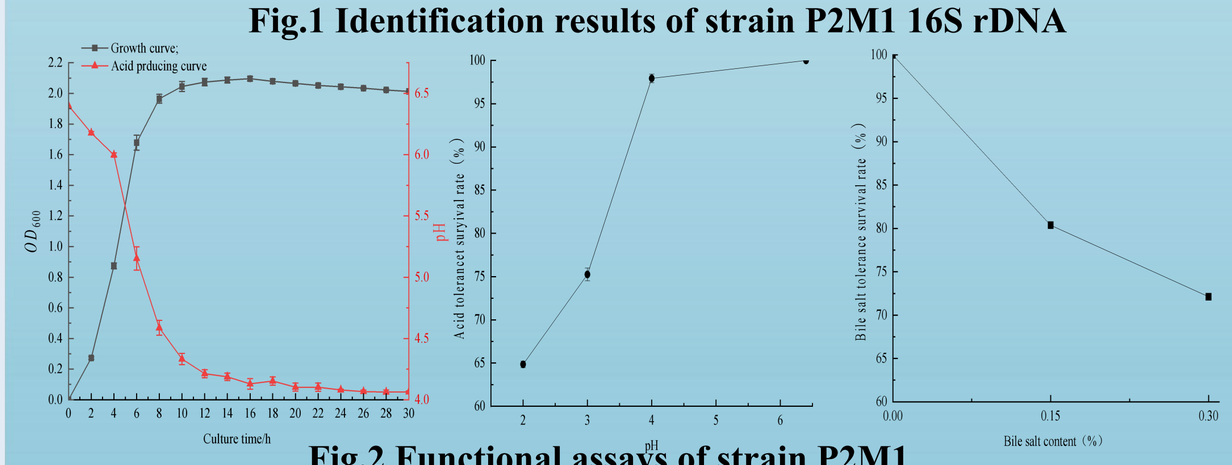

Fig.2 Functional assays of strain P2M1

Table 2 Antibiotic sensitivity of *L.reuteri* P2M1.

Antibiotic	Inhibition diameter	Sensitivity
Amikacin	8.40 ± 0.14	Resistance
Clindamycin	12.43 ± 0.19	Resistance
Erythromycin	20.52 ± 0.08	Sensitive
Lincomycin	13.49 ± 0.30	Resistance
Enrofloxacin	7.59 ± 0.25	Resistance
Roxithromycin	16.89 ± 0.16	Intermediate
Ampicillin	21.72 ± 0.47	Sensitive
Amoxicillin	32.89 ± 0.62	Sensitive
Cefuroxime	22.52 ± 1.11	Sensitive
Cefazolin	27.54 ± 0.51	Sensitive

●The strain P2M1 exhibited glycolysis ability, the logarithmic growth phase was 2-10 h, and the pH was stable between 4.0 and 4.1 after 20 h. It has good low-pH and bile salt tolerance, and is sensitive to common antibiotics.

●The DPPH, ABTS and hydroxyl radical-scavenging rates of the fermentation supernatant of the strain were significantly higher than those of the intact cell suspension (P<0.05), which were 88.79%, 93.52% and 92.15%, respectively.

●The yield of crude extracellular polysaccharide was 339.82 mg/L.

●No death or organ lesions were observed in mice after intragastric administration of 0.2 mL of viable *L. reuteri* P2M1 solution at a concentration of 1×10^9 CFU/mL per day for two weeks.

CONCLUSION

L. reuteri P2M1 has a short logarithmic growth period, rapid growth of cell concentration, high yield of extracellular polysaccharides, good probiotic and antioxidant properties, and safety *in vitro*, and can be used as a candidate strain for probiotic preparations.

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