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Screening of Lactic Acid Bacteria Inhibiting Xanthine Oxidase and Safety Evaluation

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INTRODUCTION & AIM

Hyperuricemia in poultry, characterized by elevated uric acid levels, is a metabolic disorder that can lead to gout and substantial economic losses in the poultry industry. Xanthine oxidase (XOD) is a key enzyme in uric acid biosynthesis, making it a prime target for therapeutic intervention. While chemical XOD inhibitors such as allopurinol are effective, their potential side effects and residue issues limit their use in food-producing animals.

This study aimed to **screen lactic acid bacteria (LAB) strains with potent XOD-inhibitory activity** and **probiotic potential as a safe, natural strategy to reduce uric acid levels in poultry**.

METHOD

1. **Strain Screening**

26 LAB were initially screened for **XOD-inhibitory activity *in vitro***.

Cell-free extracts (CFE) and supernatants (CFS) of the top 4 performers were quantitatively assessed.

2. **Probiotic Characterization**

GI tolerance: 3 h gastric (pH 3) → 3 h intestinal (pH 6.8).

Antibiotic susceptibility profiled (21 antimicrobials).

3. **Identification & Safety**

Strains were identified via 16S rDNA sequencing.

***In Vivo* Safety**: Mice received intraperitoneal injections(10^{10} CFU/mL, 14 days) to monitor acute toxicity.

RESULTS & DISCUSSION

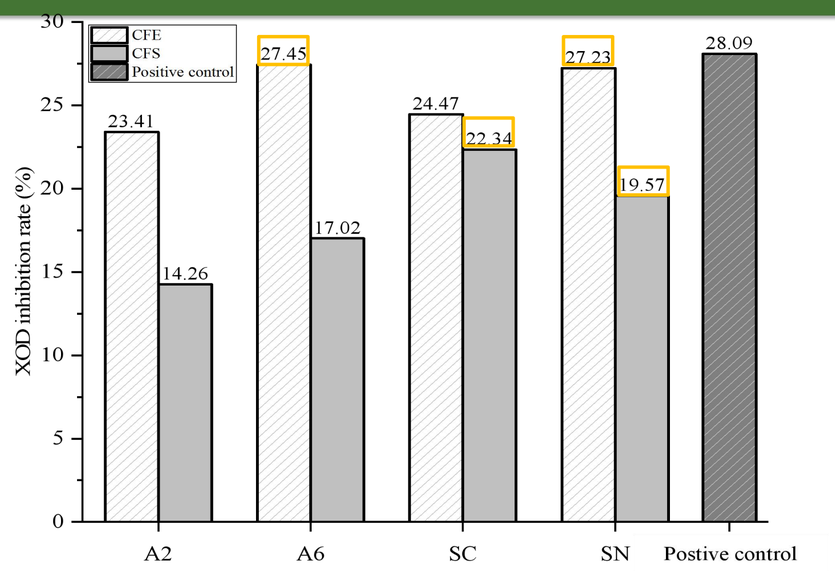

Fig.1 In vitro inhibition of XOD by 4 strains of LAB CFE and CFS.

CFE of the four finalists inhibited **23-27% XOD activity**, statistically on par with allopurinol (28%), indicating **potent enzymatic suppression**.

Table 1 The viable count and survival rate of 4 LAB strains after digestion with artificial gastrointestinal fluid.

Strain	Viable Count (10^8 CFU·mL^{-1})			Survival Rate (%)	
	Gastric fluid treatment (0 h)	Gastric fluid treatment (3 h)	Intestinal fluid treatment (3 h)	Gastric	Intestinal
A2	2.25 ± 0.10^{a}	3.13 ± 0.47^{ab}	3.62 ± 0.07^{b}	**139.11**	**115.65**
A6	2.57 ± 0.21^{a}	2.74 ± 0.07^{a}	2.71 ± 0.21^{a}	106.61	98.91
SC	4.48 ± 0.37^{a}	4.58 ± 0.30^{a}	4.31 ± 0.05^{a}	102.23	94.1
SN	2.49 ± 0.13^{a}	2.44 ± 0.38^{ab}	1.77 ± 0.29^{b}	97.99	72.54

The high survival rates and maintained viable counts after simulated gastrointestinal digestion confirm **strong probiotic potential**, which is a prerequisite for the strains to colonize the host gut and exert their function *in vivo*.

Table 2 Susceptibility test results of 4 LAB strains.

Antibiotic Class	Example Antibiotic	A2	A6	SC	SN
Penicillins	Amoxicillin	S	S	S	S
Cephalosporins	Cefoperazone	S	S	S	S
Macrolides	Erythromycin	S	S	S	S
Lincosamides	Clindamycin	S	I	S	S
Oxazolidinones	Linezolid	S	S	S	S
Aminoglycosides	Tobramycin	R	R	R	R
Quinolones	Enrofloxacin	I	I	R	I
Monobactams	Aztreonam	R	R	R	R

Sensitivity to key antibiotics indicates a **low risk of resistance transfer**, a critical safety attribute for probiotics in poultry.

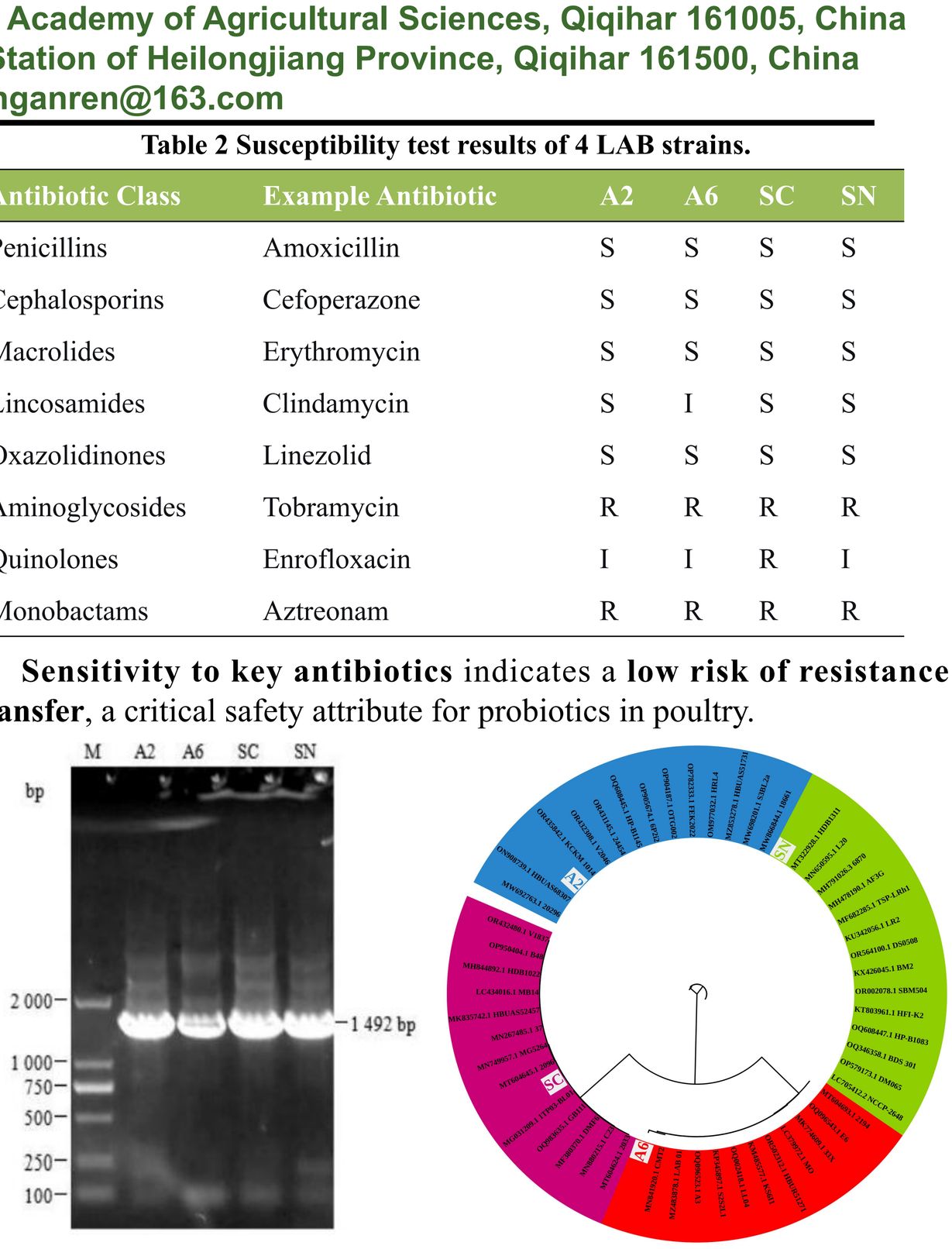

Fig.1 Molecular biological identification results of LAB strains.

Molecular identification classified them as *L. paracasei* (A2), *L. plantarum* (A6), *L. brevis* (SC), and *L. rhamnosus* (SN).

No mortality or organ lesions were observed in mice after 14-day high-dose challenge, confirming absence of acute toxicity.

CONCLUSION

Four Lactobacillus strains (A2, A6, SC, SN) showed **potent XOD-inhibition, GI-robustness, antibiotic susceptibility and no toxicity in mice**, offering ready-to-test probiotic candidates for controlling avian hyperuricemia.

FUTURE WORK

1. Elucidate the Mechanism of Action

Identify and purify the specific intracellular inhibitory compounds through LC-MS and genome mining.

Investigate the interplay with gut microbiota and host pathways (e.g., urate transporters).

2. Validate Efficacy in Poultry Production Models

Conduct a comprehensive 42-day trial with hyperuricemic broilers to confirm uric acid-lowering effects and growth performance.

3. Develop a Commercial Application

Optimize the formulation into a stable, scalable micro-encapsulated feed additive for practical use in the poultry industry.