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Optimization of solid-state fermentation process of Angelicae Pubescentis Radix stems and leaves by probiotics

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INTRODUCTION & AIM

The overuse of **antibiotics in animal husbandry** necessitates the development of **natural alternatives**. Chinese herbal residues, such as **stems and leaves of Angelica pubescentis Radix (APR)**, are often **discarded** despite containing valuable bioactive compounds.

This study aims to **valorize these residues** through **solid-state fermentation** using the probiotic strain ***Bacillus amyloliquefaciens* SSYB**. We focus on enhancing the content of **osthole**-a key bioactive compound with demonstrated **antimicrobial and anti-inflammatory properties**-to develop a **high-value feed additive** and promote **sustainable resource utilization**.

METHOD

1. Strain and Substrate APR stems and leaves were fermented with *B. amyloliquefaciens* SSYB, a cellulase-producing strain isolated from corn silage.

2.Experimental Design The fermentation process was systematically optimized through **single-factor tests** followed by an **$L_9(3)^4$ orthogonal array**, evaluating four critical parameters: fermentation time, temperature, inoculation amount, and water content.

3.Analytical Method Osthole content was quantitatively analyzed using **high-performance liquid chromatography (HPLC)** with a C18 column and UV detection at 330 nm.

RESULTS & DISCUSSION

Table 1. Effects of Fermentation Parameters on Osthole Content

Factor	Optimal Level	Osthole Content (mg/g)	Key Trend
Time	**72 h**	**1.63 ± 0.05**	Increased to a peak at 72 h, then declined.
Temperature	**37 °C**	**1.58 ± 0.05**	37 °C yielded the highest content.
Inoculation	**5 %**	**1.62 ± 0.04**	5 % was optimal for microbial efficiency
Water Content	**65 %**	**1.59 ± 0.05**	Critical for oxygen transfer and nutrient uptake.

The single-factor tests systematically identified the optimal range for each parameter. A consistent trend was **observed where osthole content initially increased to a distinct peak** before declining with further increases in factor levels. The **optimal conditions** were thus determined as **fermentation for 72 hours at 37°C with a 5% inoculation amount and 65% water content**, providing the foundation for orthogonal optimization.

Table 3. Validation of Optimized Fermentation Process

Sample	Osthole Content (mg/g)
Unfermented Control	1.24 ± 0.02
Initial Fermentation Process	1.57 ± 0.02
Optimized Fermentation Process	1.81 ± 0.03

Under the optimum conditions, the content of osthole reached **1.81** mg/g. Compared with the unfermented raw material, it was **significantly increased by 45.97% (P<0.01)**, and the optimization results were successfully verified.

Table 2. Orthogonal Test $L_9(3)^4$ Design and Results

Trial	Time (h)	Temp (℃)	Inoculation (%)	Water Content (%)	Osthole (mg/g)
	A	B	C	D	
1	60	35	4	55	1.58
2	60	37	5	60	**1.73**
3	60	39	6	65	1.58
4	72	35	5	65	**1.75**
5	72	37	6	55	1.71
6	72	39	4	60	1.64
7	84	35	6	60	1.41
8	84	37	4	65	1.51
9	84	39	5	55	1.50
k_1	1.630	1.580	1.577	1.597	
k_2	1.700	1.650	1.660	1.593	
k_3	1.473	1.573	1.567	1.613	
R	0.227	0.077	0.093	0.020	

The order of the influence of various factors on the content of osthol in orthogonal experiment is: **fermentation time>inoculation amount>fermentation temperature>water content**. The optimal combination for fermentation of Angelica sinensis stems and leaves is $A_2B_2C_2D_3$, which is fermentation time of **72 hours, fermentation temperature of 37 ℃, inoculation amount of 5%, and water content of 65%**.

CONCLUSION

The solid-state fermentation process for APR stems and leaves was successfully optimized using *Bacillus amyloliquefaciens* SSYB. The **optimal parameters of 72 h, 37°C, 5% inoculation, and 65% water content** significantly enhanced the **osthole content to 1.81 mg/g**, representing a **45.97% increase** over unfermented materials. This method effectively converts herbal residues into **high-value feed additives**, providing a **sustainable approach** for utilizing non-medical plant parts and supporting antibiotic reduction in animal production.

FUTURE WORK

Process Scale-up: Transition the optimized fermentation process from laboratory to industrial scale for commercial production.

In Vivo Validation: Conduct animal feeding trials to evaluate the efficacy and safety of the fermented product in livestock.

Mechanism Investigation: Elucidate the molecular mechanisms underlying the enhanced osthole release during fermentation.

Synergistic Effects: Explore potential synergistic relationships between osthole and other bioactive compounds in fermented material.