

Tannic acid tolerance and biocontrol activities of *Bacillus velezensis* ML122-2 isolated from Miang leaf



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Abstract

Miang leaves (*Camellia sinensis* var. *assamica* (J.W. Mast.) Kitam.) contains tannic acid, a natural phenolic compound involving flavor, aroma and biochemical properties of the leaves. The traditional fermentation process of Miang, a method of leaf preservation, affects the quantity and structure of tannic acid. This study focused on tannic acid tolerance and biocontrol potential against some bacteria during the fermentation process of *Bacillus velezensis* ML122-2 isolated from Miang leaves. To evaluate the tannic acid tolerance, *B. velezensis* ML122-2 was grown on tryptic soy agar (TSA) supplemented with tannic acid at concentrations of 0.0, 0.2, 0.4, 0.8 and 1.6% (w/v). All plates were incubated at 37°C for 24 hours. The results demonstrated that *B. velezensis* ML122-2 could tolerate all test tannic acid concentrations. Wheng rown *B. velezensis* ML122-2 in tryptic soy broth containing various concentration of tannic acid ranging from 0 - 1.6% (w/v) and incubated at 37°C for 48 hours with culture broth sample collection at time 0, 24 and 48 hours for agar well diffusion assay against *Lactiplantibacillus plantarum*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *S. epidermidis* and *Pseudomonas aeruginosa*. The results exhibited the ability of *B. velezensis* ML122-2 to inhibit growth of *S. aureus* and MRSA. Interestingly, *L. plantarum*, a significant probiotic, was not inhibited by the *B. velezensis* ML122-2 culture broth. These findings highlighted the potential of *B. velezensis* ML122-2 as a biocontrol agent.

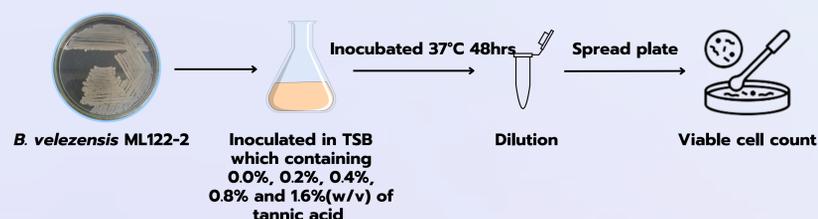
Objectives

- To evaluate the tannic acid tolerance of *B. velezensis* ML122-2 isolated from Miang tea leaves
- To investigate the growth inhibitory activity of *B. velezensis* ML122-2 on *Lactiplantibacillus plantarum*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, MRSA and *S. epidermidis*

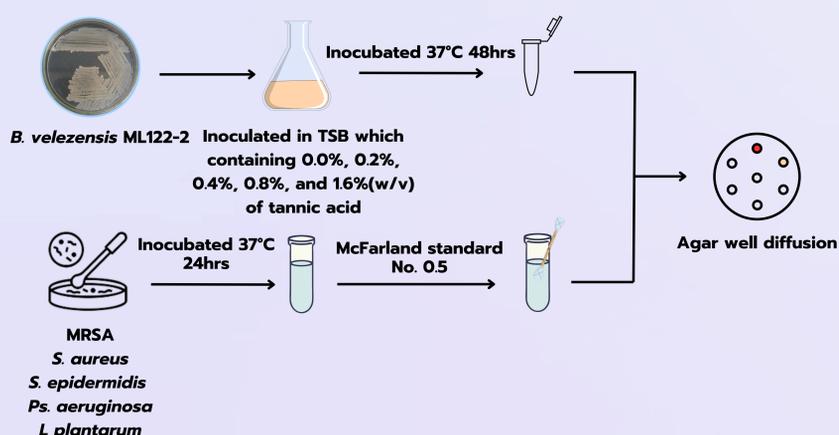
Methods

The preparation of *B. velezensis* ML122-2 began with its cultivation in Tryptic Soy Broth (TSB) at 37°C for 24 hours. The bacterial suspension was then adjusted to an optical density (OD) of 0.1 at 600 nm using a spectrophotometer. The prepared culture was subsequently used to assess its tolerance to tannic acid and its potential as a biological control agent.

Tannic acid tolerance



Antimicrobial activity (agar well diffusion)



Results and Discussions

1. Tannic acid tolerance

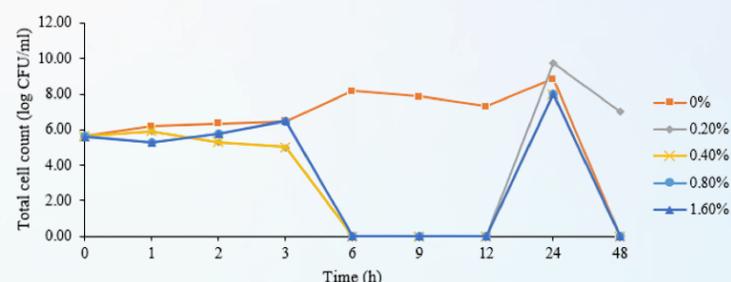


Figure 1 The survival of *Bacillus velezensis* ML122-2 cultured in TSB medium with different concentrations of tannic acid at 37°C for 48 hours

B. velezensis ML122-2 tolerated tannic acid concentrations of 0.2%–1.6% (w/v) for up to 3 hours but died after 6 hours due to toxicity. However, growth resumed after 24 hours, indicating that the bacteria survived by forming spores as a protective mechanism.

2. Biological activities

Table 1 Antimicrobial activity against pathogenic microorganisms was assessed using the culture supernatant of *Bacillus velezensis* ML122-2 incubated at 37°C for 48 hours

Tannic acid concentrations (w/v)	Inhibition zone (mm)			
	MRSA	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>Ps. aeruginosa</i>
0.0	18.8 ± 0.5	14.5 ± 0.3	0	0
0.2	18.4 ± 0.1	23.1 ± 0.6	9.5 ± 0.7	16.5 ± 0.2
0.4	15.0 ± 0.6	13.5 ± 0.3	9.7 ± 0.7	17.9 ± 0.5
0.8	21.0 ± 0.4	16.8 ± 0.7	14.5 ± 0.7	24.5 ± 0.3
1.6	24.5 ± 0.3	22.0 ± 1.0	13.6 ± 0.6	33.6 ± 0.1
Gentamicin	12.8 ± 0.2	28.0 ± 0.3	28.0 ± 0.4	32.1 ± 0.7



Figure 2 Inhibition zone of *Bacillus velezensis* ML122-2 supernatant against skin pathogens: Positive control (P) and Negative control (N)

The study found that the supernatant of *B. velezensis* ML122-2, grown in TSB with varying tannic acid concentrations, did not inhibit *L. plantarum* growth in the agar well diffusion test.

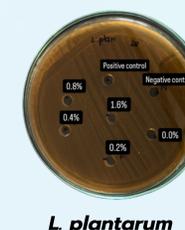


Figure 3 Inhibition zone of *Bacillus velezensis* ML122-2 supernatant against *Lactiplantibacillus plantarum*: Positive control (P) and Negative control (N)

Conclusion

The results showed that *B. velezensis* ML122-2 could tolerate tannic acid for 24 hours and produce antimicrobial compounds to inhibit MRSA and *S. aureus* without affecting the growth of *L. plantarum*. Therefore, *B. velezensis* ML122-2 can be considered a biocontrol agent in the Miang fermentation process.

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Reference

Rungsirivanich, P., et al. (2021). Simultaneous production of multiple antimicrobial compounds by *Bacillus velezensis* ML122-2 isolated from Assam tea leaf (*Camellia sinensis* var. *assamica*).