ZIBELINE INTERNATIONAL™ PUBLISHING
ISSN: 2773-6202 (Online)
CODEN: JTIOCC

Journal of Technology & Innovation (JTIN)

DOI: http://doi.org/10.26480/jtin.01.2021.20.22

RESEARCH ARTICLE

USE OF LACTIC ACID BACTERIA IN PEANUT SEED TREATMENT

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ARTICLE DETAILS

Article History:

Received 15 January 2021
Accepted 19 February 2021
Available online 2 March 2021

ABSTRACT

Three lactic acid bacteria (LAB) strains, Lactobacillus sp. L5, Lactobacillus sp. L3, Lactobacillus sp. L2N, isolated from Vietnamese traditional Nem chua grew well in cabbage broth supplemented with 12 g/L glucose and 15 g/L peptone and showed inhibitory activity ranging from 40% to 44% against Aspergillus sp. CDP isolated from mould contaminated peanuts, while Daconil 75WP – a fungicide compounds – as a positive control showed only 26.9%. LAB strains also displayed Indole-acetic acid (IAA) production, P-solubilization and biofilm formation. Soaking seeds in separate cabbage based culture broth of three LAB strains with/without heat treatment and mixed culture broths at the ratio 1:1:1 with the total bacterial count 108cfu/mL exhibited the antifungal activity of mixed cultures in both cases with or without bacterial culture heat treatment. Soaking seeds in the same mixed bacterial cultures without heat treatment increased seed germination and vigor index, compared to the control seeds without any treatment and those treated with fungicide compounds. After 75 days of sowing the length and total fresh weight of LAB-treated peanut plants increased by 22.4 % and 99.6%, higher than that of Daconil treated ones with only 15.9% and 59.7% increment. Moreover, the fresh yield of peanut pegs increased 2.5 times, compared to those of untreated and Daconil treated seeds. This study suggested that seed treatment with LAB is a novel technology towards organic farming to replace fungicide used in conventional agriculture.

KEYWORDS

Antifungal, biofilm, Indole-acetic acid, PGPB, seed treatment.

1. INTRODUCTION

LAB are usually used for starter cultures in food fermentation and have received much attention, especially because of their GRAS status. Besides that, LAB, especially lactobacilli have been used for decades in agriculture as they are the dominant group in EM products [6]. There are many studies showing the antifungal activity of LAB. Recently, there have been several reports about the plant growth promoting (PGP) traits of LAB such as the synthesis of auxin indole-3-acetic acid (IAA) or P-solubilization. Furthermore, colonizing the plant root, LAB can form biofilm due to their extracellular polysaccharides providing a physical barrier to protect root plants against pathogens (Bogino et al., 2013). In the trend to develop organic agriculture, this work aimed to isolate antifungal LAB from traditional foods, especially against aflatoxigenic moulds such as Aspergillus spp. and to use them in seed treetment, which stimulates the seed germination, improves the seed vigor index, along with promotes the plant growth.

2. MATERIALS AND METHODS

2.1 Materials

Vietnamese traditional food nem chua was used to isolate LAB; mould contaminated peanut seeds were the source for fungal isolation, Daconil Fungicide 75WP (75% Chlorothalonil, SDS Biotech, Japan) was used as positive control in antifungal assay.

2.2 Methods

2.2.1 Isolation

LAB strains were isolated from Vietnamese traditional Nem chua in MRS agar. Fungi were isolated in PDA agar. Macroscopic and microscopic of pure cultures were examined. For bacteria, gram staining, spore staining, motility test, catalase test and sugar fermentation were carried out to identify them at the genus level.

2.2.2 Selection of alternative culture medium

Cell density determination

LAB were cultured on the de Man Rogosa Sharpe (MRS) Broth and then inoculated into cbbage extract (500g bean sprout or cabbage in 1000 mL water then boiled at 100°C in 20 minutes, add 12 g glucose and 15 g peptone). To determine the number of bacteria in sample at difference time, using the bacteria standard curve, absorbance at 600nm increases as the number of bacteria increases.

IAA analysis

Quick Response Code	Access this article online	
	Website: https://jtin.com.my	**DOI:** 10.26480/jtin.01.2021.20.22

Cite the Article: Hoai Huong Nguyen and Bich Tram Tran Le (2021). Use Of Lactic Acid Bacteria In Peanut Seed Treatment. *Journal of Technology & Innovation, 1(1): 20-22.*

All the strains was grown in three culture broths and incubated at 37°C overnight, then centrifuged at 8000 rpm for 15 min and the concentration of IAA produced by the bacteria was calculated from a standard curve of IAA obtained in the range of 0-25 µg/mL by measuring the absorbance of samples and standards at 530 nm (Mohite, 2013).

Biofilm formation assay

This assay was adapted from George A. O'Toole method (O'Toole, 2011).

In vitro antifungal test assay

1cm² *Aspergillus* sp. CĐP plug was inoculated in the center of a plate on modified MRS Agar, isolated bacteria were cultured in three culture media at 37°C for 24 hrs then the antifungal asssay was performed (Giassi et al., 2016), The control was the petri dish without bacterial inoculation.

$$Inhibitory\ activity\ (\%) = \frac{(\text{mould diameter Control} - \text{mould diameter Treatment}) \times 100\%}{\text{mould diameter Control}}$$

2.2.3 Application of isolated bacteria in peanut seed treatment

In vivo antifungal assay

LAB were cultured in culture broths at 37°C for 24 hours, cell density was adjusted to 10^8 CFU/mL then treated in different conditions: No heat and 100°C in 15 minutes. Peanut seeds were washed with water and drained and soaked in bacteria mixture which were mixed in proportions 1:1:1 from three bacterial culture broths. After 15 minutes, seeds were dried and placed into 100 mL autoclaved glass bottle (12 g in each), inoculated with 10^2 CFU/g of fungal spores and stored at room temperature. The time of first appearance of mycelia was recorded.

Effects of bacterial seed inoculation on germination and development of seedlings

Peanut seeds were soaked for 15 min in bacterial mixture which were mixed in proportions 1:1:1 from three bacterial culture broths and then put in petri dishes for germination. The seedlings were sown in unsterilized Tribat natural soil pH 5.5-6.5, using NPK 30-10-10 for top dressing, the first time after 15 days and the second time and after the blossom. Plantlets including the roots were harvested and fresh shoot weight, fresh root weight, shoot length, root length were measured after 7, 14, 30, 75 days, The seedling vigour index was calculated by using the formula SVI = (mean root length + mean shoot length) × Germination percentage (Murthy et al., 2012).

2.2.4 Statistical analysis

All the experiments followed a randomized design with three replicates each. Data were compared by Least Square Difference (LSD) and Duncan's Multiple Range Test (DMRT). Statistical analysis was performed using SAS software package, version 9.4.

3. Results and Discussion

3.1 Isolation of LAB and fungal indicator

LAB strains L5, L3 and L2N were isolated from Vietnamese traditional Nem chua. The colonies of three strains were round, smooth, opaque white with convex edge. Besides, they are rod-shaped, gram-positive (Fig. 1A, 1B, 1C), non-sporulating, non-motile, Catalase-negative, changed Uffelmann's reagent from violet to yellow color, able to ferment glucose, fructose, mannitol, lactose, maltose and sucrose. These bacterial strains displayed characteristics of *Lactobacillus* genus according to Bergey's manual of Systematic Bacteriology.

Peanut seeds contaminated with green mold were used for fungal indicator isolation as they are usually contaminated with aflatoxigenic *Aspergillus flavus* and *A. parasiticus.* A fungal strain named CĐP was isolated on PDA plates from mold contaminated peanut seeds. Colonies of CĐP on PDA grew rapidly at room temperature, developing green conidia. Microscopic observation of sample taken from slide culture technique of isolated fungus suggested that CĐP strain belong to the genus *Aspergillus*, based on their presence of conidiophores, vesicles and phialides (John et al., 2009) (Fig. 1D).

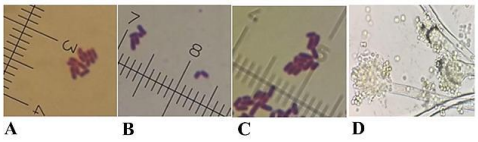

Figure 1: LAB isolated from Nem chua and fungus isolated from contaminated peanuts under microscope (A - *Lactobacillus* sp. L5, B - *Lactobacillus* sp. L3, C - *Lactobacillus* sp. L2N, D - *Aspergillus* sp. CĐP)

3.2 Screening for alternative culture media

MRS Broth is considered cost effective for biomass production scale. That was the reason the present study aimed to reduce the medium cost by replacing vitamin source from yeast extract by plant extract, as sugested from the literature (Chen et al., 2015). In this study, we used mungbean sprout broth and cabbage broth supplemented with glucose and peptone.

3.2.1 Cell density determination

Compared to MRS Broth, bacterial culture density in mungbean sprout broth and cabbage broth reached approximately 92.67% and 91.0%, respectively. There was no difference between cell densities in two alternative media.

3.2.2 IAA synthesis

Figure 2 shown that all three strains L5, L3, L2N unanimously gave the best IAA production when being cultured in MRS Broth, this result was clearly better than that stated by Giassi et al, 2016, whose highest production concentration was recorded at 5.46 µg/mL. The ability to produce IAA of three strains was found to be mostly better in cabbage broth than that in mungbean sprout broth. The IAA production depends on the Trp concentration in the media, so it depends on protease activities of strains and free amino acid concentration in culture broths.

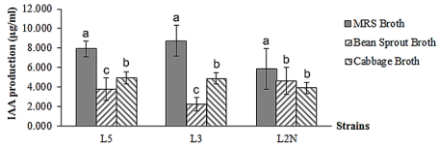

Figure 2: IAA synthesis (µg/mL) of LAB strains in three types of culture media

3.2.3 Biofilm formation

The biofilm formation of PGPB is expected to support them to adhere onto the seed and root surface, therefore to protect the root against the invasion of pathogens and abiotic stress from the environment. Results after 24 hours of culture affirmed that Cabbage broth could boost biofilm formation of all strains based on the absorbance of samples at 550nm (Fig. 3). LAB biofilm exopolysaccharides are usually heteropolysaccharide (Ryan et al., 2015), therefore their formation depends on the composition of monosaccharides in the culture media, which are evidently more abundant in plant extract than MRS containing only glucose as monosaccahride.

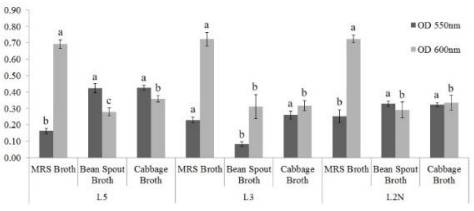

Figure 3: OD 550 nm (biofilm) and OD 600 nm (free cells) of LAB strains in three types of culture media

3.2.4 In vitro antifungal test

All three strains cultured in 3 media MRS, mungbean sprout broth and cabbage broth showed remarkably higher antifungal activity against *Aspergillus* sp. CĐP than the possitive control – Daconil 75WP 0.5g/L (Fig. 4). There were no significant difference in antifungal activities neither among strains nor among culture media.

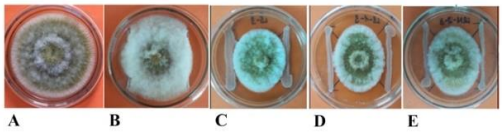

Figure 4: *In vitro* antifungal assay: A) negative control (fungus without antifungal agent), B) fungus with positive control with Daconil, C) fungus with L5,D) fungis with L3, E) fungis with L2N (LAB were cultured in MRS Broth).

The IAA production and biofilm formation were clearly better when bacteria were cultured in cabbage broth. Combining with the fact that no significant difference between the two plant extract media in terms of antifungal activity and cell density, the cabbage broth stands out as the most suitable alternative culture medium.

3.2.5 In vivo antifungal assay

Table 1: The time of mycelia appearance in in peanut seeds after treatment with bacterial culture broth from cabbage broth (days)

Formula	Without heat treatment	Heat treatment 100°C, 15 min
Negative control – *Sterillized water*	$1,7^{cd} \pm 0,6$	
Positive control - Daconil	$5,2^{a} \pm 1.2$	
L5	$4,7^{ab} \pm 2,9$	$1,0^{d} \pm 0,0$
L3	$2,0^{cd} \pm 0,0$	$2,0^{cd} \pm 0,0$
L2N	$1,3^{cd} \pm 0,6$	$2,0^{cd} \pm 0,0$
L5:L3:L2N (1:1:1)	$4,3^{ab} \pm 0,6$	$3,0^{bc} \pm 0,0$

Data are presented as average values ± standard deviation

Means in the same column with different letters are significantly different at $p < 0.05$, DRMT

Table 1 displayed different antifungal activities of 3 strains in vivo assay when seeds were artificially infected with 10^2 conidia/g seeds in spite of similar in vitro test results. After heat treatment, the antifungal activity of L5 decreased sharply, however that of L3 and L2N remained nearly the same, leading to the rather high antifungal activity of the mixture of 3 culture broths at the ratio 1:1:1 in both cases with and without heat treatment. In fact, antifungal compounds are in a mixture which can action in synergy. From that point of view, the mixture of 3 broths, cultured in cabbage broth medium and mixed at the ratio 1:1:1 was used for seed priming before germination.

3.2.6 Effects of bacterial seed inoculation on germination and development of seedlings

Seed germination ratio improved 15.0% and 21.7% compared those soaked in Daconil solution and water, respectively. Meanwhile, seedling vigor index (SVI) increased 87.1% when seeds were primed in LAB mixture compared to untreated.

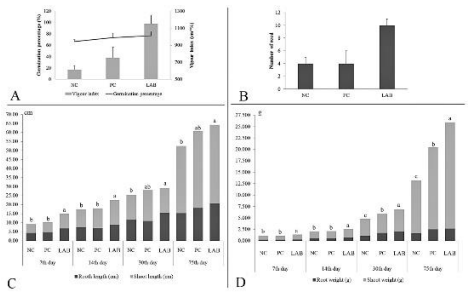

Figure 5: Development of peanut seedlings. A) Germination and seed vigor index, B) Quantity of pegs after 75 days, C) Root and shoot length (cm), D) Root and shoot fresh weight (g). (NC: negative control: seeds soaked in distilled water, Da: seeds soaked in Daconil solution; LAB: seeds soaked in LAB culture. *Means in same day with different letters are significantly different at p < 0.05*

The results are similar to those reported by Murthy *et al.* [8] using lactic acid bacteria to inoculate tomato seeds with the increase of germination ratio from 76,1% to 79,7%, and the seed vigor index from 700 to 1130. Meanwhile, inoculation of seeds with *Trichoderma viride* and *Pseudomonas fluorescens* (Dolas et al., 2018) augmented the germination ratio and seed vigor index only over 10,37% and 27,15%, respectively. Therefore, it is clear that LAB culture broth contains different compounds expressing different PGP mechanisms. The role of LAB in seed treatment was demonstrated better during the development of plantlets, which are displayed in Fig. 6B, C, D with the difference compared to the controls in root and shoot legnth, root and shoot weight and especially the quantity of peanut pegs after 75 days. After 75 days of sowing the length and total fresh weight of LAB-treated peanut plants increased by 22.4 % and 99.6%, higher than that of Daconil treated ones with only 15.9% and 59.7% increment. Moreover, the fresh yield of peanut pegs increased 2.5 times, compared to those of untreated and Daconil treated seeds. These results could be explained by the capacity of IAA production and biofilm formation of LAB leading to the exploitation of LAB in the future for production of PGPB products in order to replace chemical compounds used in agriculture.

4. Conclusion

Cabbage broth (supplemented with 12 g/L glucose and 15 g/L peptone g) was chosen as an alternative medium to culture LAB strains of L5, L3 and L2N with similar antifungal activity, IAA production, and biofilm formation as obtained in MRS broth. The seed inoculation with culture broth mixture at the ratio 1:1:1 of L5: L3: L2N significantly stimulated the germination, growth and fruit yield potential leading to LAB application in replacement of chemical compounds used in conventional agriculture. Further studies should be carried out to assess the effect of LAB on other plant hosts and elucidate the nature of antifungal compounds, in order to facilitate the selection of PGP LAB strains.

References

Bogino, P.C., Oliva Mde, L., Sorroche, F.G., Giordano, W. (2013). The role of bacteria biofilms and surface components in plant bacterial associations. Int J Mol Sci, 14:15838-15859.

Chen, B., Wang, X., Zhang, L. 2015. United States Patent No US 9,040,037 B2 https://patents.google.com/patent/US9040037B2/en

Dolas, R.M., Gawade, S.B., Kasture, M.C. 2018. Efficacy of seed treatment of fungicides, bio agents & botanicals on seed mycoflora, seed germination and seedling vigour index of mung bean. *J Pharmacogn Phytochem*, 7: 1074 – 1077.

Giassi, V., Kiritani, C., Kupper, K.C. 2016. Bacteria as growth-promoting agents for citrus rootstocks. *Microbiol Res*, 190: 46-54.

John, I.P., Hocking, A.D. 2009. *Aspergillus* and related telemorphs. In: Fungi and food spoilage, 3rd edn. Springer Dordrecht Heidelberg London New York, pp 275-338

Lamont, J., Wilkins, L., Bywater-Ekegärd, M., Smith, D.L. 2017. From yogurt to yield: Potential applications of lactic acid bacteria in plant production. Soil Biol Biochem, 111:1-9.

Mohite, B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr, 13: 638-649.

Murthy, K.N., Malini, M., Savitha, J., Srinivas, C. 2012. Lactic acid bacteria (LAB) as plant growth promoting bacteria (PGPB) for the control of wilt of tomato caused by *Ralstonia solanacearum*. Pest man hort eco, 18: 60-65.

Ryan, P.M., Ross, R.P., Fitzgerald, G.F., Caplice, N.M., Stanton, C. 2015. Sugar-coated: exopolysaccharide producing lactic acid bacteria for food and human health applications. Food Funct. 6:679-93. http://doi: 10.1039/c4fo00529e.

O'Toole, G.A. 2011. Microtiter dish biofilm formation assay [online], viewe d 28th September, 2018, from < https://www.ncbi.nlm.nih.gov/pmc/ articles/PMC3182663/ >.