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Investigation of the Antifungal Mechanism Activity of *Lactobacillus plantarum* NCDN4 against *Aspergillus niger* and Its Application in Brown Rice Germination

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Abstract

Germinated brown rice has a higher nutritional value than brown rice due to the germination process, which enhances nutrient content. However, this product is easily contaminated by mold during germination, leading to food safety concerns. The aims of this study were to evaluate the antifungal ability of Lactobacillus plantarum NCDN4 lactic acid bacteria against Aspergillus niger CBS 76997 during the germination of brown rice. The in vitro antifungal activity of L. plantarum NCDN4 was assessed in agar and liquid medium. The effect of the bacterial cell-free supernatant and/or biomass on the growth of A. niger was determined based on the mycelial growth diameter of fungi over time. The research results indicated that the mycelial diameter of A. niger treated with L. plantarum ranged from 15 – 30 mm, lower than the control sample (85mm) after five days of incubation. L. plantarum also delayed the formation of fungi spores. The preliminary results showed that the antifungal mechanism of L. plantarum related to the lactic acid produced during bacteria metabolism, delayed and reduced spore formation, nutrient competition and damaged mycelium of A. niger. L. plantarum reduced the mould contamination rate during the germination of brown rice without affecting the germination of rice, with a germination ability of 94.7%. The mould contamination rates in the samples non-treated and treated with LAB were 100.0% and 15.7%, respectively. The results demonstrated the potential application of L. plantarum in food technology as a biocontrol agent in inhibiting fungi.

Keywords: *Aspergillus niger*, antifungal activity, germination, *lactobacillus plantarum.*

1. Introduction

Germinated brown rice is nutritionally valuable due to its high content of nutrients and biological activities such as ferulic acid, γ-oryzanol, and gamma-aminobutyric acid. The germination process of brown rice positively impacts the amino acid composition and the available protein content, increasing the total sugar content and bioactive components while reducing the levels of anti-nutritional factors. Germinated brown rice and its products help prevent certain diseases such as obesity, hyperlipidemia, cancer, diabetes, cardiovascular diseases, and Alzheimer's [1].

Moisture is a crucial factor in the germination process of brown rice. However, the presence of some fungal spores belonging to *Aspergillus* and *Penicillium* in the rice, along with the germination process at high moisture levels, makes germinated brown rice susceptible to fungal contamination during germination, potentially leading to the formation of mycotoxins harmful to human health.

In the context of a demand for "chemical preservative-free" food products, bio-preservation appears as a promising alternative to replace or reduce chemical preservatives. In this scenario, scientific interest in microbial cultures with bioprotective functionality has been intensified, and the market for this type of culture is continuously rising [2]. When bioprotective cultures are added with the aim of bio-preservation, the use of chemical preservatives in food can be reduced or even eliminated.

Among microorganisms most used for bio-protection purposes, lactic acid bacteria (LAB) stand out based on their capacity to produce several antimicrobial compounds, such as organic acids, fatty and volatile acids, reuterin, diacetyl, bacteriocins, peptides and/or low molecular weight proteinaceous compounds. For antifungal effects, many molecules

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are likely to act synergistically as lactic acid and acetic acids; in cheese and yoghurt, LAB produces a vast array of antifungal compounds [3].

However, in Vietnam as well as globally, the use of lactic acid bacteria as preservatives remains limited. *Lactobacillus plantarum* NCDN4 showed probiotic activities such as survival in intestinal and antibacterial activities against pathogenic bacteria such as *Salmonella* Typhimurium [4]. However, the antifungal capability of this strain has not been reported. Therefore, this study was conducted with the aim of (1) evaluating the antifungal activity of *L. plantarum* NCDN4 against *Aspergillus niger*, and (2) applying the results obtained in the germination process of brown rice.

2. Materials and Methods

2.1. Materials

Lactobacillus plantarum NCDN4 was collected from the Department of Food Technology, Hanoi University of Science and Technology laboratory. *Aspergillus niger* CBS 76997 was provided by Food Industries Research Institute. These strains were kept in 20% glycerol solution and stored at –80 ºC. Huyet Rong brown rice (Vietnam) was used to produce germinated brown rice.

2.2. Methods

2.2.1. Preparation of LAB culture and cell-free supernatant

L. plantarum NCDN4 was activated by inoculation twice in De Man–Rogosa–Sharpe (MRS) broth (HiMedia, India) at 30 ºC for 24 hours. After incubation, the cell culture was determined for pH, total acid content and optical density at wavelength 600 nm.

Bacteria culture after the second activation was centrifuged at 6000 g, at 6 °C for 10 minutes to separate the biomass and supernatant (Hermle, Germany) then removed the bacterial biomass deposited below after centrifugation, and filtered the supernatant through 0.22 µm pore size (Millipore, Merck) to obtain the cell-free supernatant (CFS).

A. niger was activated in Yeast Peptone Dextrose (YPD) agar (HiMedia, India). After 5 days of culture, *A. niger* mature mould spores were collected with saline water NaCl 0.9% and diluted to a concentration of 10^7 spores/ml using a hemocytometer.

2.2.2. Assessment of antifungal activity of L. plantarum by double layer method and spot inoculation method

The antifungal activities of *L. plantarum* NCDN4 against *A. niger* were determined as previously described [5]. Three samples were conducted, including:

- CFS: cell-free supernatant of *L. plantarum* NCDN4 using the spot inoculation method
- S: biomass of *L. plantarum* NCDN4 using double layer method
- CFS-S: combination of CFS with biomass of *L. plantarum* NCDN4 using double layer method

For CFS-S sample, a combination of spot inoculation and double-layer methods was used. Specifically, the activated *L. plantarum* NCDN4 was evenly spread on the surface of MRS agar and incubated for 24 hours at 37 °C. Subsequently, the second agar layer containing 10% v/v CFS of *L. plantarum* NCDN4 was mixed into soft YPD agar, and the fungal was inoculated.

The mycelial growth diameter was used to evaluate the antifungal activity of LAB. The assays were performed in triplicate.

2.2.3. Determination of the minimum inhibitory concentration and the minimum fungicidal concentration of CFS by microdilution method

The microdilution method was used to determine the minimum inhibitory concentration (MIC) value with slight modification [6]. A total volume of 600 µL solution consisting of *L. plantarum* NCDN4 CFS, spore suspension and YPD broth was added to each microwell. First, 540 µL of diluted CFS was put into different concentrations using YPD medium, and YPD broth was pipetted into each microwell. Then, 60 µL of prepared spore suspension (10^7 cells/mL) was pipetted into each microwell so that the final spore density of all microwells was 10^6 cells/mL. Positive and negative controls were prepared under the same conditions. Positive control microwell consisted of 540 µL of YPD broth and 60 µL spore suspension. The negative control microwell comprised 540 µL YPD broth and 60 µL sterile distilled water. Amphotericin B at the concentration of 1.6 mg/L was used to compare the antifungal activity. After that, the microplates were incubated at 30 °C for five days. The concentration of bacterial CFS in the microwell in which there was no visible fungal growth was the minimum inhibitory concentration – MIC value.

To determine the minimum fungicidal concentration (MFC) value, 10 µL of the concentration corresponding to the MIC and higher concentrations evaluated were recultivated on YPD agar. The recultivated cultures were then incubated again at 30 °C for 3 days for observation. The MFC value was determined as the concentration of CFS that avoided any visible fungal growth of the recultivated culture [6].

2.2.4. High-performance liquid chromatography method for organic acid analysis

The high-performance liquid chromatography

(HPLC) method was used to determine the organic acid profile of bacterial CFS. The CFS was filtered through a 0.20 µm microbiological membrane (Sartorius) before undertaking HPLC analysis. The sample was then diluted with HPLC grade water (Merck) in the appropriate ratio so that the concentration of targeted substances in the sample is greater than 0.1 g/L and less than 1 g/L. Standard samples of lactic acid, acetic acid, propionic acid, butyric acid and formic acid at concentrations of 0.1, 0.5, and 1 g/L were used to establish the standard curve. MRS broth, after sterilization and centrifugation at 10000 rpm, 4 °C for 4 minutes, was used as the control sample.

HPLC analysis was performed using an RID detector with 10 mM H_2SO_4 as eluent at a flow rate of 0.5 mL/min through an Aminex HPX-87H column (Bio-Rad) maintained at 60 °C. The time to analyze a sample was 30 minutes. The sample volume included in the analysis was 20 µL. The peak signal of the substances was shown on the chromatogram. The chromatography of tested samples was compared to that of standard substances for the qualitative and quantitative analysis of the targeted compounds.

2.2.5. Influence of LAB on fungal biomass production in liquid mMRS medium

0.1 mL of activated *L. plantarum* NCDN4 cell culture at 10^6 CFU/mL and 0.1 mL of spore suspension at 10^6 spores/mL were added in 50 mL of sterilized mMRS medium (Table 1) in a conical flask and were incubated for 10 days at 30 °C. Subsequently, on days 3rd, 5th and 10th, the mycelium was separated from the medium by filtration on filter paper (No 1. Whatmann), thoroughly washed with ethyl acetate, dried in an oven at 95 °C until constant weight, and the dry mass was determined. Cultures of the fungus grown without LAB were used as the control [7].

Table 1. mMRS medium composition

Composition	Ratio
Peptone	10g/L
Beef extract	10g/L
Yeast extract	5.0g/L
Dextrose (Glucose)	20g/L
$MgSO_4$	0.1g/L
$MnSO_4$	0.05g/L
K_2HPO_4	2g/L

2.2.6. Evaluation the effect of L. plantarum NCDN4 during germination of brown rice

One hundred grains of Huyet Rong brown rice were soaked in sterilized distilled water at 44 °C for 12 hours, with water changed every 6 hours before germination. After soaking, the grains were dried and evenly distributed in a Petri dish (diameter 90 mm) lined with two sterilized filter papers. They were then treated with different treatments and allowed to germinate in a chamber with constant temperature and humidity (HWS-150, China) at 30 °C for 72 hours. During germination, the grains were sprayed with distilled water every 6 hours to maintain moisture levels [1].

The treatment samples consisted of **Control** (untreated brown rice); **M1** (Sprayed with 340 µL of CFS + 10^6 CFU/mL of *L. plantarum* NCDN4); **M2** (sprayed with 340 µL of CFS + 10^6 CFU/ml of *L. plantarum* NCDN4 + 10^6 spores/ml of *A. niger* CBS 76997) and **M3** (Sprayed with 10^6 spores/ml of *A. niger* CBS 76997).

The germination rate of the rice grains and the mould contamination rate were evaluated during the germination process. The germination rate was calculated using the formula:

$$\text{GR} = \frac{N}{M} \times 100\% \quad (1)$$

Where: *N* is the number of germinated grains and *M* is the total number of grains.

The mould growth status and the number of mould-infected grains were counted daily and monitored continuously for three consecutive days. The mould contamination rate was calculated using the formula:

$$MR\ (\%) = \frac{N}{M} \times 100\% \quad (2)$$

Where: *N* is the number of mold-infected grains and *M* is the total number of grains.

3. Results and Discussion

3.1. Characteristics of L. plantarum NCDN4

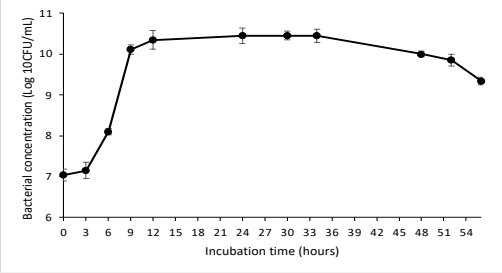

Fig. 1. *L. plantarum* NCDN4 growth curve

The growth curve of *L. plantarum* NCDN4 was established (Fig. 1), and the bacteria entered the stationary phase after 24h of incubation, reaching optical density of 2.585 (equivalent to 2.83. 10^{10} CFU/mL). At this point, the *L. plantarum* NCDN4 cell culture had pH 4.02, total acid content 21.6 g /L (lactic acid equivalent by titration method).

Sathe *et al*. (2007) reported that the antifungal activity of CFS from *L. plantarum* depends on the distinct phases of the growth curve of the microorganism, reaching highest at the end of the exponential (log) phase and gradually decreasing in the late stationary phase [8]. Therefore, selecting the time point of 24 hours after inoculation, when the bacteria begin the equilibrium phase, for conducting experiments was appropriate.

The CFS of *L. plantarum* was analyzed using HPLC method with lactic acid, acetic acid, propionic acid, and butyric acid as the standard substances. The organic acid profile of the bacteria was presented in Table 2. Indeed, the bacterial CFS contained a large amount of lactic acid (18.86 g/L), followed by acetic acid (0.17 g/L) and propionic acid (0.02 g/L). This aligns with the findings of Russo *et al*. (2017), who demonstrated that lactic acid levels produced by two *L. plantarum* strains increased exponentially and reached about 25 g/L after 24 hours [9].

Table 2. Organic acid profile of *L. plantarum* NCDN4 supernatant

Organic acid	Content (g/l)
Lactic acid	18.86
Acetic acid	0.17
Propionic acid	0.02
Butyric acid	0

These organic acids are the antifungal metabolites [3], which can explain the mold-inhibitory effects of this bacteria. The most reported antifungal activity of LAB was generally associated with producing organic acids, which results from the primary metabolism of carbohydrate fermentation. Numerous works have reported the production of organic acids with antifungal ability, mainly lactic and acetic acid, but also formic, propionic, butyric, phenylacetic, and hydroxyphenyl lactic acids, among others. Production of organic acids lowers the pH of the media and creates adverse conditions for the growth of potentially pathogenic microorganisms in food products.

3.2. Antifungal Activity of L. plantarum NCDN4 against A. niger CBS 76997 on Agar Medium

The mycelial growth diameter of *A. niger* within five days of co-incubation with different treatments of *L. plantarum* were presented in Fig. 2 and Fig. 3.

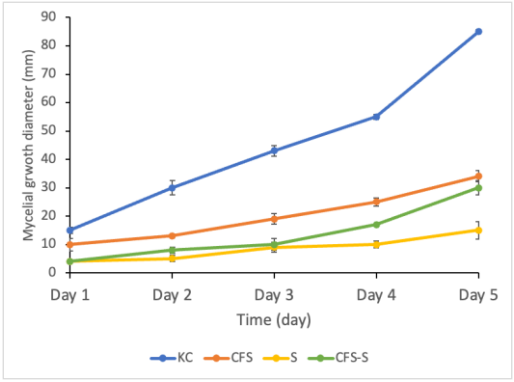

Fig. 2. Mycelial growth diameter of *A. niger* CBS 76997 within 5 days of incubation with *L. plantarum*

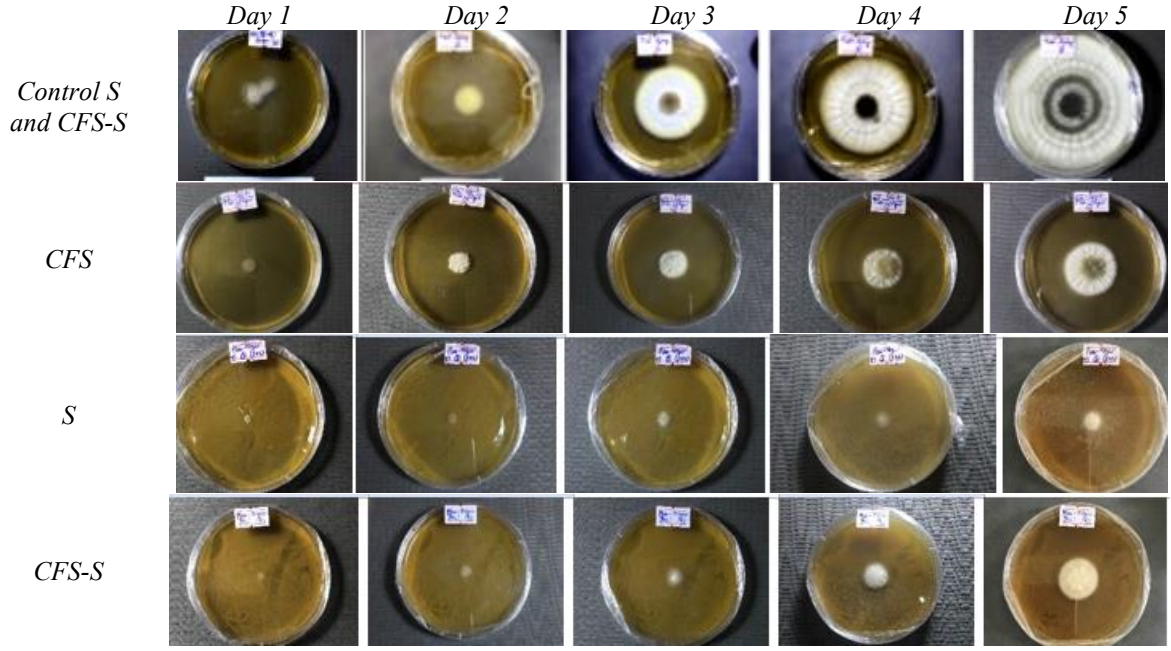

Fig. 3. Antifungal activity of *L. plantarum* NCDN4 on *A. niger* CBS 76997 on agar medium

In the control sample, the black spore was formed after 3 days of incubation, and the mycelial fungi reached 85 mm after five days. The lower mycelial growth, the higher antifungal activity and vice versa. The fungi diameter of the sample treated with *L. plantarum* ranged from 15.0 – 30.0 mm after five days of incubation. The sample treated with a combination of CFS and biomass of *L. plantarum* NCDN4 showed the best antifungal effect against *A. niger* among treated samples, followed by biomass and CFS samples. Interestingly, no spore forming was observed in the samples presenting biomass of *L. plantarum*, whereas the CFS-treated sample appeared the spore on day four of incubation with lower density compared to the control sample. Therefore, *L. plantarum* can delay the spore-forming of fungi from 4 to 5 days.

Russo *et al*. (2017) investigated the antifungal activity of 88 strains of *L. plantarum* against *A. niger, A. flavus, Fusarium culmorum, Penicillium roqueforti, Penicillium expansum* and *Penicillium chrysogenum*. The study revealed that increasing the supplementing of CFS of *L. plantarum* in the medium could increase the antifungal activity. The best antifungal effect was found at 12% CFS supplemented. However, none of the tested strains *L. plantarum* could completely inhibit the growth of fungi [9]. Among LAB, *L. plantarum* is most frequently reported for its strong antifungal activity with a broad spectrum of inhibition against various types of fungi. Compared to the other LAB species in our previous study, *L. plantarum* showed the best antifungal effect [5].

3.2. MIC and MFC Value of CFS of L. plantarum NCDN4 against A. niger CBS 76997

The antifungal activity of CFS of *L. plantarum* against *A. niger* in a liquid medium was determined by using the microdilution method. The mould growth on microplates was observed after five days of incubation. The concentration of CFS from *L. plantarum* NCDN4 increased progressively from top to bottom and left to right (Fig. 4).

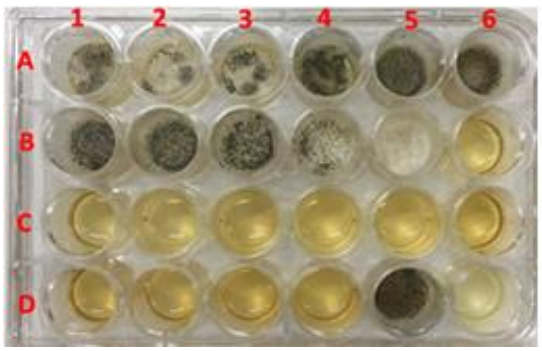

Fig. 4. Growth of *A. niger* CBS 76997 in 24-well microplates after 5 days of incubation

In microwell D6 (negative control), no mould growth was observed, whereas *A. niger* formed a spore of black colour on the surface of microwell D5 (positive control). In addition, no mould growth was observed when treated with amphotericin B at 1.6 mg/L (microwell D4). Mould growth appeared in the form of turbid residues at the bottom and/or black spores on the surface of each microwell. No growth of fungi was observed at the concentration of 340 µL/mL and higher. So, the MIC value of CFS was 340 µL/mL, and the MFC value of CFS was 340 µL/mL. This result indicated that CFS of *L. plantarum* showed a fungicidal effect against *A. niger* CBS 76997.

These findings were consistent with the results of Dopazo *et al*. (2022). The CFS from *L. plantarum* BN17, *L. plantarum* BN16, *L. plantarum* E3, and *L. plantarum* E4 also exhibited inhibitory activity against the *Aspergillus* sp., with MIC values ranging from 12.5 to 100 g/L [10].

3.4. Effect of L. plantarum NCDN4 on the Formation of Fungal Biomass

In the co-incubation liquid medium, the biomass was increased, and the fungal biomass was significantly inhibited after 10 days of cultivation (Fig. 5 and Table 3). Indeed, after 3 days of co-cultivation, the biomass in the treated sample was reduced by 50.84 ± 6.61% compared to the control sample. These results were 35.53 ± 5.96% and 10.51 ± 5.02% on the 5th and 7th days of cultivation, respectively. Thus, the biomass of *L. plantarum* had a significant impact on the biomass formation ability of the *A. niger*.

The most effective inhibition was observed in the first 3 days of cultivation, followed by a gradual decrease in the subsequent days of cultivation. Kim *et al*. (2005) also reported that *Lactobacillus* sp. isolated from kimchi products could inhibit fungal biomass of *A. fumigatus* from 40 – 95% after 2 weeks of incubation [7].

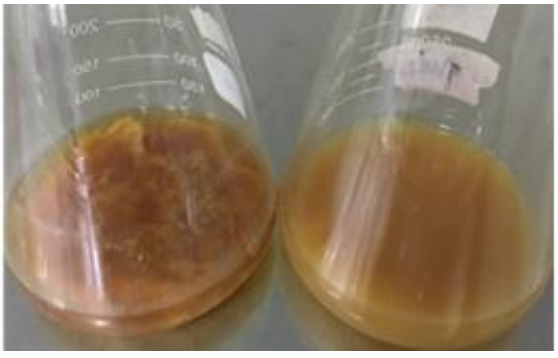

Fig. 5. Mold biomass after five days of incubation in mMRS medium (Left: control sample; Right: treated sample in mMRS medium)

Table 3. Effect of *L. plantarum* NCDN4 on the formation of *A. niger* biomass

***Aspergillus niger* CBS 76997**	**Incubation days**		
	3	**5**	**7**
Control sample (g)	0.1128 ± 0.0070	0.2944 ± 0.0104	0.3772 ± 0.0240
Treated sample (g)	0.0554 ± 0.0082	0.1898 ± 0.0109	0.3376 ± 0.0062
Weight reduction (%)	50.84 ± 6.61%	35.53 ± 5.96%	10.51 ± 5.02%

LAB may possess the ability to inhibit the growth of microorganisms by consuming available nutrients such as carbon and nitrogen sources, thereby limiting or even depleting essential nutrients in the medium. Apart from the metabolic products of LAB, competition for nutrients could be associated with inhibiting the growth of moulds. Honore *et al.* (2016) measured the consumption of metabolic products by three strains of *L. paracasei* and its correlation with mould inhibition activity [11]. The results showed that this Lactobacilli glucose and glutamine consumption was inversely correlated with mould growth. Similarly, competition mechanisms for carbon sources (specifically glucose and glycerol) were also highlighted by Toplaghaltsyan *et al*. (2017) when studying the antifungal capability of *L. rhamnosus* R-2002 against two strains, *Mucor plumbeus* and *Penicillium aurantioviolaceum* [12].

L. plantarum and their CFS had effects on the morphology of mould. When observed under a microscope, the samples supplemented with both CFS and bacterial biomass show significant alterations in the morphology of the mould compared to the control sample (Fig. 6). The density of spores in the control sample appears thicker, which is evident when viewed under a 40x magnification. Additionally, a mould of the same age in the control sample exhibited robust spore growth, forming long chains of spores that were absent in the tested sample. Concerning the conidiophore stalks, the treated sample showed smoother and more delicate stalks compared to the control sample. Therefore, the conidiophore stalks in the treated sample displayed signs of damage. These observations demonstrated the antifungal activity properties of *L. plantarum* against *A. niger*.

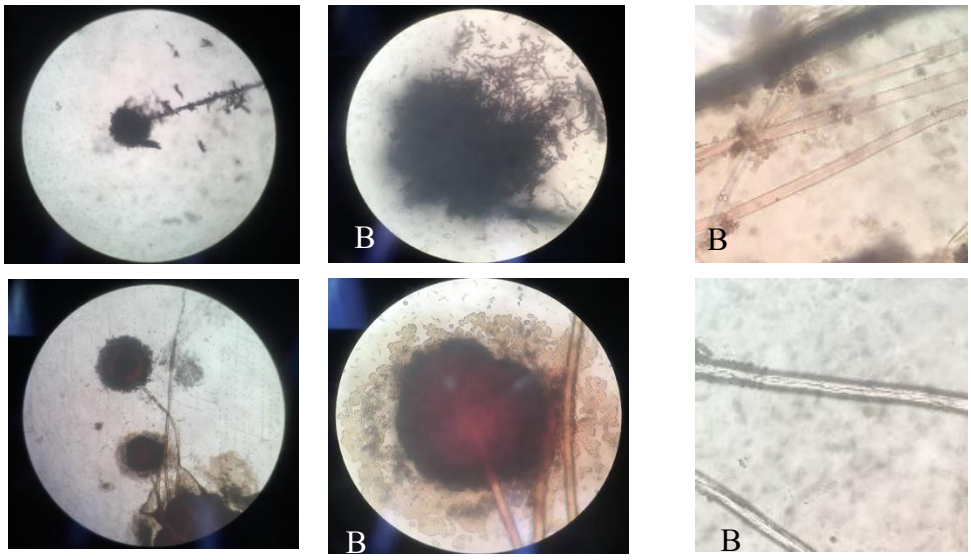

Fig. 6. Morphology of *A. niger* CBS 76997 under microscope

(Top row – Control sample; Bottom row – treated sample supplemented with 10% v/v *L. plantarum* NCDN4 bacterial cell supernatant + 10^6 CFU/mL bacterial; A – Under 10x objective; B – Under 40x objective)

Therefore, the strong antifungal activity of *L. plantarum* was thanks to the production of organic acid, especially lactic acid. In addition, nutrient competition was one of the reasons for the contribution of LAB to antifungal activity. Last but not least, *L. plantarum* influenced the spore formation process by slowing down the spore formation process, reducing the density of spores, and damaging fungi mycelial.

Changes in morphology in 7-day-old *A. flavus* and *A. parasiticus* upon exposure to the CFS from *L. plantarum* K35 at MIC concentration were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were reported. SEM observations revealed that the fungal mycelium of untreated *A. flavus* and *A. parasiticus* retained their intact hyphae with the regular appearance of barrel-shape and smooth cell walls. However, exposure to the CFS from *L. plantarum* K35 at a concentration of 5.87 mg/mL caused severe damage to the cell wall and cytoplasmic membrane, leading to a massive loss of cytoplasmic content, the formation of membrane-bound vesicles, and complete destruction of membranous organelles including mitochondria and nucleus [13].

3.5. Antifungal Effect of L. plantarum in the Germination Process of Brown Rice

Since the combination of biomass and CFS of *L. plantarum* showed the best antifungal effect among tested samples, this treatment was chosen for application in brown rice germination. The results was presented in Table 4 and Fig. 7.

On the third day of germination, the germination rate was 95.7 ± 0.6 %, and 24.0 ± 4.2 % of rice was contaminated with fungi during germination in the control sample. The sample showed the presence of green and black long mycelium, and the length of the brown rice sprouts was approximately 0.5 cm.

Table 4. The germination ability of brown rice and the antifungal effect of *L. plantarum* NCDN4 on *A. niger* on brown rice

Sample	Germination ability (%)	Mold contamination rate (%)
Control	95.7 ± 0.6[a]	24.0 ± 4.2
Spray LAB – M1	95.3 ± 0.6 [a]	9.7 ± 1.5
Spray fungi – M2	88.7 ± 1.5[b]	100 ± 0.0
Spray fungi + LAB – M3	94.7 ± 2.5[a]	15.7 ± 3.7

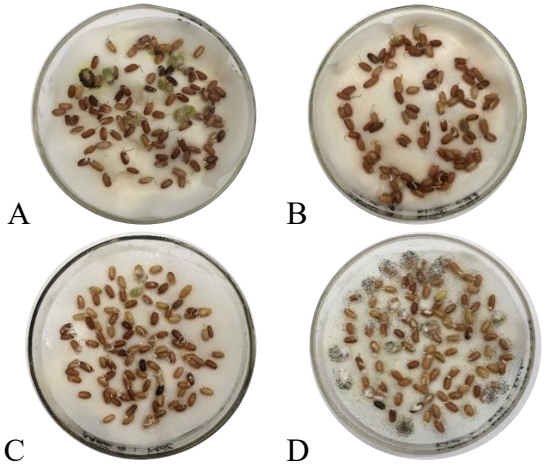

Fig. 7. The appearance of mold on brown rice after 3 days of germination (A – Negative control sample: sprayed with distilled water; B – Treated sample: sprayed with 340µL of CFS + 10^6 CFU/mL of *L. plantarum* NCDN4; C – Treated sample: sprayed with 340µL of CFS + 10^6 CFU/ml of *L. plantarum* NCDN4 + 10^6 spores/ml of *A. niger* CBS 76997; D – Positive control sample: sprayed with 10^6 spores/ml of *A. niger* CBS 76997)

In positive control sample M2 – sprayed with 10^6 spores/mL of *A. niger*, all grains were infected by mould after three days of germination. The fungi exhibited vigorous growth, with most moulds showing spore formation and long mycelium. The germination rate decreased to 88.7 ± 1.5%, and short nascent sprouts were observed.

In the sample sprayed with 340 µL of CFS + 10^6 CFU/mL of *L. plantarum* – M1, short black and green mycelium was observed on the brown rice with a lower contamination rate (9.7 ± 1.5 %). The mould did not exhibit dense spore formation. The brown rice sprayed with LAB maintained a germination ability of 95.3 ± 0.6 %, indicating that LAB did not affect the germination ability of brown rice. In terms of brown rice sprout length, the sprouts reached approximately 0.5 cm, like the control sample.

In the M3 sample – sprayed with 10^6 spores/ml of *A. niger* + 340 µl of CFS + 10^6 CFU/ml LAB, the growth of mould was still observed after three days of germination with a contamination rate was 15.7 ± 3.7%. Additionally, spore formation time inhibition was noted, with a few grains beginning to form spores. No significant difference was found between the control sample and M1 and M3 samples in germination ability. The length of the brown rice sprouts remained unchanged at approximately 0.5 cm. Therefore, the presence of *L. plantarum* demonstrated a strong inhibitory effect on A. *niger* during the germination process of brown rice. The number of mold-infected grains significantly decreased without a major impact on the germination ability of the grains.

Some studies also investigated the antifungal effect of LAB as a biocontrol for grains and seeds. Yang *et al*. (2010) tested the antifungal effect of CFS of *L. plantarum* AF1 against *A. flavus* ATCC 22546 in soybeans. The results showed no mould growth was observed in soybeans treated with four times sprayed CFS of *L. plantarum* AF1. *A. flavus* was not found in germinated soybeans in these samples even after over a week [14]. The use of *L. plantarum* and its CFS in the germination process of brown rice demonstrated an effective antifungal ability. Furthermore, the results indicated that brown rice's germination ability was not affected compared to normal germination processes. The sprouts can be dried to obtain a commercial food product. Therefore, the application of LAB in the germination process of brown rice showed potential as a biocontrol method.

4. Conclusion

In conclusion, this study showed the strong antifungal effect of biomass and CFS of *L. plantarum* NCDN4 against *A. niger* CBS 76997. The mycelial diameter of *A. niger* treated with *L. plantarum* ranged from 15 – 30 mm, lower than the control sample (85 mm) after five days of incubation. The MIC and MFC values of *L. plantarum* were equal and reached 340 µL/mL. The preliminary results showed that the antifungal mechanism of *L. plantarum* related to the lactic acid produced during bacteria metabolism delayed and reduced spore formation from 4 to 5 days, nutrient competition by reducing fungi biomass of 50.8% after three days of cultivation, and damage mycelium of *A. niger*. *L. plantarum* reduced the mould contamination rate during the germination of brown rice without affecting the germination of rice, with a germination ability of 94.7%. The mould contamination rates in the samples non-treated and treated with LAB were 100.0% and 15.7%, respectively. The results demonstrated the potential application of *L. plantarum* in food technology as a biocontrol agent in inhibiting fungi.

Acknowledgements

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