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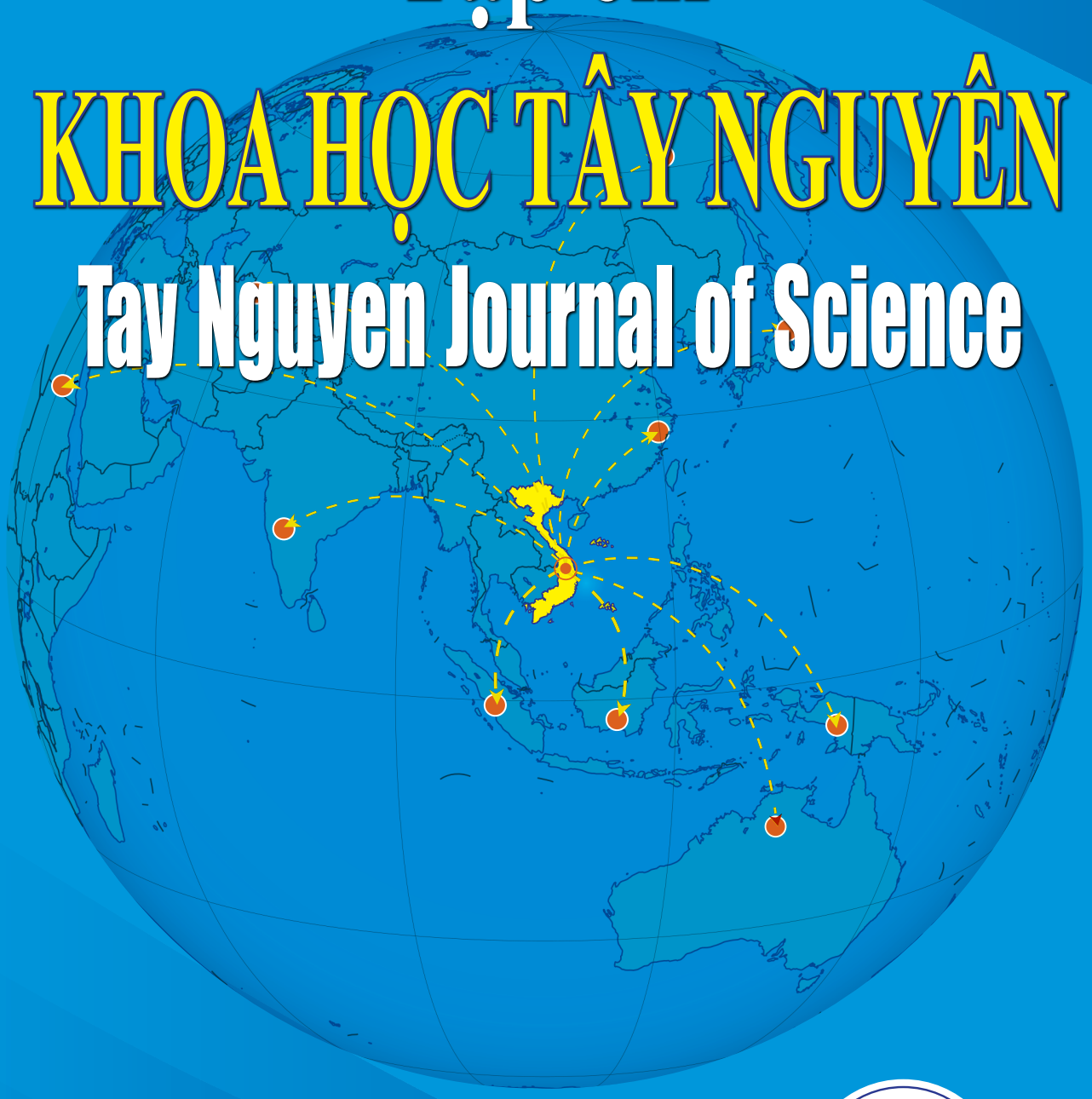

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REUTILIZATION OF SOYBEAN BY-PRODUCT FOR BIOMASS PRODUCTION OF *Bacillus amyloliquefaciens* AND EVALUATION OF ITS ANTIFUNGAL POTENTIAL AGAINST *Fusarium*

Tran Thi Ha Trang[1], Ngo Van Anh[1], Nguyen Van Bon[1], Nguyen Anh Dung[1]

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ABSTRACT

Recently, *Bacillus amyloliquefaciens* has been recognized as a potential biocontrol agent for protecting crops against significant diseases. Thus, biomass production of this bacterium has received much interest. However, most previous studies have used commercial nutrient broth as a C/N source for fermentation. This study evaluated the potential reuse of various agricultural by-products, and soybean pulp by-product (SPBP) was identified as a suitable substrate for the fermentation of *B. amyloliquefaciens* EB.CK9. Experimental results showed that strain EB.CK9 grew best in a culture medium containing 1.75% SPBP, 0.05% $MnSO_4$, and 0.1% KH_2PO_4, with an initial pH of 7. Cultivation was carried out at a temperature of 36°C, 200 rpm for 36 hours, and the biomass concentration reached up to 14.29×10^8 CFU/ml (shake flask condition). The production of *B. amyloliquefaciens* EB.CK9 in a bioreactor system resulted in a higher biomass concentration of 9.56×10^{11} CFU/ml with a reduced fermentation time (10 hours). After fermentation in the bioreactor system, the antifungal activity of EB.CK9 biomass was evaluated and demonstrated promising antifungal potential against *Fusarium incarnatum* (56.25%), *Fusarium solani* (68.75%), and *Fusarium oxysporum* (67.50%). The study results highlight the potential for reusing agricultural by-products in the development of *B. amyloliquefaciens* EB.CK9-based products, open up opportunities for the development of indigenous bioproducts from endophytic bacteria, which can support the sustainable cultivation of durian.

Keywords*: Bacillus amyloliquefaciens, soybean pulp by-product, Fusarium incarnatum, Fusarium solani, Fusarium oxysporum, antifungal activity.*

1. INTRODUCTION

Durian (*Durio zibethinus* L.) leads fruit and vegetable exports, accounting for 55.4% of the total fruit export value in Vietnam. The Central Highlands region accounts for approximately 47% of the country's total durian cultivation area (Nguyen et al., 2021). However, fungal diseases have affected severely this crop (Ngo et al., 2024).

Fusarium spp. commonly causes disease in soil and affects a wide range of crops. *Fusarium solani* and *Fusarium incarnatum* are major pathogens responsible for dieback disease in durian trees (Pongpisutta et al., 2023). The spores of *Fusarium* are capable of persisting in soil, plant tissues, and plant debris for many years, making it difficult to control (Nelson et al., 1981). Current strategies for managing *Fusarium* wilt include cultural practices, chemical treatments, and biological control methods. Among these, biocontrol agents, such as antagonistic bacteria, offer a promising and sustainable alternative (Hamed et al., 2009; Miller et al., 2020; Rahman et al., 2021).

Bacillus amyloliquefaciens is an effective biocontrol agent for sustainable crop production. Notably, *B. amyloliquefaciens* has been evidenced as an effective fungicidal bio-agent against *F. oxysporum* causing diseases in watermelon, banana, and cucumber (Al-Mutar et al., 2023; Yuan et al., 2012; Han et al., 2019). Besides that, *B. amyloliquefaciens* has been reported with control postharvest fruit Yugu melon rot caused by *F. incarnatum* (Liu et al., 2023). Additionally, it has the potential to control various pathogens such as *F. solani* and *Alternaria alternata* (Pham et al., 2024). However, its potential against *Fusarium* spp. disease on durian plants has not been recorded. *B. amyloliquefaciens* reached an interesting point in mass production via fermentation for application. Almost all previous studies have used commercial media for cultivation. For cost-effective production, several studies have explored fermentation by-products for cultivating *B. amyloliquefaciens*, such as molasses, bran, corn starch, wheat bran, grape seed powder, banana peels... (Table 2). In recent years, soybean flour has been used in *B. amyloliquefaciens* fermentation. However, the potential reuse of soybean pulp has not yet been investigated. Furthermore, almost all the previous works reported biomass production in the minor scale of flasks.

Soybean pulp by-product (SPBP) is a secondary product generated during soybean processing,

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commonly used as animal feed or fertilizer. SPBP contains approximately 14.85% protein, 10% lipid, and other essential minerals such as Ca, Mg, K, P_2O_5, and Zn (Table 1). Due to its high nutritional content, SPBP has also been utilized as a C/N source for microbial fermentation to produce enzymes, and bioactive secondary metabolites (Soussi et al., 2019; Doan et al., 2021; Ahsan et al., 2022).

In this study, we use *B. amyloliquefaciens* EB.CK9 was isolated from root durian (Ngo et al., 2024). Regarding the eco-friendly production of this potential strain, various agricultural processing by-products were used as C/N sources for fermentation. In which, soybean processing by-products as a novel substrate of *B. amyloliquefaciens* achieved higher biomass on a high-level scale fermentation (14-L bioreactor system). Additionally, the biocontrol potential of this strain against durian fungal pathogens was also investigated, including its antagonistic activity against several pathogenic *Fusarium* spp. including *F. incarnatum, F. solani,* and *F. oxysporum.*

2. MATERIALS AND METHODS

2.1. Materials

The three pathogenic fungal strains—*Fusarium incarnatum*, *F. solani*, and *F. oxysporum*—along with the endophytic durian-associated bacterial strain *Bacillus amyloliquefaciens* EB.CK9: were preserved at the Institute of Biotechnology and Environment, Tay Nguyen University, Vietnam.

Potato Dextrose Agar (PDA) was used to activate the fungal strains, while Luria-Bertani (LB) medium was used to activate the bacterial strain. Agricultural processing by-products, including soybean pulp by-product (SPBP) (Table 1), rice bran pulp by-product (RBPBP), and cassava pulp by-product (CPBP), were collected from Buon Ma Thuot, Dak Lak, Vietnam.

Table 1. The nutrient content of soybean pulp by-product (SPBP)

Nutrient content	Ratio (%)
Protein	14.846
Total sugar	0.412
Reducing sugar	0.176
Lipid	10.12
Total ash	11.23
Ca	0.152
Mg	0.046
K	0.103
P_2O_5	0.057
Zn (ppm)	4.09

2.2. Methods

2.2.1. Method for assessing bacterial density after culture

The microbial density was determined by counting the number of colonies growing on the agar medium (Mai et al., 2011)

2.2.2. Investigation of the fermentation process of Bacillus amyloliquefaciens EB.CK9 in conical flasks

- Screening for an appropriate C/N source to culture the endogenous bacterium *B. amyloliquefaciens* EB.CK9 from three by-products of agricultural processing (SPBP, RBPBP, and CPBP). The EB.CK9 strain was cultured in liquid media (30 mL) containing 1.5% C/N, 0.5% NaCl, and pH = 7, 36°C, with shaking at 200 rpm for 36 hours (Tran et al., 2024). The control medium consisted of 1% peptone, 0.5% yeast extract, and 0.5% NaCl. After 36 hours, the fermentation broth was collected, and bacterial density was determined according to method 2.2.1 (experiments were repeated three times).

- The effect of C/N concentration on the growth of *B. amyloliquefaciens* EB.CK9: Concentrations of 1, 1.25, 1.5, 1.75, and 2 % of SPBP were added to a liquid medium containing 0.5% NaCl, and pH = 7, 36°C, with shaking at 200 rpm for 36 hours. The optimal concentration was determined based on bacterial density after incubation (according to method 2.2.1).

- The influence of some types of mineral salts on the growth of *Bacillus amyloliquefaciens* was determined in previous studies (Table 2; Ngo et al. 2024). In this study, some other sources of sulfate and phosphate salts were investigated:

+ The effect of different sulfate salts: $MgSO_4$, $CaSO_4$, $MnSO_4$, $ZnSO_4$, and $FeSO_4$. The culture medium (30 mL) contained 1.75% SPBP and 0.05% sulfate salt, with a control medium using 0.5% NaCl instead of 0.05% sulfate salt, pH = 7, 36°C and cultures were incubated with shaking at 200 rpm for 36 hours. The optimal sulfate salt was determined using method 2.2.2.

+ Similarly, the effect of different phosphate salts was investigated, including K_2HPO_4, KH_2PO_4, Na_2HPO_4, $Ca_3(PO_4)_2$, and 0.05% $MnSO_4$. The culture medium (30 mL) contained 1.75% SPBP and 0.1% phosphate salt, with a control medium containing 0.5% NaCl instead of 0.1% phosphate salt, pH = 7, 36°C and cultures were incubated with shaking at 200 rpm for 36 hours. The most suitable phosphate salt was determined by the result of bacterial density after culture (according

to method 2.2.1).

- Method for constructing the growth curve of *B. amyloliquefaciens* CK9 in a medium: 1.75% SPBP, 0.1% KH_2PO_4, and 0.05% $MnSO_4$ at pH 7 with 36°C. The OD600nm value of the suspension is determined every 2 hours, continuously for 40 hours, to monitor the growth curve.

2.2.3. Scaling up of the B. amyloliquefaciens EB.CK9 fermentation in a 14-L bioreactor system

The parameters of the bioreactor system (Bioreactor 14 L, BiFlo, Brunswick, USA) were adjusted similarly to the experimental results in the Erlenmeyer flask, including 1.75% SPBP, 0.1% KH_2PO_4, 0.05% $MnSO_4$, 36°C, dissolved oxygen at 15 vvm, and a stirring speed of 200 rpm. Bacterial density was monitored every 2 hours, from 2 to 10 hours after inoculation (CFU/mL) according to method 2.2.1.

2.2.4. Screening for antifungal activity against Fusarium

Based on the method of Ngo (2020), the endophytic bacterial strain EB.CK9 was evaluated for antifungal activity on the PDA medium, with each experiment repeated three times. In the experimental setup, the pathogenic fungus was placed in the center of a petri dish, while the antagonistic endophytic bacterium was inoculated 3 cm away from the pathogen, with a bacterial streak of 1 cm. The experimental plates were incubated at 30°C and monitored for approximately five days (until the leading edge of the pathogenic fungus in the control group reached the edge of the plate). In the control setup, only the pathogenic fungus was inoculated into the petri dish.

The inhibition rate of fungal growth was calculated as follows:

The inhibition rate of mycelial growth (%) = [(D1 – D2)]/D1] × 100, where D1 is the radius of fungal growth on the control plate (cm), and D2 is the radius of fungal growth on the co-cultured plate with bacteria (cm).

2.2.5. Statistical analysis

All experiments were designed randomly, and the results were processed using Microsoft Office Excel and analyzed statistically by ANOVA with SPSS 22.0 software. Data are presented as the mean of three replicates ± standard deviation, with a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Screening of suitable substrates and mineral salts for biomass production of Bacillus amyloliquefaciens EB.CK9

Almost all previous studies have used commercial media to cultivate *B. amyloliquefaciens*. For cost-effective production, several studies have explored fermentation by-products for cultivating it (Table 2). According to the study by Berikashvili (2018), corn cob was used to ferment *B. amyloliquefaciens* B-1895, achieving 82×10^{10} spores g^{-1} of biomass. According to Li (2021), corn starch was used to culture *B. amyloliquefaciens* B7, reaching a high density of 7.0×10^9 CFU/mL. Wang (2023) used 1-3% brown sugar and 1% yeast extract in the cultivation of *B. amyloliquefaciens* PMB04 and gained 4.83×10^8 CFU/mL. In addition, various agricultural by-products such as molasses, bran, rice husk powder, corn gluten, soybeans, corn starch, wheat bran, banana peels, and grape seed powder have also been used to culture *B. amyloliquefaciens*. In this study, *B. amyloliquefaciens* EB.CK9 exhibited good growth on all three agricultural by-product sources (SPBP, RBPBP, and CPBP) (Figure 1a). Among them, soybean by-product (SPBP) was the most suitable substrate for culturing *B. amyloliquefaciens* EB.CK9, achieving a high density of 6.65×10^8 CFU/mL (Figure 1a), which is quite similar to previous studies. This is because soybean by-products have a high nutritional content, containing approximately 14.85% protein, 10% lipid, and other essential minerals such as Ca, Mg, K, P_2O_5, and Zn (Table 1). Notably, this is the first report of the ability to reuse these three agricultural processing by-products for the *B. amyloliquefaciens* strain cultivation.

When the SPBP component was increased in the medium, EB.CK9 showed an increase in density; therefore, the appropriate ratio of soybean by-products was determined. Figure 1b shows that the bacterial density remained relatively high, at $8.20\text{-}8.27\times10^8$ CFU/mL, and was nearly equivalent in the two treatments using 1.75% SPBP and 2% SPBP. When the SPBP content in the medium was reduced, the EB.CK9 density decreased accordingly (Figure 1B). Thus, 1.75% SPBP was selected for use in the culture medium for subsequent studies to save production costs.

Sulfate and phosphate salts have been reported to positively affect the growth and development of *B. amyloliquefaciens* (Table 2). Berikashvili (2018) reported that KH_2PO_4 and $MgSO_4.7H_2O$ are positive aspects in *B. amyloliquefaciens* B-1895 fermentation. According to Doan (2021), *B. amyloliquefaciens* TKU050 went up significantly with K_2HPO_4, KH_2PO_4, and KNO_3. Meanwhile, $MgSO_4$ is the best option for the development of

B. amyloliquefaciens 3-5 (Ma et al., 2021) and *B. amyloliquefaciens* BAM (Ahsan et al., 2022). In this study, sulfate salts significantly promoted the biomass growth of *B. amyloliquefaciens* EB.CK9 (Figure 1C) compared to the initial medium containing NaCl. Among them, *B. amyloliquefaciens* EB.CK9 exhibited the highest growth in the medium supplemented with $MnSO_4$, reaching a cell density of 11.66×10^8 CFU/mL. Additionally, phosphate salts also positively affected the fermentation process of strain EB.CK9 (Figure 1d), with the optimal bacterial density observed in the medium supplemented with KH_2PO_4 (14.29×10^8 CFU/mL). Based on the results, the growth curve of *B. amyloliquefaciens* EB.CK9 in the medium was constructed (Figure 1. E). The bacterium shows continuous growth and reaches the stationary phase at 28 hours. After 28 hours, growth slows, likely due to nutrient depletion. The growth time differs from previous studies: Ngo (2024) reported optimal growth of *B. amyloliquefaciens* EB.CK9 in LB medium at 48 hours, while Tran (2024) found optimal growth after 36 hours using fishmeal to ferment *B. amyloliquefaciens* EB.CK9.

Table 2. The reports on C/N source and fermentation parameters of Bacillus amyloliquefaciens

Bacteria	Culture medium	Culture conditions	Bacterial density	References
B. amyloliquefaciens LPL061	22 g/L sucrose and 18.4 g/L yeast extract	pH 7.0, 28°C, 220 rpm for 24 h		Li et al., 2013
B. amyloliquefaciens B-1895	15 g of corn cob, 10 g peptone, 2 g KH_2PO_4, 1 g $MgSO_4.7H_2O$, 1 g NaCl		82×10^{10} spores g -1 biomass	Berikashvili et al., 2018
B. amyloliquefaciens C5	0.2% grape seed flour			Soussi et al., 2019
B. amyloliquefaciens TKU050	0.5% wheat bran powder, banana peels, 0.2% K_2HPO_4, 0.2% KH_2PO_4, 0.2%, KNO_3, 0.3% yeast extract	pH 6–9, 150 rpm for 4 days		Doan et al., 2021
B.amyloliquefaciens B7	Peptone 20 g/L, corn starch 10 g/L, rice protein powder 20 g/L, Mn 2+ 1.0 mmol/L	48 hours	$7,0\times10^9$ CFU / mL	Li et al., 2021
B. amyloliquefaciens 3-5	35% bran, 40% rice husk powder, 20% corn gluten, 15% soybeans, 1.5% corn starch, 1.5% $MgSO_4$	32°C, pH 7.0 for 44 h		Ma et al., 2021
B. amyloliquefaciens BAM	15 gm/L semolina, 12.5 gm/L beef extract and 0.5 gm/L $MgSO_4$			Ahsan et al., 2022
B. amyloliquefaciens J2V2AA	Molasses			Kumar et al., 2022
B. amyloliquefaciens S8TS	Wheat bran	pH 7.0, 37°C for 72 h		Shahzadi et al., 2023
B. amyloliquefaciens PMB04	1-3% brown sugar, 1% yeast extract		$4,83\times10^8$ CFU/ mL	Wang et al., 2023
B. amyloliquefaciens EB.CK9	LB	pH 7, 36°C, 200rpm for 10h	$8,25x10^{11}$ CFU/ml	Ngo et al., 2024
B. amyloliquefaciens EB.CK9	1.25% fish head powder, 0.5% NaCl	pH 7.25, 36°C, 200 rpm for 8h	9.85×10^{11} CFU/ml	Tran et al., 2024
B. amyloliquefaciens EB.CK9	1.75% SPBP, 0.1% KH_2PO_4, 0.05% $MnSO_4$	pH 7, 36°C, 200rpm for 10h	2.56×10^{11} CFU/ml	This study

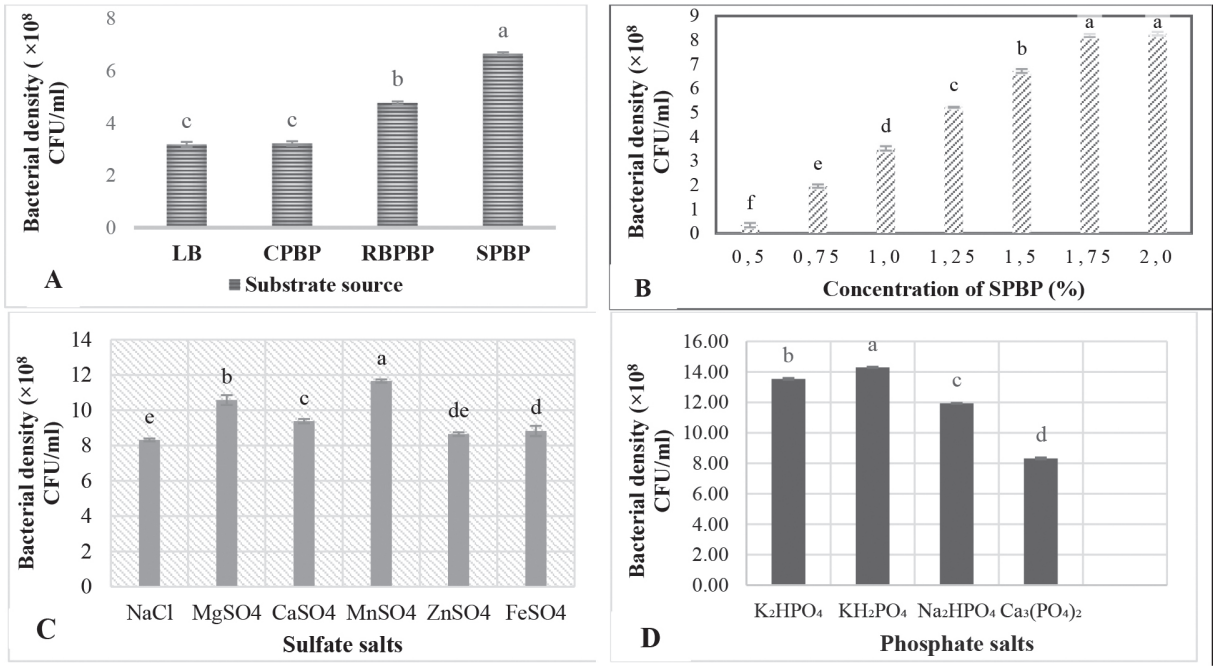

Figure 1. Screening of C/N sources (1A), the concentration of SPBP (1B), the effect of sulfate salts (1C), and the effect of phosphate salts (1D) on the growth of B. amyloliquefaciens EB.CK9 in Erlenmeyer flasks. Statistical analysis was performed using ANOVA, α ≤ 0.05 was considered significant. The different symbols (a-e) indicate statistical significance.

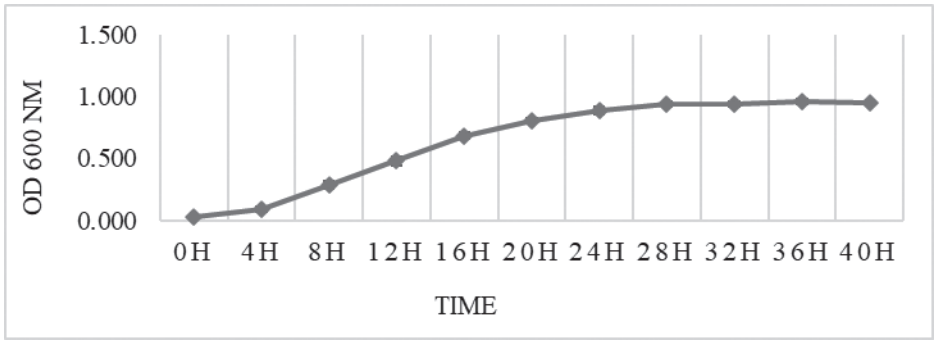

Figure 1. E. The growth curve of B. amyloliquefaciens EB.CK9 in a medium containing 1.75% SPBP, 0.1% KH_2PO_4, and 0.05% $MnSO_4$ at an initial pH of 7, and with 36°C.

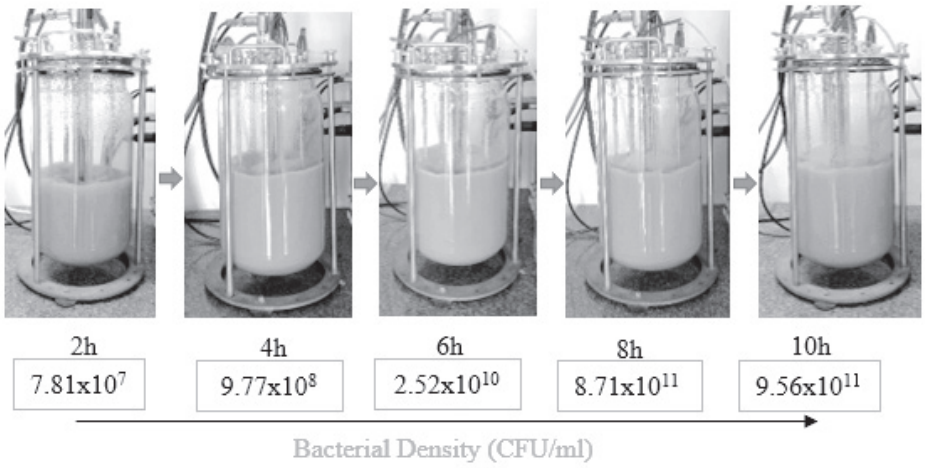

Figure 2. The results of B. amyloliquefaciens EB.CK9 biomass in a larger fermentation system

3.2. Scaling up the fermentation of Bacillus amyloliquefaciens EB.CK9

The study successfully fermented *B. amyloliquefaciens* EB.CK9 bioproduct in a 14L

bioreactor to shorten fermentation time. There was a continuous increase in biomass from 0 to 10 hours (Figure 2), reaching its peak at 10 hours of fermentation (9.56×10^{11} CFU/ml). The bacterial density increased rapidly in a short time in the bioreactor showing a clear difference compared to cultures in shake flasks. Additionally, the results differed from those reported by Lima (2019), *B. amyloliquefaciens* IT45 was fermented in a medium containing glucose syrup, yeast extract, and calcium chloride, and reached 3.0×10^9 CFU/ml in a 40L bioreactor after 48 hours of fermentation. The results are consistent with the study by Ngo (2024), in which *B. amyloliquefaciens* EB.CK9 cultured in commercial LB medium for 10 hours reached a density of 8.25×10^{11} CFU/mL. Additionally, fermentation of *B. amyloliquefaciens* EB.CK9 using fish head powder achieved a density of 9.85×10^{11} CFU/mL for 8 hours (Tran et al., 2024). In general, the growth of *B. amyloliquefaciens* strains depends on the substrate and the bacterial strain.

This is evidence of the successful reuse of SPBPs in the production of *B. amyloliquefaciens* bioproducts, contributing to cost savings, safety, and environmental friendliness.

3.3. The evaluation of the antifungal activity of Bacillus amyloliquefaciens EB.CK9 against certain Fusarium pathogens

B. amyloliquefaciens is a promising candidate for biological control with high antifungal efficacy due to its ability to produce various antifungal lipopeptides (Al-Mutar et al., 2023). *B. amyloliquefaciens* strain PT14 produces peptides PT14-3 and PT14-4a, which exhibit broad-spectrum antifungal activity against *Fusarium solani* and *Fusarium oxysporum*, with minimum inhibitory concentrations of 3.12 mg/L (*F. solani*) and 6.25 mg/L (*F. oxysporum*), causing severe morphological deformation in spores and hyphae (Kim et al., 2015). According to Al-Mutar (2023), *B. amyloliquefaciens* DHA55 produces various antifungal lipopeptides, including iturin, surfactin, and fencing, demonstrating high efficiency against *F. oxysporum* f. sp. niveum (74.9%). Liu (2023) reported that *B. amyloliquefaciens* Baf1 produces surfactin C13–C15 and bacillomycin C17 L, achieving over 75% control efficacy against *Fusarium incarnatum* fruit rot in postharvest Yugu melons. Moreover, *B. amyloliquefaciens* also produces volatile organic compounds (VOCs) that inhibit fungal growth and spore germination. Yuan (2012) found that *B. amyloliquefaciens* NJN-6 generates 11 VOCs that suppress *F. oxysporum* f. sp. cubense. According to Han (2019), *B. amyloliquefaciens* B1408, isolated from cucumber rhizosphere soil, reduced *Fusarium* wilt disease incidence (*F. oxysporum* f. sp. cucumerinum) by 59.0% and promoted plant growth. Pham (2024) reported that *B. amyloliquefaciens* TV2.5 exhibited strong antagonistic activity against *F. solani*, *F. oxysporum*, and *Alternaria alternata*. In this study, the endophytic durian *B. amyloliquefaciens* EB.CK9 demonstrated potent antifungal activity, with inhibition rates of *F. solani*: 68.75%, *F. oxysporum*: 67.5%, and *F. incarnatum*: 56.25% (Figure 3).

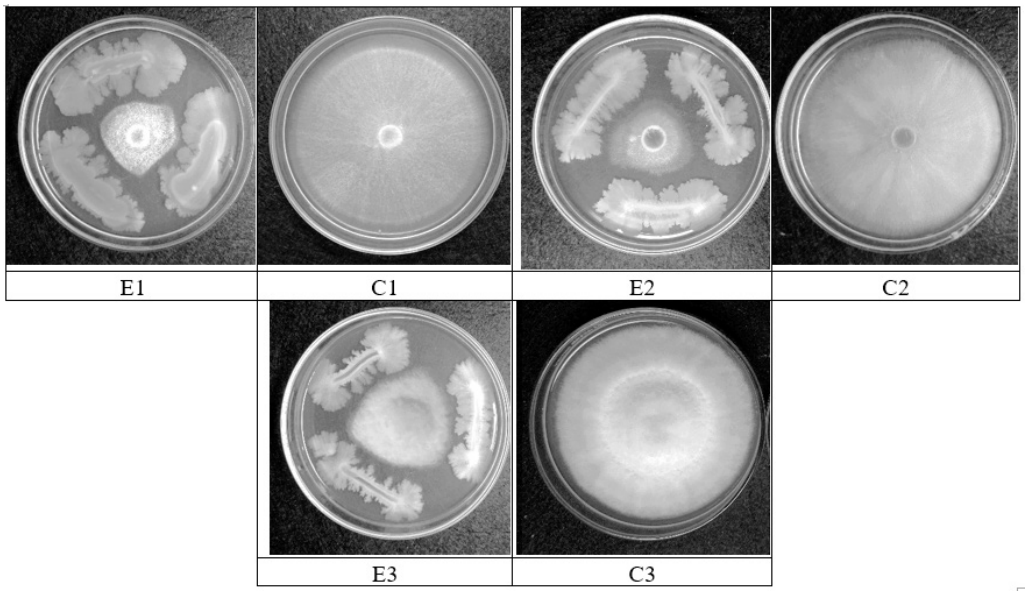

Figure 3. Evaluation of the antifungal potential of B. amyloliquefaciens EB.CK9 against several pathogenic Fusarium species. The control plate is represented by the symbol C and the experimental plate is represented by the symbol E. E1. B. amyloliquefaciens EB.CK9 against F. solani (C1), E2. B. amyloliquefaciens EB.CK9 against F.oxysporum (C2) and E3. B. amyloliquefaciens EB.CK9 against F. incarnatum (C3).

4. CONCLUSION

The study determined the optimal parameters for fermenting *B. amyloliquefaciens* EB.CK9 at the Erlenmeyer flask scale: 1.75% SPBP, 0.05% $MnSO_4$, 0.1% KH_2PO_4, at 36°C, pH 7, 200 rpm for 36 hours (14.29×10^8 CFU/ml). The successful large-scale production of *B. amyloliquefaciens* EB.CK9 in a 14L bioreactor achieved a high density (9.56×10^{11} CFU/mL) in a short time (10 hours). *B. amyloliquefaciens* EB.CK9 demonstrated antifungal potential against *F. solani*, *F. oxysporum,* and *F. incarnatum*, with inhibition rates of 68.75%, 67.5%, and 56.25%, respectively. This study provides evidence for the reuse of soybean by-products in biomass production of *B. amyloliquefaciens*. Additionally, it explores the potential of *B. amyloliquefaciens* for biological control of *Fusarium* fungi in laboratory conditions, which could contribute to the future development of safe and sustainable bioproducts for durian cultivation.

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TÁI SỬ DỤNG PHỤ PHẨM ĐẬU NÀNH TRONG SẢN XUẤT SINH KHỐI *Bacillus amyloliquefaciens* VÀ ĐÁNH GIÁ TIỀM NĂNG CHỐNG NẤM *Fusarium*

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TÓM TẮT

Bacillus amyloliquefaciens là tác nhân kiểm soát sinh học tiềm năng, bảo vệ nhiều loại cây trồng quan trọng. Do đó, sản xuất sinh khối của vi khuẩn này đã nhận được nhiều sự quan tâm. Tuy nhiên, hầu hết các nghiên cứu trước đây sử dụng môi trường thương mại làm nguồn C/N chính trong lên men. Trong nghiên cứu này, khả năng tái sử dụng nhiều phụ phẩm nông nghiệp khác nhau của *B. amyloliquefaciens* EB.CK9 đã được đánh giá. Trong đó, phụ phẩm đậu nành (SPBP) được xác định là chất nền phù hợp cho quá trình lên men của *B. amyloliquefaciens* EB.CK9. Kết quả thực nghiệm cho thấy chủng EB.CK9 phát triển tốt nhất trong môi trường nuôi cấy chứa 1,75% SPBP, 0,05% MnSO4, 0,1% KH_2PO_4, nuôi cấy ở nhiệt độ 36°C, pH 7, 200 rpm trong 36 giờ và mật độ sinh khối đạt $14,29 \times 10^8$ CFU/ml (điều kiện bình tam giác). Nhằm rút ngắn thời gian lên men, *B. amyloliquefaciens* EB.CK9 được nhân nuôi trong hệ thống Bioreactor với mật độ sinh khối cao hơn ($9,56 \times 10^{11}$ CFU/ml) trong 10 giờ. Sau khi lên men trong Bioreactor, hoạt tính kháng nấm của sinh khối EB.CK9 đã được đánh giá và cho thấy tiềm năng kháng nấm hứa hẹn: *Fusarium incarnatum* (56,25%), *Fusarium solani* (68,75%) và *Fusarium oxysporum* (67,5%). Kết quả nghiên cứu là minh chứng cho tiềm năng tái sử dụng các sản phẩm phụ nông nghiệp trong nhân nuôi *B. amyloliquefaciens* EB.CK9 và mở ra triển vọng ứng dụng vi khuẩn nội sinh trong phát triển các sản phẩm sinh học bản địa nhằm hỗ trợ canh tác sầu riêng bền vững.

Từ khóa: *Bacillus amyloliquefaciens, phụ phẩm đậu nành, Fusarium incarnatum, Fusarium solani, Fusarium oxysporum, hoạt tính kháng nấm.*

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