Innovative use of polymeric biosurfactants for boost germination of Lettuce (*Lactuca sativa*)

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ABSTRACT

Agriculture is crucial for global food security and has continuously improved production. However, it faces significant challenges from crop losses due to phytopathogenic fungi and the overuse of agrochemicals, which has led to resistance and environmental contamination. This work aims to utilize lignocellulosic by-products from the agro-industry to produce second-generation biosurfactants and apply them as seed germination promoters. Sugarcane bagasse was processed through acid hydrolysis, concentration, and detoxification, and the resulting hydrolysates were fermented with *Scheffersomyces shehatae* 16-BR6-2AI. Germination assays with lettuce seeds treated with various biosurfactant concentrations showed significant improvement in germination and early growth at concentrations between 50 and 2000 mg/L, while higher concentrations were phytotoxic. In conclusion, polymeric biosurfactants can enhance lettuce seed germination at moderate concentrations, highlighting their potential in sustainable agriculture by improving water and nutrient absorption in plants.

Keywords: Sustainable agriculture, Biosurfactants, Seed germination, Lignocellulosic biomass

1 INTRODUCTION

Agriculture is crucial for global food security, continuously modernizing to improve production (1). However, it faces significant challenges from phytopathogenic fungi, causing substantial losses, especially in tropical and subtropical regions (2). Despite the use of pesticides and fertilizers, crop losses remain significant, and the overuse of agrochemicals has led to problems like resistance and environmental contamination (3, 4). Seed germination is fundamental for agriculture, starting with imbibition and culminating in the emergence of the radicle (5). The dependence on agrochemicals and the need for efficient germination have driven interest in using microorganisms and their metabolites as viable alternatives, promoting sustainability (6). Among these, biosurfactants stand out for their low toxicity, high biodegradability and biocompatibility, making them useful as antifungal agents and germination promoters (7, 8). In this context, the use of lignocellulosic by-products from the agro-industry can be an intriguing alternative for the production of biosurfactants for agricultural applications, significantly contributing to sustainable development, an emerging necessity in today's world. This work aims to utilize lignocellulosic biomass (sugarcane bagasse) to produce second-generation biosurfactants for use as seed germination promoters. This approach provides promising strategies for agricultural use, greatly enhancing environmental sustainability.

2 MATERIAL & METHODS

Sugarcane bagasse was subjected to acid hydrolysis, concentration, and detoxification with activated carbon. Subsequently, sugars (glucose, xylose, arabinose, acetic acid, and furans) were quantified in the sugarcane bagasse hemicellulosic hydrolysate (SBHH), concentrated SBHH, and detoxified SBHH using high-performance liquid chromatography (HPLC), while phenolic compounds were quantified using spectrophotometry.

The yeast strain Scheffersomyces shehatae 16-BR6-2AI was obtained from the Center for the Study of Social Insect Collections at the Institute of Biosciences, Rio Claro Campus, São Paulo State University (UNESP). For biosurfactant production, fermentation was carried out in a specific medium containing peptone, ammonium nitrate, SBHH xylose, and soybean oil. After incubation, cultures were centrifuged to remove cells, and the biosurfactant was extracted through precipitation and resuspension in ethanol. Biosurfactant characterization involved phenol-sulfuric acid and DNS methods for sugar quantification and the Lowry method for total protein quantification.

Finally, germination assays were conducted using biosurfactant solutions at various concentrations (0, 50, 100, 150, 200, 500, 1000, 2000, 5000, 10000 mg/L). Green crisp lettuce seeds were disinfected and placed in Petri dishes with the solutions. Several germination parameters were evaluated, including germination time, germination percentage, and seedling size (radicle, hypocotyl, and cotyledon). Data were statistically analyzed using ANOVA and Tukey's multiple comparison test to determine the effectiveness of the biosurfactant in the germination process.

3 RESULTS & DISCUSSION

In the pretreatment process of the hemicellulosic hydrolysate, an initial concentration of 20.58 g/L of xylose was obtained in the reactor, which increased to 71.7 g/L through vacuum evaporation. During detoxification with activated carbon, xylose concentration decreased to 66.26 g/L, with significant reductions in total phenolic compounds to 0.0560 mg/L and furans to 42 mg/L. These results are consistent with previous studies using acid hydrolysis of sugarcane bagasse (9-11). The pH decrease observed during the concentration of the hemicellulosic hydrolysate is attributed to the increase in H+ ions from the sulfuric acid used in the hydrolysis (12).

The production of polymeric biosurfactant (BE) was carried out in triplicate using xylose in HHBCA and soybean oil. After fermentation, the cultures were centrifuged and the cell-free medium was washed with hexane, resulting in a surface tension of the supernatant of 57.5 mN/m. The extracted biosurfactant was resuspended in ethanol, yielding 8-11 grams of dry white mass per 600 ml of medium. The concentration of reducing sugars was 167.15 µg/ml, total sugars 52.27 µg/ml, and total proteins 121.86 µg/ml, consistent with studies by Maneerat et al. (13) and Kiran et al. (14), which reported surface tensions of 55 mN/m and biosurfactant productions of 9-12 grams.

Germination assays of *Lactuca sativa* showed that biosurfactant concentrations between 50 and 2000 mg/L significantly improved germination parameters such as radicle, hypocotyl, and cotyledon length, as well as the presence of absorbent hairs on the radicle and seed coat shedding. These results suggest greater efficiency in water and nutrient absorption, consistent with previous research on the benefits of biosurfactants for seed water and nutrient availability (15, 16). Specifically, the 500 mg/L treatment (Figure 1) showed optimal radicle growth, similar to findings by Wang et al. (17), who also observed improved seed germination with biosurfactant application. However, extremely high concentrations (5000 and 10000 mg/L) resulted in significant decreases in germination rate and radicle length, likely due to phytotoxic effects as discussed by Basra et al. (18).

Table 1. Parameters measured for germination tests of green curly lettuce seeds.

Treatments (BE mg/L)	Number of seeds	Germination percentage (%)	Radicle size mm (mean ± SD)	Hypocotyl size mm (mean ± SD)	Cotyledon size mm (mean ± SD)	Number of absorbent hairs	Seed coverage
T0 (0)	60	100	33,81 ± 2,473	2,99 ± 0,475	6,15 ± 0,874	+	Presence
T1 (50)	60	100	32,24 ±2,804	3,01 ± 0,174	6,61 ± 0,642	+	Presence
T2 (100)	60	100	34,92 ± 3,124	3,01 ± 0,222	6,78 ± 0,719	+	Presence
T3 (150)	60	100	36,61 ± 2,031	3,05 ± 0,235	6,58 ± 0,476	++	Absence
T4 (200)	60	100	37,40 ± 3,108	2,95 ± 0,276	7,06 ± 0,740	++	Absence
T5 (500)	**60**	**100**	**40,59 ± 2,776**	**3,01 ± 0,308**	**6,67 ± 0,653**	**++**	**Absence**
T6 (1000)	60	100	38,90 ± 1,900	2,99 ± 0,211	6,54 ± 0,587	++	Absence
T7 (2000)	60	100	31,55 ± 2,685	2,90 ± 0,166	6,65 ± 0,664	++	Absence
T8 (5000)	54	85	9,52 ± 1,232	2,60 ± 0,310	5,80 ± 0,533	++	Absence
T9 (10000)	21	35	9,31 ± 1,914	2,23 ± 0,183	5,16 ± 0,706	++	Absence

+: low number of absorbent hairs
++: higher number of absorbent hairs

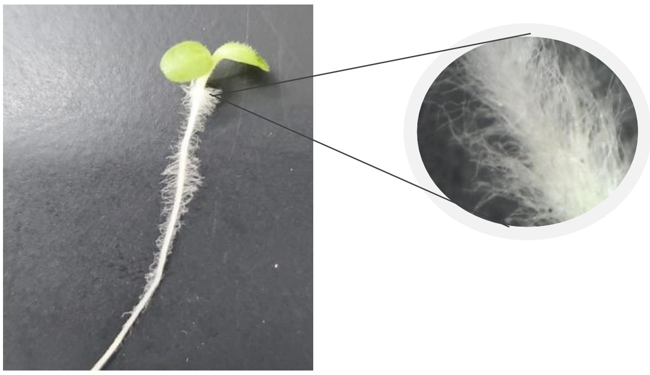

Figure 1. Visualization of the absorbent hairs of the radicle of *Lactuca sativa* applied to T5 (500 mg/L) observed with the digital microscope.

4 CONCLUSION

In conclusion, this promising study on the use of polymeric biosurfactants suggests that they likely enhance the germination and early growth of lettuce seeds at moderate concentrations. However, it is crucial to determine the optimal dosage to avoid adverse effects. These findings underscore the importance of biosurfactants in sustainable agriculture, aligning with previous studies that highlight their potential to improve water and nutrient absorption efficiency in plants.

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