FUNGAL SCREENING FOR GIBBERELLIC ACID PRODUCTION BY SUBMERGED FERMENTATION

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ABSTRACT

Gibberellic acid is a plant hormone that has a beneficial effect on various agricultural crops when used in their management. Its use can promote better vegetative development of plants, nutrient absorption and, consequently, increased productivity. In this context, the production of gibberellic acid can be a viable alternative in the search for greater efficiency in food production. The objective of this work was to evaluate the GA_3 production potential of eight fungal strains provided by Embrapa Agroenergia, of the genus *Fusarium* and *Aspergillus*, through submerged fermentation with a standard cultivation medium. Through the results obtained, it was observed that the strains Bioenzi F321 (*Fusarium fujikuroi*), Insumicro 175 (*Fusarium fujikuroi*) and LGB-034-2015 (*Aspergillus niger*) have the highest production capacity of gibberellic acid, with values of 420.3 ppm, 324.2 ppm and 262.73 ppm, as well as greater productivity and yield in fermentation. The Insumicro 20 and Insumicro L1RC3 strains do not have the potential to produce this metabolite.

Keywords: Keyword 1. Keyword 2. Keyword 3. Keyword 4. Keyword 5.

1 INTRODUCTION

Gibberellic acid (GA_3) has been widely studied due to its promising effects on the growth, quantity and quality of various crops. This hormone has the ability to regulate enzymes and antioxidants in plants, helping to mitigate stresses such as heat and soil salinity [1, 2].

The use of products that contain bioactive compounds has been a strategy adopted by producers for better plant growth and development, and, consequently, greater nutrient absorption. Organomineral fertilizers, a category that encompasses biofertilizers, have grown exponentially in Brazil. In 2022, sales exceeded R$4.0 billion, and are growing at an average of 20% per year [9].

Therefore, the production of gibberellic acid, a plant hormone responsible for the growth and development of plants, affecting processes such as stem elongation, seed germination and flowering, becomes a convenient strategy, since its use associated with traditional fertilizers can favor plant development and productivity [3, 4, 5, 7].

Therefore, the objective of this work was to evaluate the gibberellic acid production potential of eight fungal strains.

2 MATERIAL & METHODS

The fungi *Fusarium fujikuroi* (5 isolates) and *Aspergillus niger* (3 isolates) were evaluated in this study as potential producers of gibberellic acid (GA_3). The eight isolates were provided by Embrapa Agroenergia to the Biotechnological Processes Center (NUCBIO) of the Faculty of Chemical Engineering of the Federal University of Uberlândia and are kept in an ultrafreezer at -70ºC. The microorganisms were collected from different Brazilian biomes, and their identifications are presented in Table 1.

Table 1 Identification of the microorganisms used in the work.

Embrapa Code	Others code	Taxonomy
BRM 052099	GBF 27/13	*Aspergillus niger*
BRM 057297	LGB-034-2015	*Aspergillus niger*
BRM 028977	CTAA 54	*Aspergillus niger*
BRM 057631	Bioenzi F321	*Fusarium fujikuroi*
BRM 052451	Insumicro 175	*Fusarium fujikuroi*
BRM 063350	Insumicro L1RC3	*Fusarium fujikuroi*
BRM 063349	Insumicro EP639	*Fusarium fujikuroi*
BRM 058680	Insumicro 20	*Fusarium fujikuroi*

The 08 fungi were subjected to submerged fermentation tests, in the Research and Development Laboratory of the company Satis Indústria e Comércio LTDA, to evaluate their productive capacity of gibberellic acid, where, initially, a screening of the strains in

question was carried out (Figure 1), using the sacrificial bottle method, in which 5 containers were arranged for each fungus, totaling 40 batches.

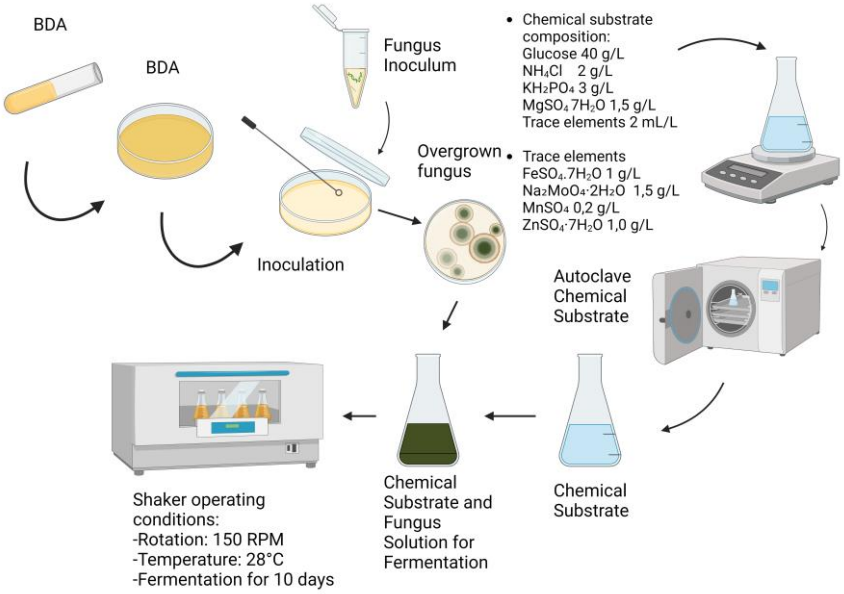

Figure 1 Fermentation process for fungal screening

After the sampling stage, filtration was carried out to separate the biomass and a 10 mL aliquot of the fermented product was collected, which was subjected to the clarification process to precipitate macromolecules using 0.5 mL of a 30% Zinc Acetate solution and 0.5 mL of a 15% Potassium Ferrocyanide solution. Subsequently, the mixture was centrifuged and 1 mL of the supernatant was pipetted and dispensed into a 10 mL volumetric flask, 1 mL of ethanol was added and the volume was completed with a 30% HCl solution. The solution was kept at rest for 75 min and then read on a spectrophotometer at 254 nm, previously calibrated [6]. The pH readings were taken with the aid of a pH meter from the manufacturer Hanna Instruments.

Using data obtained from biomass and gibberellic acid concentration analyses, several kinetic parameters were calculated to evaluate fermentation performance, thus facilitating the understanding of the kinetics of the fermentation process [10]. To calculate the parameters, the maximum GA_3 production value, the time in which this concentration was obtained, as well as the biomass value at that same moment and the initial value at time 0 were considered.

3 RESULTS & DISCUSSION

For fungi of the genus *Aspergillus*, gibberellic acid production varied according to the strain used. For the inoculum GBF 27/13, the maximum production was 31.51 ppm, in 240 hours, while the production achieved by the fungus LGB-034-2015 was 262.73 ppm in the same fermentation period, while the microorganism CTAA 54 reached a maximum production of 102.24 ppm, also in 240 hours. The three strains showed a high pH reduction in the culture media, with values of 2.5, 1.86 and 1.85 at the end of fermentation for each fungus, respectively. For the production of dry dough, the LGB-034-2015 strain demonstrated greater development capacity compared to the others, reaching an average of 18.9 g/L during the fermentation period. The fermentative process of fungi to produce gibberellic acid demonstrated that the metabolite under study is primary and the product is directly associated with growth. Some fermentations using other strains of *Aspergillus niger*, in Modified Czapek Dox culture medium, reaching a maximum production of 2.61g/L, differing from the strains studied in this work [8]. In another study using *Aspergillus niger* in solid state fermentation, using orange residue as substrate, the authors obtained results between 0.27 and 2.44 g/L of GA_3, which are closer to those found in this work [11].

For fermentations using fungi of the genus *Fusarium*, different concentrations of GA_3 were obtained in the fermentation media, however, similar performances in relation to pH reduction. The fungus Bioenzi F321 reached a maximum production of 420.3 ppm in 240 hours, however, at other times production was low, not exceeding 100 ppm, which can be explained by the use of the sacrifice method. For the biomass variable, the strain reached 17.7g/L also in 240h of fermentation and the pH was 1.87 in the same time. The fungus Insumicro 175 presented a more linear performance in terms of GA_3 production and biomass growth. The maximum metabolite concentration achieved was 324.2 ppm in 240h of fermentation, however, the maximum biomass was reached in 192h, with a value of 11g/L, and the pH was 2.05 in the same time. Insumicro EP639 also showed linearity in acid production and biomass growth, however, the maximum value obtained was 191.51 ppm and 18.7 g/L of biomass, and, like other fungi, it reduced the pH of the medium to 1.77. The fungi Insumicro 20, L1RC3 (68-1) did not demonstrate a good capacity for producing gibberellic acid under the conditions studied. Despite having good growth and pH reduction capacity, 12 g/L and 1.96; 22 g/L and 1.81, respectively, GA_3 production for the 2 tests were below 45 ppm.

The kinetic parameters were calculated for each of the fungi. The fungus Bioenzi F321 of the genus *Fusarium*, obtained the highest productivity of the fungus in relation to the product, the highest specific speed of formation and the highest yield in product, both when compared with other fungi of the same genus and with fungi of the genus *Aspergillus*. Insumicro 175 also stood out in the product yield and specific speed indices, followed by Insumicro EP639, Insumicro 20 and Insumicro L1RC3 (68-1). For microorganisms of the genus *Aspergillus*, LGB-034-2015 obtained the highest calculated kinetic indices, followed by CTAA 54 and GBF 27/13. When the evaluation is made comparatively to *Fusarium*, LGB-034-2015 obtained the highest productivity in relation to biomass and also the highest yield in biomass, while for the specific speed of product formation the performance was low in relation to the too much. The calculated data for all parameters for each of the fungi are described in Table 2.

Table 2 Productivity of each fungus in relation to product (P_P), biomass (P_X), specific rate of product formation (μ_p), biomass yield ($Y_{X/S}$) and product yield ($Y_{P/S}$).

Fungi	P_P (mg/L.h)	P_X (g/L.h)	μ_p (g product/g cells.h)	$Y_{X/S}$ (g cells/g substrate)	$Y_{P/S}$ (mg product/g substrate)
GBF 27/13	0,45	0,038	0,021	0,09	0,79
LGB-034-2015	1,09	0,097	0,036	0,58	6,57
CTAA 54	0,43	0,072	0,079	0,43	2,56
Bioenzi F321	1,75	0,070	0,554	0,42	10,51
Insumicro 175	1,35	0,042	0,220	0,12	8,11
Insumicro L1RC3 (68-1)	0,92	0,103	0,003	0,46	0,17
Insumicro EP639	0,80	0,070	0,049	0,42	4,79
Insumicro 20	0,18	0,066	0,038	0,24	1,00

4 CONCLUSION

Through the results obtained, it is concluded that the fungi Bioenzi F321 (*Fusarium fujikuroi*), Insumicro 175 (*Fusarium fujikuroi*) and LGB-034-2015 (*Aspergillus niger*) have the greatest potential for producing gibberellic acid among the strains evaluated. The fungi Insumicro 20 and Insumicro L1RC3 have the lowest potential. Therefore, studies are needed to optimize fermentation conditions and increase the production of the metabolite in question.

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