HERBICIDE RESISTANCE AND INDOLE-3-ACETIC ACID PRODUCTION BY YEASTS ISOLATED FROM POLLINATING INSECTS

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ABSTRACT

Yeasts are known for their interactions with the environment and with other living beings, generating gains for those involved, such as the production of volatile organic compounds (VOCs) that attract pollinators to the flowers in which the yeasts inhabit. Furthermore, yeasts can produce phytohormones that stimulate plant development and growth, such as indole-3-acetic acid (IAA). These characteristics can be changed by environmental disturbances. Thus, in this work, six strains of yeast isolated from pollinating insects (the beetle *Astylus variegatus* and the bees *Scaptotrigona postica* and *Tetragonisca angustula*) were tested for the production of IAA, with promising results for three strains (CHAP-237, CHAP-242, and CHAP- 248). Furthermore, resistance to Glyphosate and 2,4-D herbicides, the most common on the market, was tested at concentrations of ¼, ½, 1, and 2 times that indicated in the leaflet. Strains CHAP-237, CHAP-242, and CHAP-245 showed resistance to 2,4-D in cultures with 25% and 50% of the label dosage. The other strains, however, did not resist. These strains were identified as belonging to the species *Kurtzmaniella* sp. (CHAP-237) and *Meyerozyma caribbica* (CHAP-242, CHAP-245 and CHAP-248). Therefore, while demonstrating the negative impact of these herbicides on wild microorganisms, these data suggest that some of these yeasts can be used in bioremediation strategies for areas contaminated by pesticides.

Keywords: 2,4-D. Glyphosate. *Kurtzmaniella*. *Meyerozyma*.

1 INTRODUCTION

Indole-3-acetic acid (IAA) is the main phytohormone of the auxin class, responsible for plant growth. Naturally, plants produce IAA through different metabolic routes, depending or not on the availability of L-tryptophan (Trp), and the main one is the indole-3-pyruvic acid (IPyA) pathway, in which Trp is deaminated into IPyA, which is decarboxylated to IAA[1]. Auxin levels are regulated by biosynthesis and inactivation processes, as well as by polar and lateral transport — which are responses to light and gravity — maintaining the gradient necessary for plant development[2]. The regulation of the IAA gradient is very important, since high doses of this auxin can have a negative effect on plant organisms, disturbing their growth. It is in this logic that herbicides with 2,4-dichlorophenoxyacetic acid (2,4-D), a molecule analogous to IAA, act and are mainly used to control weeds[3,4].

However, microorganisms such as bacteria and fungi are also capable of synthesizing IAA[4,5]. This can be useful for treating and optimizing the growth of agricultural crops, but it should be noted that these ecological relationships are sensitive to environmental imbalances. For example, yeasts play an important role in plant reproduction, as, by growing and fermenting flower nectar, they produce volatile organic compounds that attract pollinators[6]. The application of herbicides for pest control, however, can alter the microbiological biodiversity of ecosystems, as well as affect the health and behavior of pollinators, as seen for Glyphosate (N-(phosphonomethyl)glycine) [7,8].

In Brazil, herbicides with the active ingredients 2,4-D and Glyphosate are the most common on the market. Therefore, in this work, the growth of yeasts isolated from pollinating insects was evaluated in relation to different concentrations of two herbicides with 2,4-D and Glyphosate. These yeasts were also tested for IAA synthesis, verifying the concern about the decrease in the biodiversity of microorganisms necessary for maintaining life.

2 MATERIAL & METHODS

After being collected, beetles and bees were inoculated into flasks with liquid YNB (6.7 g/L Yeast Nitrogen Base) medium with 10 g/L xylose and 0.2 g/L chloramphenicol. After 3–5 days of cultivation at 30 ºC, 10 µL loops were removed from the medium and streaked on solid media with the same composition (added with 20 g/L of agar). After growth, colonies with typical yeast morphology were isolated. The strains CHAP-223, CHAP-224, and CHAP-237 were isolated from the beetle *Astylus variegatus*, the strains CHAP-242 and CHAP-248 from the bee *Scaptotrigona postica*, and CHAP-245 from the bee *Tetragonisca angustula* (bee species popularly known as Mandaguari and Jataí, respectively).

Cell culture of the control groups of the strains was carried out in cotton-plugged Erlenmeyer flasks with YPD liquid medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose, pH 5.0) at 145 rpm and 30°C for 48 h. Cultivation under the same conditions was carried out by adding ¼, ½, 1, and 2 times the dosage recommended in the leaflet for each herbicide to the crops, that is,

0.525%, 1.05%, 2.1% and 4.2% for the Zapp IQ 620 and 0.1875%, 0.375%, 0.75% and 1.5% for Aminol 806, whose active ingredients are glyphosate and 2,4-D, respectively (percentages by volume). Cell growth was determined by absorbance on a spectrophotometer at a wavelength of 570 nm, twice a day.

The IAA standard curve was made with 10% IAA solution (50 mg/L), distilled water, and Salkowski Reagent (2 mL of 0.5 M Iron III Chloride and 98 mL of 35% Perchloric Acid)[9]. For the IAA production curve, the strains were cultivated in a YPD liquid medium, under the same conditions as those cultivated with herbicides, protected from light, according to a protocol adapted from Fu *et al*. (2016)[10]. Triplicate samples were collected twice a day and centrifuged at 5000 rpm for 3 minutes, saving 500 µL of supernatant for each sample. To determine the production of IAA by yeast, 500 µL of Salkowski Reagent was pipetted onto the supernatant, allowing the reaction to proceed for 30 minutes, protected from light. If positive for IAA production, the mixture was turned pink. Finally, the absorbances were measured on a spectrophotometer at 530 nm.

The taxonomic identification of the strains CHAP-237, CHAP-242, CHAP-245, and CHAP-248 was carried out by analyzing the sequences of the Internal Transcribed Spacers (ITS) between the small- and large-subunit rRNA genes, as described in a previous work of this group[11].

3 RESULTS & DISCUSSION

The growth curves obtained in media with herbicides are shown in Figure 1. No strain was able to grow in the presence of Glyphosate, regardless of its concentration. For the herbicide with 2,4-D, the strains CHAP-237, CHAP-242, CHAP-245, and CHAP-248 showed, to a greater or lesser extent, cell growth, with higher values for lower concentrations of the herbicide (¼ and ½ dose). Through the analysis of their ITS sequences, these strains were identified as *Kurtzmaniella* sp. (CHAP-237) and *Meyerozyma caribbica* (CHAP-242, CHAP-245, and CHAP-248).

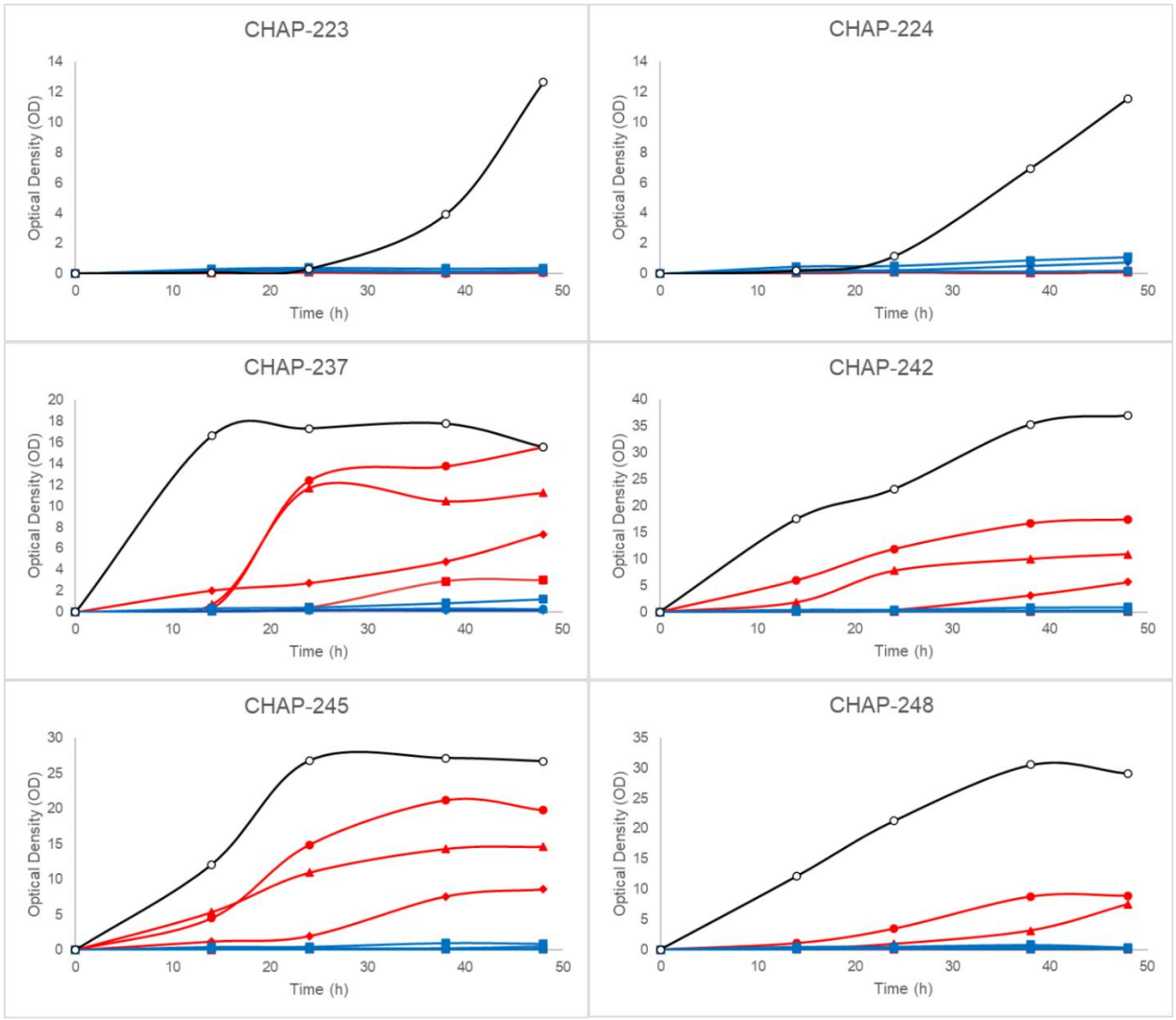

Figure 1. Yeast growth profiles under different herbicide concentrations. The colors black, red, and blue represent, respectively, the control group (YPD), growth with 2,4-D and with Glyphosate. The closed symbols represent the herbicide concentrations with ¼ (circle), ½ (triangle), 1 (diamond), and 2 (square) times the dosage indicated in the commercial leaflet.

The strains were also tested for IAA production during 48 h, as shown in Figure 2. The strains that displayed the best results at the end of cultivation were CHAP-242 (2.559 mg/L), CHAP-248 (2.468 mg/L), and CHAP -237 (2,394 mg/L). The identified strains presented IAA synthesis values similar to the literature[12]. Interestingly, the IAA production curves followed the profiles of the respective cell growth curves. Given that CHAP-223 and CHAP-224 did not reach the stationary phase of growth during the 48-h incubation period, future works could test the production of IAA by these strains over a longer period, to verify their potential compared to the others.

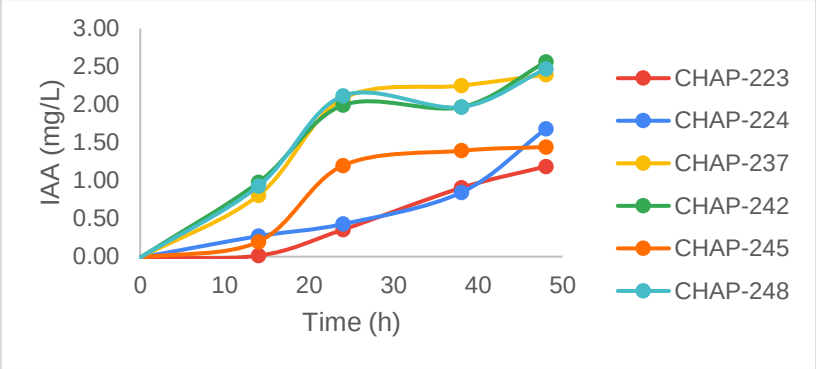

Figure 2. Production of indole acetic acid over 48 h of cultivation in YPD medium.

4 CONCLUSION

In this work, yeasts isolated from pollinating insects were tested for the production of IAA, as well as for cell growth against two herbicides with the most common active ingredients on the market (Glyphosate and 2,4-D). The results indicate that strains CHAP-237 (*Kurtzmaniella* sp.), CHAP-242, and CHAP-248 (*M. caribbica*) synthesized almost twice as much IAA compared to the other strains. Furthermore, the improvement of this yield can be tested in new crops with the addition of extra Tryptophan.

The results obtained also indicate that yeasts do not tolerate the presence of glyphosate in the culture medium. Regarding 2,4-D, however, four strains showed different degrees of tolerance. Our data, therefore, demonstrate that these herbicides can affect microbial biodiversity and the interactions of these organisms with the environment. On the other hand, the resistance presented by some strains suggests the potential use of these yeasts as bioremediation tools.

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