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ACTIVITY OF MYCOCINES PRODUCED BY *Wickerhamomyces anomalus* ABOUT STRAPS OF *Escherichia coli*

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INTRODUCTION

Mycocins are glycoprotein compounds secreted by some yeasts, such as *Wickerhamomyces anomalus,* these substances have antimicrobial action causing the death of susceptible microorganisms (KURTZMAN et al., 2011, CAPELLI et al., 2014). The production of mycocins by some microorganisms is considered a form of survival strategy in the habitat, because this way they stand out in the environment in which they live, causing the inhibition of non-mycocin producers (CAPELLI et al., 2014).

Escherichia coli is a Gram negative bacterium that belongs to the Enterobacteiaceae family, its natural habitat is the gastrointestinal tract of humans and animals, but its presence in water indicates fecal contamination and possible presence of other pathogenic microorganisms (MORATO, 2009; WEINTRAUB, 2007; WHO, 2011).

Considering the need for new alternatives for antimicrobial agents and the power of action of mycocins, this study aimed to evaluate the activity of mycocins produced by *W. anomalus* WA40 about the development of E. coli strains.

MATERIAL AND METHODS

MICROORGANISMS

Mycocins were obtained from the environmental strain of yeast: *W. anomalus* WA40, and the strains of *E. coli* were obtained from different clinical samples (1 tracheal secretion, 1 abscess secretion and 7 urine), in addition to using a standard strain of E. coli ATCC25922.

MYCOCIN PRODUCTION

For the production of mycocins, strain WA40 of W. anomalus was inoculated on modified Sabouraud agar (2% agar; 1% peptone; 2% glucose; 1.92% citric acid and 3.48% potassium phosphate dibasic – pH 4.7) and incubated at 32°C for 48 hours. After this period, a yeast suspension was inoculated in 200 mL of growth broth (1% peptone; 2% glucose; 1.92% citric acid and 3.48% dibasic potassium phosphate - pH 4.7). The flasks were incubated at 25°C for 5 days. After this period, the growth broth was centrifuged at 6000 rpm for 10 minutes to obtain the broth with supernatant containing mycocins produced by the yeast, then the supernatant was sterilized by 0.22 μm membrane filtration and stored at 4°C.

MYCOCIN SUSCEPTIBILITY TEST BY MICRODILUTION

The susceptibility test was performed using the broth microdilution method. Mycocins were subjected to serial dilutions 1:2, 1:4, 1:8, 1:16, and 1:32 in sterile water and tested against E. coli strains. Suspensions of 103 CFU/mL of each bacterial strain were added to 96-well microplates. Then, the mycocin dilutions were added to evaluate their activity. The plates were incubated at 36°C/48h.

ANTIMICROBIAL ACTIVITY IN SOLID MEDIUM

To determine the activity of mycocins in solid medium, the agar diffusion method was used. 20 ml of 4% Bacteriological Agar was added in a Petri dish as a pre-layer, and after solidification another layer (20 ml) of Brean Heart Infusion (BHI) medium was added over the solidified medium. Two 5 mm holes were made equidistantly only in the BHI medium. Then, 100 μL of a suspension of E. coli ATCC25922, standardized according to turbidity 0.5 on the McFarland scale, was added onto the BHI medium and dispersed with the aid of a sterile swab. In one of the holes were added 50 μL of the supernatant with mycocins from *W. anomalus* (WA40)

and in the other hole, as a control, only the culture medium used for the production of mycocins. The system was incubated at 37 °C for 48 hours. The test was considered positive when there was the formation of a yeast inhibition halo around the orifice containing the mycocins.

RESULTS AND DISCUSSION

The mycocins showed antimicrobial activity against strains of E. coli, and all strains of *E. coli* used were inhibited by mycocins at 1:2 and 1:4 dilutions. the strains of *E.coli*: EC08, EC09, EC11 and EC13 (isolated urine samples) were inhibited at 1:8 dilution (Table 1). Mycocins are more active against other yeasts, but they present antagonistic behavior to other microorganisms such as bacteria, which allows their use as an alternative antimicrobial agent.

E. coli	Myokines (dilutions)				
	1:2	1:4	1:8	1:16	1:32
ATCC25922	+	+	-	-	-
EC01	+	+	-	-	-
EC05	+	+	-	-	-
EC06	+	+	-	-	-
EC07	+	+	-	-	-
EC08	+	+	+	-	-
EC09	+	+	+	-	-
EC10	+	+	-	-	-
EC11	+	+	+	-	-
EC13	+	+	+	-	-

(+) positive inhibitory action (-) negative inhibitory action.

Table 1. Inhibitory action of mycocins obtained from the strain of *W. anomalus* WA40 against bacterial strains:*E. coli*.

In the test of antimicrobial activity in solid medium, the inhibition of the growth of E.coli was verified when it was seeded close to the hole of the agar containing WA40 mycocins (Figure 1). The results found were similar to those obtained by Junges et al (2020), Calazans et al (2021) and Rosseto et al (2022) who, through the test in solid media, confirm the effectiveness of mycocins acting on strains of: *Acinetobacter baumanni, Staphylococcus aureus* and *Candida albicans*, respectively. Thus, they confirm the effectiveness for the susceptibility of these microorganisms to the action of mycocins.

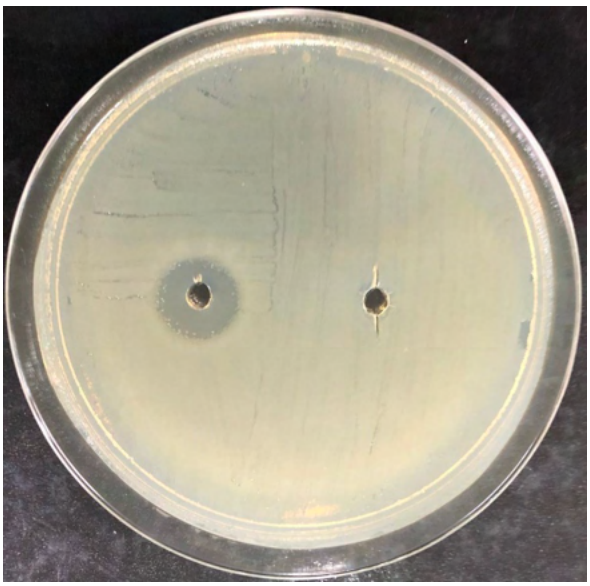

Figure 1. Antimicrobial activity test of mycocins in solid medium. (A) Well containing supernatant with mycocins from W. anomalus (WA40) with E. coli inhibition halo formation. (B) Orifice with culture broth, without formation of inhibition halo.

CONCLUSION

This work showed through tests that mycocins have antimicrobial action against E.coli strains. This way, they become an alternative agent with antibacterial action.

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