LACTOBACILLUS SSP. ARE INHIBITED BY EXTRACELLULAR METABOLITES PRODUCED BY *SACCHAROMYCES CEREVISIAE* IN THE CONTEXT OF ALCOHOLIC FERMENTATION

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1. INTRODUCTION

The genus *Lactobacillus* are reported as the main contaminant in bioethanol production (LUCENA et al., 2010). Considering that contaminants are harmful to *Saccharomyces cerevisiae* fermentation efficiency and consequently to industrial yield (BASSO et al., 2014; XU et al., 2021), the referring study aimed to verify whether contaminating bacteria isolated from the bioethanol industry have their growth inhibited by *S. cerevisiae* (PE-2) during alcoholic fermentation.

2. MATERIALS AND METHODS

The bacterial contaminants *L. fermentum* I3a (heterofermentative) and *L. plantarum* I4a (homofermentative) were isolated from a distillery in the State of São Paulo. The cultivations were carried out with sugarcane molasses (170 $g.L^{-1}$ total reducing sugar) in three conditions: T_1- Pure culture of bacteria in fed-batch; T_2- Co-cultivation with PE-2 ($1.07x10^9$ $Cel.mL^{-1}$) mimicking the industrial process in fed-batch (RAGHAVENDRAN et al., 2017); T_3 - Pure culture in wine (123 $g.L^{-1}$ total reducing sugar) previously fermented by yeast for 7 hours (Fermentation "in stages"). All treatments were performed as three biological replicates, with an incubation at 32°C, without agitation, of 24 hours. Bacteria growth was determined by drop plating and lactic acid was quantitated by HPLC analysis.

3. RESULTS AND DISCUSSION

As shown in Figure 1, we observed that both strains showed high values of CFU/mL after 24h when cultured alone (T_1). In T_2, however, both strains showed a lower cell concentration after 24h when compared to T_1, possibly because it competed with PE-2 for nutrients and/or was inhibited by extracellular metabolites accumulation in the must (BAYROCK; INGLEDEW, 2004). Interestingly, in T_3 there was no growth at all, leading to the assumption that the yeast had already consumed essential nutrients present in the must. In addition, based on these results it seems that PE-2 can produce metabolites capable of inhibiting bacterial growth and such production was not induced by the presence of contaminants.

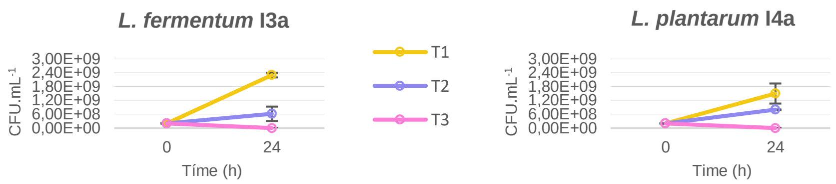

Figure 1. *Lactobacillus growth for 24 hours.*

Growth results are corroborated by lactic acid concentrations and final pH values (Table 1).

Table 1. Latic acid concentration and pH after 24 hours

Treatment	Strain	Lactic acid at 24h ($g.L^{-1}$)	pH at 24h
T_1	*L. fermentum* I3a	10.62 ±0.11[a]	4.01 ±0.02[c]
	L. plantarum I4a	10.3 ±0.08[a]	4.17 ±0.17[b]
T_2	*L. fermentum* I3a	5.95 ±0.08[b]	4.23 ±0.02[b]
	L. plantarum I4a	6.19 ±0.30[b]	4.28 ±0.01[b]
T_3	*L. fermentum* I3a	2.23 ±0.01[c]	5.00 ±0.05[a]
	L. plantarum I4a	2.07 ±0.14[c]	5.00 ±0.05[a]

Note: pH in the beginning of the cultivation (time 0h) was 5.0. Different lower case letters in columns designate statistically significant differences ($p < 0.05$) between fermentation trials (Tukey's test, at a significance level of 0.05).

4. CONCLUSION

Based on these results, the PE-2 was able to produce extracellular metabolites that inhibit the growth of to both Homo- and heterofermentative *Lactobacillus*.

5. REFERENCES

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6. ACKNOWLEDGMENTS

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