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Effect of Quinine Sulphate on *Saccharomyces cerevisae* Yeast Strains. Preliminary Note.

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SUMMARY. While the effects of *Cinchona spp.* alkaloids on certain microorganisms have been well studied, their activity on yeasts has received little attention. This could be interesting, as the use of natural products offers one other possibility of selecting new yeast strains that can have industrial use.

RESUMEN. "Efecto del Sulfato de Quinina sobre Cepas de la Levadura *Saccharomyces cerevisae*. Nota Preliminar". Mientras que los efectos de los alcaloides de *Cinchona spp.* sobre ciertos organismos han sido bien estudiados, no hay conocimiento de su actividad sobre las levaduras. Esto puede ser interesante, ya que la acción de los productos naturales representa una posibilidad de seleccionar cepas con nuevas características de valor industrial.

INTRODUCTION

The biological action of alkaloids has been extensively studied in terms of their cellular mechanisms of action. Of the alkaloids present in *Cinchona spp.* bark, quinine has been shown to have a wide spectrum of uses, including antimalarial, antitetanic, analgesic as well as other properties [1-7]. Quinine and its synthetic derivative, chloroquine, have been shown to form molecular complexes with DNA, and studies on the action of similar alkaloids on the growth cycle of *Plasmodium knowlesi* in parasitized erythrocytes has demonstrated that their primary inhibitory action is directed at the mechanisms of DNA replication, rather than at RNA replication or protein synthesis [1,4,5].

In this paper was report the action of quinine sulphate on three strains of *S. cerevisae* in terms of cell growth.

KEY WORDS: Metabolism; Quinine; Yeasts.
PALABRAS CLAVE: Metabolismo; Quinina; Levaduras.

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MATERIALS AND METHODS

Strains

Three diploid strains of *S. cerevisae* from the Collection held at the Instituto de Biotecnología of the Universidade de Caxías do Sul were used: *KI,* a carrier of the "killer" trait; *Zymasil,* a commercial strain used in the wine industry; *1A,* a native strain, isolated from musts from the grape-growing region near Caxías do Sul, Rio Grande do Sul, Brasil.

Culture media

The media used for strain maintenance and for experiments were: a) *Complete YEPD:* 1% Bacto-Yeast extract (DIFCO), 2% Bacto-Peptone (DIFCO), with 2 or 5% glucose); *Complete YEPG:* 1% Bacto-Yeast extract (DIFCO), 2% Bacto-Peptone (DIFCO), with 5% glycerol. Media were solidified with 2% Bacto-Agar (DIFCO) to work on Petri dishes.

Reagents

Quinine sulphate was Merck (Darmstadt). Alkaloid solutions were prepared immediately prior to their use, and added to liquid or solid media without autoclaving.

Methodology

Yeast cultures were used in the stationary phase, obtained by growth in grooves on complete YEPD media, after incubation for 48 h at 28 °C.

Appropriate suspensions for each strain were prepared by inoculation on Complete YEPD media (2% glucose) containing different concentrations of quinine sulphate, from 0.0 to 2.0 mg/ml.

In a second stage, strains were dispersed with a Drigalski loop on plates of Complete YEPD (5% glucose) and Complete YEPD with and without quinine sulphate. Each experiment was completed in triplicate using populations of 100 cells/plate.

RESULTS AND DISCUSSION

The inhibition of yeast growth on complete media containing 2% glucose and different concentrations of quinine sulphate (Table 1) can be ascribed to the possible cytotoxic effect of the alkaloid. This has been already observed for eukaryote cells *(Plasmodium gallinaceum* and *P. berghei* [6]. The KI and Zymasil strains show variations in their sensitivity to the alkaloid.

The yeasts also show different sensitivity in relation to the carbon source available. In Table 2 inhibitions observed using Complete YEPD (5% glucose) and Complete YEPD media are listed. As can be observed, with similar alkaloid concentrations, inhibition is higher when the carbon source is glycerol. Comparison of Tables 1 and 2 shows that the strains had a higher resistance in the presence of higher concentrations of glucose.

Strain	mg/ml alkaloid	growth (%)
KI	0.0	100.0
	1.0	60.8
	1.5	0.0
	2.0	0.0
Zymasil	0.0	100.0
	1.0	35.5
	1.5	0.0
	2.0	0.0

Table 1. Growth of *Saccharomyces cerevisae* strains on YEPD 2% glucose and different quinine sulphate concentrations. The figures relate to the % number of colonies growing in alkaloid free medium (standard), based on an average of 3 plates per concentration studied.

Strain	Media			
	YEPD	YEPD + Q	YEDG	YEPG + Q
KI	100.0	87.3	100.0	0.0
Zymasil	100.0	100.0	100.0	0.0
1A	100.0	1.8	100.0	0.0

Table 2. Yeast growth in presence of quinine sulphate (1.5 mg/ml) in Complete YEPD (5% glucose) and Complete YEPG media (Q: quinine sulphate). The values correspond to the % of colonies growing using pure media as standard.

The results of Table 2 can be interpreted as due to two different mechanisms. Quinine sulphate could affect the membrane permeability processes or energy-related sugar metabolism.

If we assume that the alkaloid inhibits glucose transport through the membrane, higher sugar concentrations will still allow the utilization under these unfavorable conditions, if the blockade is in some way overwhelmed or alternative routes are made possible. The permeability of glycerol seems to be impired in a more effective way, as not even higher external glycerol concentrations made the cells viable.

Quinine sulphate may also inhibit the enzymatic steps related to sugar metabolism, as cell development was inhibited with 1.5 mg/ml when glucose and glycerol concentrations were low (2%). This has been observed for lycorine and other alkaloids [8,9]. The hypothesis is supported by the fact that growth occured when the cells were cultured in the presence of genuine sulphate with higher glucose concentrations, which permits fermentative metabolism.

Of the strains studied, 1A was most sensitive in all conditions, while Zymasil was capable of normal growth when the adequate carbohydrate concentrations

were present. The differential resistance of yeast strains to quinine sulphate indicates that the use of this and other alkaloids, as well as other natural products, offers a possibility os isolating resistant mutants, which when isolated could be studied for other technically valuable properties.

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