STEROL PROTECTION AGAINST PIMARICIN IN SACCHAROMYCES CEREVISIAE

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Pimaricin, a member of the polyene group of antibiotics, was isolated by Struyk et al. (1957-1958) from Streptomyces natalensis and its structure determined by Patrick et al. (1958). This antibiotic is chiefly inhibitory for yeasts and molds. Since microbial sterols are generally confined to yeasts and molds rather than bacteria, it was of interest to extend the observations of Gottlieb et al. (1958) to sterol protection of Saccharomyces cerevisiae against pimaricin inhibition.

Although the mode of action of polyene antibiotics is presently unknown, it has been reported that some fatty acids slightly depress the antifungal activity of ascosin (Hickey, 1953); K^+ and NH_4^+ ions reduce the activity of nystatin (Marini, 1959); and Mn^{++}, Ca^{++}, and Mg^{++} reverse the inhibitory effect of pimaricin on acetate oxidation by S. cerevisiae (Bach et al., 1960). Also Gottlieb et al. (1958, 1959) showed that certain sterols variably reduced the inhibitory action of nine polyenes: filipin, fungichromin, amphotericin B, trichomycin, rimocidin, candicidin A, candicidin B, ascosin, and nystatin. It was further observed by Gottlieb et al. that cholesterol, ergosterol, sitosterol, and stigmasterol exerted a protective effect against the antifungal activity of filipin upon Hansenula subpelliculosa, while lanosterol manifested slight protection. This effect of sterols may indicate an interference by polyene antibiotics in the biogenesis of sterols among fungi.

EXPERIMENTAL

Saccharomyces cerevisiae ATCC 2366 was grown in a medium containing 2% dextrose, 1% peptone (Difco) at pH 5.6. Cultures were incubated 20 hrs at 28° on an Eberbach rotary shaker (180 rpm). Cells from 200 ml of medium were harvested and washed twice with KH_2PO_4 buffer at pH 4.5 and resuspended in buffer for use as inoculum with the cell concentration adjusted to an optical density of 0.30 at 630 mμ for a 10^{-1} dilution.

Sterols were dissolved in ethyl acetate and 0.1 ml of this solution was added to 100 ml of sterile culture medium then placed on a shaker at 28° for 1 hr to evaporate excess ethyl acetate. Controls containing the solvent only were similarly treated.

Pimaricin (96% pure) was then added to the test medium in the form of an acetic acid solution (pH 4.5) to a final concentration of 140 mg/100 ml. All flasks were inoculated with 0.5 ml of yeast suspension and incubated 20 hrs at 28° on a rotary shaker. Comparative growth was measured as optical density at 630 mμ and reversal expressed as the amount of pimaricin inhibition which was suppressed by sterols according to equation (1).

$$\% \text{ reversal} = \frac{\text{O.D. with sterol \& pimaricin}}{\text{O.D. with sterol}} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

The growth of S. cerevisiae was completely inhibited during incubation for 20 hrs in the presence of 1.4 μg/ml of pimaricin. This effect was almost completely reversed by cholesterol (Fig. 1) and similar results were achieved with ergosterol, sitosterol and stigmasterol. When a mole ratio of cholesterol to pimaricin of 5:1 was employed 96% of the pimaricin activity was abolished. Concerning the decrease in reversal at levels of cholesterol above 4 μg/ml (Fig. 1) no adequate explanation seems appropriate since sterol controls were non-inhibitory. Under the same conditions no reversal was attained with mevalonic acid, squalene or lanosterol. These results are similar to those reported by Gottlieb et al. (1958, 1959) on sterol protection of fungi against filipin.

Thus the possibility exists that S. cerevisiae possesses a sterol require-

ment for growth. The data may be interpreted as suggesting that pimaricin is an

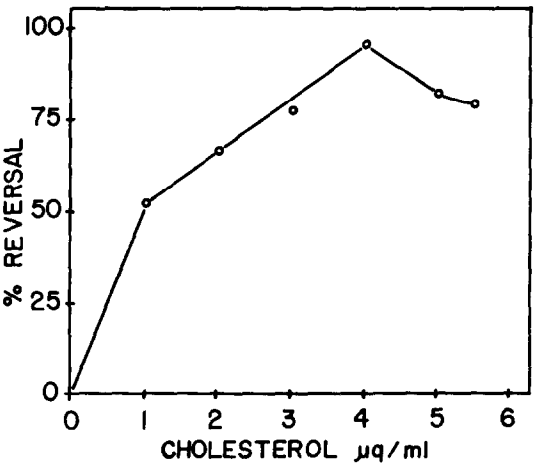

Fig. 1 Cholesterol reversal of pimaricin inhibition of growth of Saccharomyces cerevisiae ATCC 2366. Reaction system: 1.4 mcg/ml pimaricin, cholesterol, 0.5 ml washed yeast, in 100 ml of 2% dextrose-1% peptone broth (pH. 5.6) incubated with shaking 20 hrs at 28°.

antimetabolite in the pathway of sterol biosynthesis beyond lanosterol in as much as the latter was ineffective. While it has not been established that yeasts and molds have a general autotrophic requirement for sterols, such compounds are known to be synthesized by many of these organisms (Kieber et al., 1955; Klein, 1955; Fiertel, and Klein, 1959). On the other hand, ergosterol or cholesterol is required for the anaerobic growth of a strain of S. cerevisiae (Andreasen and Stier, 1953). Also, pleuropneumonialike organisms require cholesterol or other 3- OH sterols (Edward and Fitzgerald, 1951; Smith, 1959).

An alternative interpretation of the sterol effect may be the result of an interaction between sterols and polyene rendering the latter inactive. However, Gottlieb et al. (1959) were unable to demonstrate an extracellular interaction between sterols and filipin. On the other hand the predominant reaction may occur intracellularly wherein a polyene specifically reacts with a β-3-OH sterol intermediate in ergosterol synthesis.

Further evidence is required before a conclusive mechanism of sterol pro-

tection against polyenes can be suggested.

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SUMMARY

The Δ^5-β-3-OH sterols, cholesterol, ergosterol, sitosterol, and stigmasterol, reversed the antifungal activity of pimaricin on S. cerevisae while mevalonic acid, squalene and lanosterol were inactive in this respect. Several explanations for this effect were discussed.

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