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Inhibition of respiratory enzyme synthesis in yeast by chloramphenicol: Relationship between chloramphenicol tolerance and resistance to other antibacterial antibiotics

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1. INTRODUCTION

The inhibitory effect of a number of antibacterial antibiotics on the synthesis of the respiratory system of yeast has recently been investigated in this laboratory. It was observed that those antibiotics which are known inhibitors of bacterial protein synthesis, for example chloramphenicol, tetracycline, lincomycin and the macrolides also inhibited protein synthesis in isolated yeast mitochondria and the synthesis of the mitochondrial cytochromes a, a_3, b and c_1 in intact yeast cells (Clark-Walker & Linnane, 1966, 1967; Huang *et al.*, 1966). This *in vivo* effect on mitochondria is apparently specific since the yeast cells can grow and divide in the presence of the drugs provided there is an ample supply of fermentable substrate, that is, under conditions in which the respiratory system can be dispensed with.

With the original diploid *Saccharomyces cerevisiae* strain used in our studies, the concentrations of antibiotics required to inhibit cytochrome formation were high compared to the amounts normally found to inhibit bacterial protein synthesis and growth (see Reeve, 1966). In the present investigation the analysis of a number of haploid strains of this species has shown that the degree of sensitivity to each of the antibiotics tested is strain-dependent, i.e. genetically determined. Strains sensitive to 50 μg./ml. chloramphenicol have been isolated, while sensitivity to 10 μg./ml. erythromycin has been seen in other cases. Sensitive strains give rise spontaneously to stable resistant mutants both of the recessive and dominant types and the resistance levels observed in some cases corresponds to the maximum solubility of the drug.

Having characterized a number of haploid strains regarding chloramphenicol tolerance in the first instance, cross-resistance and cross-sensitivity to tetracycline, lincomycin, and some members of the macrolide group of antibiotics, erythromycin, carbomycin, spiramycin and oleandomycin were investigated.

2. METHODS

Tests were made by dropping out cell suspensions on yeast extract, peptone (YEP) agar plates (Wilkie & Lee, 1965) containing the antibiotics in the concentrations 0·001, 0·01, 0·05, 0·1, 0·25, 0·5, 1, 2, 3, 4, 5, 8, 10, 20 mg./ml. depending on individual solubility. Chloramphenicol (chloromycetin) solubility in water can be greatly increased by heating to boiling before adding to the medium. Chloramphenicol succinate is very soluble and is as active on yeast cells as chloramphenicol itself: presumably the ester is hydrolysed by yeast cells as in mammals to liberate chloramphenicol. This enzyme reaction is not available apparently in bacterial cells which are largely unaffected by the succinate. Glycerol (4%)

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was used as the energy source for growth, since ability to grow on a non-fermentable substrate in the presence of the drug is dependant on normal mitochondrial respiratory activity. The ability of a strain to grow on glycerol in the presence of a particular drug concentration is the criterion of resistance. Strains listed as resistant to one concentration are completely or greatly inhibited at the next higher concentration. The drop-out technique on solid medium is quite adequate in discriminating between these different levels of resistance. A more accurate measure of antibiotic activity is available in liquid culture where optical density readings can be taken at intervals. Where this has been done, strains have shown inhibition by amounts of antibiotics of the same order as those required on solid medium.

The tolerance levels listed were found to be stable features of these strains and not altered in any way by culture in the absence of the particular drug.

Spontaneous resistant mutants appeared on antibiotic-containing plates as small colonies against a background of inhibited cells.

For further details of procedure see Wilkie & Lee (1965).

3. RESULTS

In the first series of tests, strains classified on the basis of chloramphenicol tolerance were cross-checked for resistance to erythromycin and tetracycline. The results are given in Table 1 from which it is apparent that there is a good correlation between chloramphenicol and tetracycline, but no correlation between the resistance levels of these two and that of erythromycin. Stain S-878 is exceptional, showing resistance to chloramphenicol but sensitivity to tetracycline.

Table 1. *Resistance levels of some haploid yeast strains to antibiotics that inhibit respiratory development*

Strain	Resistance level (mg./ml.)		
	Chloramphenicol (CM)	Tetracycline (TC)	Erythromycin (ER)
45	4	2	0·25
10-1A	4	2	0·1
10-20B	2	1	0·25
4C1	1	0·5	0·1
13-2A	1	0·5	0·1
41	0·1	0·25	0·1
40	0·05	0·1	0·25
10-3C	0·05	0·1	0·05
10-14B	2	1	1
10-13A	4	2	1
S-878	1	< 0.05	0·05

The yeast strains were cultured on YEP-glycerol medium in the presence of the various concentrations of chloramphenicol (0–4 mg./ml./medium), erythromycin (0–10 mg./l. medium) and tretracycline (0–3 mg./ml/ medium). Growth of the individual strains occurred in the presence of the concentration of antibiotic indicated.

In a second series of experiments, a number of spontaneous mutants, showing higher levels of resistance to one or other of the antibiotics, were tested for a change in level of resistance to any of the other antibiotics. The number of antibiotics was increased to include lincomycin, spiramycin, carbomycin and oleandomycin. In general, the results obtained (Table 2) provided good supporting evidence for the conclusions reached in the

first series. Thus spontaneous mutants resistant to 8 mg./ml. erythromycin were mainly unchanged with respect to chloramphenicol and tetracycline tolerance, while spontaneous resistance to chloramphenicol usually resulted in simultaneous increase in resistance to tetracycline but not erythromycin. Within the macrolide group, spontaneous increase in resistance to erythromycin is frequently accompanied by increased resistance to carbomycin, oleandomycin and spiramycin strongly suggesting a similarity in mode of action of these chemically related compounds. Furthermore, the members of this group as a whole show little or no change in their inhibitory effects following changes in chloramphenicol and tetracycline tolerances in the various mutants.

Table 2. *Resistance levels of yeast strains and mutants derived from them to various antibiotics that inhibit respiratory development*

	Resistance level (mg./ml.)*						
Strain	CM	TC	ER	CA	OL	SP	LI
21-4B	< 0·1	0·1	< 0·1	< 0·5	2·0	< 0·1	0·1
21-4B-CMR	1·0	1·0	< 0·1	< 0·5	2·0	< 2·0	10·0
41	< 0·1	0·1	0·1	< 0·5	5·0	0·5	0·5
41-CMR	2·0	1·0	0·1	0·5	5·0	0·5	20·0
41-ERR	< 0·1	0·1	8·0	2·0	20·0	5·0	10·0
D243-1A	1·0	0·5	0·1	< 0·5	< 5·0	< 2·0	2·0
D243-1A-ERR	1·0	0·5	12·0	0·5	20·0	5·0	10·0
D243-F2	< 0·1	0·25	0·1	< 0·5	0·5	< 2·0	< 2·0
D243-F2-ERR	1·0	1·0	8·0	< 0·5	0·5	2·0	10·0
10-19B	1·0	4·0	0·5	< 0·5	5·0	< 2·0	10·0
10-19B-ERR	1·0	4·0	8·0	0·5	10·0	2·0	10·0
10 (diploid)	3·0	2·0	0·1	< 0·5	5·0	< 2·0	10·0
10-ERR	3·0	2·0	12·0	5·0	20·0	8·0	10·0
M (diploid)	1·0	0·5	0·1	0·5	5·0	10·0	10·0
M-CM	4·0	2·0	0·1	0·5	5·0	10·0	10·0
4C1	0·5	0·5	0·5	< 0·5	5·0	< 2·0	5·0
4C1-TCR	4·0	2·0	0·5	0·5	20·0	2·0	5·0
4C1-CM	4·0	0·5	0·5	< 0·5	5·0	2·0	5·0

Growth conditions similar to those cited in Table 1 legend.

Abbreviations: CA, carbomycin; OL, oleandomycin; SP, spiramycin. CMR, ERR, TCR denote spontaneous resistant mutants to chloramphenicol, erythromycin and tetracycline respectively.

* Range of concentrations used (mg./ml.); CM, 0·1–4; TC, 0·1–4; ER, 0·1–12; CA, 0·5–2·0; OL, 0·5–2·0; SP, 2–10; LI, 2–20.

4. DISCUSSION

It is well established and generally recognized that protein synthesis can proceed in mitochondria in eucaryotic cells including yeast, independently of the protein synthesis that goes on in the rest of the cell (Brock, 1961). The latter is based on a microsomal system having ribosomes of the 80S type. Mitochondria as well as having an intrinsic DNA, also contain RNA. Preparations of mitochondrial RNA of yeast have been fractionated by centrifugation to yeild fractions corresponding to 23S, 16S and 4S units (Wintersberger, 1966). The 4S fraction could be transfer RNA and the 23S and 16S, sub-units of ribosomes. From the present investigation and that of Clark-Walker and Linnane and Huang *et al.* previously mentioned, it is apparent that specific inhibitors of bacterial

protein synthesis are specific inhibitors of mitochondrial synthesis, suggesting there are affinities between the two systems. It is of interest then, to compare the relationships of the antibiotics as revealed by their effects on the yeast mitochondria to that already described for bacteria.

Similarity between the two systems is indicated by the findings of Taubman *et al* (1966). They report that erythromycin is bound more tightly than chloramphenicol to the ribosomes of a protein-synthesizing cell extract of *Bacillus subtilis*, and that the binding of tritiated erythromycin is not affected by the addition of chloramphenicol or tetracycline, even though binding of chloramphenicol to ribosomes can also occur. Although not established by these investigators, it seems likely that erythromycin and chloramphenicol are not competing for the same site on the ribosome (see also Tanaka *et al.*, 1966). On the other hand, Vasquez (1966) reports that the binding of ^{14}C-chloramphenicol to ribosomes in a cell-free system from *B. megaterium* is prevented by a number of macrolide antibiotics including erythromycin, but not by tetracycline. These results are consistent if it be considered that the more strongly bound erythromycin, having complexed with the ribosomes, can prevent access of binding sites to chloramphenicol, although the sites may be different in the two cases. It must be emphasized that the binding sites alluded to are the 70S ribosomes of the bacteria. Vasquez (1966) has shown that chloramphenicol does not bind to the 80S ribosome of the yeast *Crithida oncopelti*. It may be speculated that mitochondria have ribosomes resembling the 70S type and that this is the basis of the similarity of action of antibiotics in the two systems.

Although a similarity in the relationship of the antibiotics to the bacterial and yeast mitochondrial systems is indicated, it must be considered that the resistance patterns in the *in vivo* yeast system could arise from changes in the permeability of the yeast cells or of the yeast mitochondria. This question of permeability is at present being investigated. However, preliminary studies with one erythromycin-resistant mutant have shown that the amino acid incorporating activity of mitochondria isolated from this strain is resistant to erythromycin, whereas the activity of mitochondria isolated from the parent sensitive strain is markedly inhibited by this drug.

It is clear that a good deal remains to be learnt of the mechanism of action of these antibiotics in bacteria. The system described provides a convenient tool for extending and supplementing these investigations. The techniques are also of importance in that they may provide respiratory mutants for the study of mitochondrial biogenesis.

SUMMARY

Individual yeast strains show characteristic differences in the amount of chloramphenicol required to inhibit the synthesis of the respiratory system. This varies from 0·05 mg./ml. to 4 mg./ml. A correlation between chloramphenicol and tetracycline resistance appears likely but no correlation between chloramphenicol and erythromycin resistance was observed. These relationships were emphasized by cross-checking spontaneous resistant mutants for each antibiotic. Nearly all mutants showing spontaneous resistance to chloramphenicol had a simultaneous increase in tetracycline resistance but no increase in erythromycin resistance. Spontaneous resistance to erythromycin on the other hand, had no striking effect on tolerance levels to chloramphenicol and tetracycline. Increases in erythromycin resistance were commonly accompanied by an increase in resistance to other macrolide antibiotics. There are similarities between these effects and those described for certain bacterial systems.

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