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Effect of tetraethylammonium ions and 4-aminopyridine on osmotic stability of *Saccharomyces cerevisiae*

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Tetraethylammonium bromide (TEA) and 4-aminopyridine (4-AP) are lethal to *Saccharomyces cerevisiae*. However, the growth of yeast cells is restored on addition of osmotic stabilizers, i.e. 0.2 M KCl, 0.2 M NaCl or 0.4 M mannitol, to the plates containing inhibitory concentration of TEA and 4-AP. 4-AP induces osmotic stability defects instead of blocking K^+ channels in *S. cerevisiae* as reported in animal cells. As a result, this drug causes cell lysis whereas TEA seems to cause lethality by bringing about some other changes in the plasma membrane of the yeast cells.

ALL organisms require K^+ ions. In plants and fungi, intracellular K^+ ions serve a variety of vital functions, including the control of cell shape and turgor pressure[1]. In yeast, one of the important functions of K^+ is suggested to neutralize the negative charge of cellular anions[2]. Like all other organisms, *Saccharomyces cerevisiae* accumulates K^+ ions from the external medium to fulfil the cellular requirements. The concentration of K^+ inside the cells may be 10–20 times higher than in the surrounding medium. The transport of K^+ ions in the cell takes place through various transporters/channels. Various pharmacological drugs including tetraethylammonium bromide (TEA) and 4-aminopyridine (4-AP) have been reported to block K^+ channels in animal cells[3,4]. The present study is an attempt to investigate the effects of TEA and 4-AP on K^+ uptake and viability of *S. cerevisiae*.

Yeast cells were grown on yeast extract, peptone, dextrose (YPD) medium and synthetic complete medium agar plates having different concentrations of TEA and 4-AP. The growth of yeast cells was completely inhibited at 800 mM concentration of TEA and 15 mM concentration of 4-AP on YPD medium agar plates. However, on synthetic complete medium agar plates, the lethal concentration of TEA and 4-AP was 150 and 2 mM, respectively (Table 1). High inhibitory concentration of TEA and 4-AP in YPD medium as compared to synthetic complete medium suggests the presence of high concentration of ions and other molecules (nutrients) in the YPD medium which may be diluting the effect of these drugs. TEA and 4-AP are known to block K^+ transporters in animal cells[3,4]. The inhibition of growth of *S. cerevisiae* in the presence of TEA and 4-AP might also be because of blocking of K^+ transporters in yeast cells.

Further, the growth of *S. cerevisiae* on synthetic complete medium agar plates, containing inhibitory

Table 1. Effect of TEA and 4-AP on viability of *S. cerevisiae* on YPD medium and synthetic complete medium agar plates

Medium used	Drug	Concentration of drug (mM)	Viability (%)
YPD medium	TEA	100	100 ± 1.16
		200	100 ± 2.64
		400	68 ± 6.51
		600	64 ± 5.86
		800	0
	4-AP	2.5	100 ± 3.51
		5	100 ± 2.52
		10	75 ± 6.03
		15	0
Synthetic complete medium	TEA	50	100 ± 6.43
		100	100 ± 4.04
		150	0
		200	0
	4-AP	0.25	100 ± 4.72
		0.50	100 ± 5.01
		1	50 ± 3.05
		2	0

Results are mean ± SD of three independent experiments.

Table 2. Effect of KCl, NaCl and mannitol on the growth of *S. cerevisiae* on synthetic complete medium agar plates containing TEA and 4-AP

Drug	Concentration of drug (mM)	Per cent viability		
		KCl (0.2 M)	NaCl (0.2 M)	Mannitol (0.4 M)
TEA	100	100*	100*	100 ± 2.52*
	150	100	100	100 ± 2.08
	200	0	0	0
4-AP	1	100	100	100 ± 4.58
	2	100	100	100 ± 2.00

Results are mean ± SD of three independent experiments.
*Early appearance of colonies.

Table 3. Effect of 4-AP on K^+ uptake in *S. cerevisiae*

Concentration of 4-AP (mM)	Time (min)	Intracellular K^+ content (μmole/mg dry dw)
0	0	0.504 ± 0.201
0	30	0.656 ± 0.280
0	60	0.679 ± 0.199
10	30	0.665 ± 0.150
10	60	0.623 ± 0.188

Results are mean ± SD of three independent experiments. Method: ref. 8.

Table 4. Effect of TEA and 4-AP on membrane integrity of *S. cerevisiae*

	Extinction at 280 nm		
Time (hours)	Normal saline	4-AP (2 mM)	TEA (0.2 M)
1	0.166 ± 0.012	0.542 ± 0.060	0.122 ± 0.003
2	0.306 ± 0.019	1.012 ± 0.042	0.172 ± 0.002

Results are mean ± SD of three independent experiments. Method: ref. 9.

concentration of TEA and 4-AP, was restored on supplementing the plates with 0.2 M KCl (Table 2). The presence of 0.2 M KCl in the medium might be helping to overcome the lethal effect of these drugs. Furthermore, intracellular K^+ content of the untreated (control) as well as 4-AP treated yeast cells was of the same order (Table 3). These results suggest that 4-AP might not be inhibiting K^+ uptake by blocking K^+ transporters, but it may be causing lethality in *S. cerevisiae* by acting at some other level. The restoration of growth on supplementation of KCl (0.2 M) to the synthetic complete medium agar plates suggested another possibility that 4-AP and TEA may be causing some osmotic stability defect and the presence of 0.2 M KCl has osmotic stabilizing effect. The growth of *S. cerevisiae* was also restored when the plates containing inhibitory concentration of TEA and 4-AP were supplemented with NaCl (0.2 M) or mannitol (0.4 M). Hence, the lethality conferred by TEA and 4-AP to *S. cerevisiae* cells may be attributed to the osmotic stability defect caused by these drugs.

Mutants with the osmotic stability defect have been reported in *S. cerevisiae*[5]. These mutants showed normal growth on the medium containing osmotic stabilizer, i.e. 10% sorbitol or mannitol and 1.6% NaCl, but lysed on transfer to hypotonic solution. These mutants had defects in both cell wall and cell membrane[6,7]. So, it is possible that TEA and 4-AP may be causing cell lysis in *S. cerevisiae* by inducing some alterations either in cell wall or cell membrane.

4-aminopyridine caused lysis of *S. cerevisiae* cells (Table 4). It may be possible that 4-AP interacts with some protein involved in the maintenance of cell integrity, thereby inducing some osmotic stability defect. In the presence of osmotic stabilizer (Table 2), this defect might be restored. It may be noted that in the presence of normal saline (control), some material absorbing at 280 nm radiations is released. During normal growth, yeast cells release a number of metabolites in the medium. Some of these might be absorbing at 280 nm and it may not be the result of cell lysis.

On the other hand, TEA-treated yeast cells did not show any lysis (Table 4), indicating that it may be causing lethality by resulting in some other defects in the plasma membrane. TEA having both polar as well as non-polar groups might have replaced amphipathic membrane lipids, thereby affecting the functioning of membrane proteins, which led to the lethality of yeast cells. The restoration of growth of *S. cerevisiae* cells on addition of KCl, NaCl or mannitol in the synthetic complete medium agar plates containing TEA can be explained by considering that the presence of osmotic stabilizer somehow inhibited the incorporation of the drug in the membrane, but this proposal needs further investigation.

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Comparative studies on the radula teeth of two species of *Conus* from the Indian coast

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The scanning electron microscope (SEM) has been used to determine the surface morphology of the radula teeth of the two species of marine gastropod of the genus *Conus*. The teeth are adapted to the cap-