Pathogenicity of Sorghum Ergot Fungus (*Sphacelia sorghi* McRae)[1]

During our pathogenicity studies, we found that the conidia of *Sphacelia sorghi* from artificial cultures were inferior in pathogenesis on sorghum florets compared to the conidia from the honeydew of the infected spikelets. In order to compare their relative pathogenicity, conidial suspensions were prepared from 15-day-old cultures maintained on KIRCHHOFF's[2] medium, as well as from the honeydew of the infected spikelets, and sprayed on the unpollinated inflorescences of msCK 60-A sorghum variety. The inflorescences were covered with selfing-bags to provide adequate humidity. After 20 days, the percentage of infection was recorded by counting 2000 spikelets at the top of the inflorescence.

The results revealed that the conidia from artificial cultures were less pathogenic compared to the honeydew conidia. Therefore, presence of some stimulatory substance in the honeydew was suspected. To ascertain this, a spore suspension from artificial cultures was prepared in a conidium-free honeydew extract obtained by repeated centrifugation and used for inoculation on sorghum florets.

It was found that the virulence of conidia from artificial cultures was increased by the addition of honeydew extract prepared from the infected spikelets, indicating the presence of some stimulatory principles in the honeydew. This was further confirmed by spore germination studies also. Addition of honeydew extract to the washed conidia from artificial cultures increased the spore germination, while the germinability of the honeydew conidia was reduced when they were washed free of the honeydew. In the case of other ergotial fungi also, the conidia from artificial cultures were shown to be less pathogenic than the conidia from the infected spikelets[3,4]. The nature of the stimulatory substance in the honeydew of sorghum ergot fungus remains to be studied.

Relative virulence and germinability of conidia of *Sphacclia sorghi* from the honeydew and the artificial cultures

Nature of conidia	% of infection	Nature of conidia	% of spore germination
Conidia from artificial cultures	22.5	Unwashed honeydew conidia (diluted suspension)	77.1
Honeydew conidia	87.5	Washed honeydew conidia	30.3
Conidia from artificial cultures + honeydew extract	61.5	Unwashed conidia from artificial cultures	33.8
Uninoculated inflorescence	0.0	Washed conidia from artificial cultures	30.5
		Washed conidia from artificial cultures + honeydew extract	54.5

Zusammenfassung. Die Konidien einer in vitro aufgezogenen Kultur von *Sphacelia sorghi* sind weniger infektiös als die Konidien aus dem Honigtau der auf Hirse gewachsenen Pilze. Es wird daher eine Substanz im Honigtau vermutet, welche die Keimung der Konidiensporen fördert.

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Temperature Sensitivity in the Respiratory-Deficient Mutants of Yeast (*Saccharomyces cerevisiae*)

In connection with the study of cancer cells as temperature-sensitive mutants[1], and analysis of their heat sensitivity with the aid of microbial models of cancer cells[2], an investigation of temperature sensitivity in the respiratory-deficient mutants of yeast is of considerable interest.

Yeast mutants with impaired respiration and enhanced glycolysis are more heat sensitive than the original parent cells[3]. In these experiments yeasts were grown on nutrient agar plates under aerobic conditions, suspended in sterile distilled water and heated at 54 °C for various intervals of time. The curves of survival for the normal strain and its respiratory-deficient mutants were compared and it was recorded that the mutant strain showed greater heat sensitivity than the wild type. These observations were confirmed in the work with another strain of yeast, *Saccharomyces cerevisiae* S (parent culture), and 2 respiratory-deficient mutants (c and d) derived from it[1].

In the course of these studies we observed that respiratory-deficient mutants of yeast are temperature sensitive only when grown under aerobic conditions. In this work, parent culture *S. cerevisiae* strain S and 2 respiratory-deficient mutants c and d were grown under anaerobic conditions upon the nutrient medium containing 10% of beerwort and 0.6% of glucose, as described previously[4]. For aerobic cultivation the same medium was used in shaking flasks. Yeast cells in the logarithmic phase of growth were washed on the centrifuge and suspended in m/15 phosphate buffer at pH 5 as previously described[4].

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For the study of the curves of survival, the suspensions of yeast cells were heated at 50 °C for various intervals of time, plated upon the nutrient agar, incubated at 28 °C, and the number of colonies counted. Figure 1 shows the increased percentage of dead cells with the increased exposure to 50 °C. These data represent the averages for 3 series of experiments.

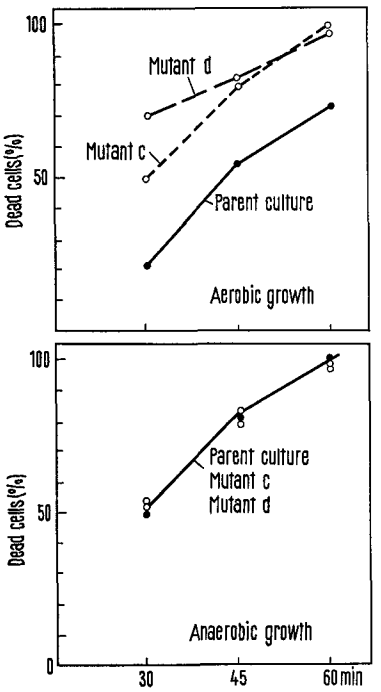

Fig. 1. Sensitivity to heating at 50 °C in the parent culture of *S. cerevisiae* and its mutants after aerobic and anaerobic growth.

The effect of short hyperthermy at 50 °C upon subsequent aerobic glycolysis at 28 °C in the parent culture of *S. cerevisiae* S. and 2 respiratory-deficient mutants, c and d

Strain	Qo_2 (aerobic culture)	Qco_2 Aerobic culture		Anaerobic culture	
		Control	Hyperthermy	Control	Hyperthermy
Parent	25.6	28.2	16.5	142.2	31.2
Mutant c	1.0	59.4	16.5	153.9	32.5
Mutant d	0.5	121.9	29.7	185.0	43.8

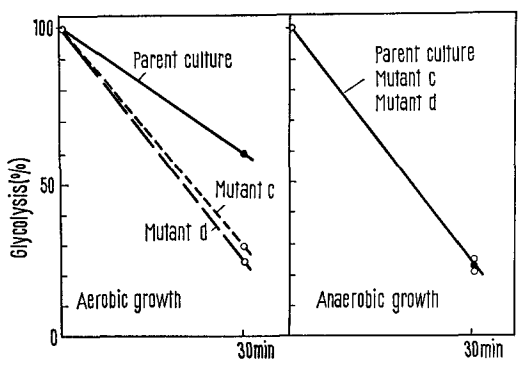

Fig. 2. The effect of hyperthermy at 50 °C upon subsequent aerobic glycolysis at 28 °C in the parent culture of *S. cerevisiae* and its mutants after aerobic and anaerobic growth.

It is entirely clear that the respiratory-deficient mutants are temperature sensitive and are killed at 50 °C more rapidly than parent cells only when grown under aerobic conditions. After anaerobic cultivation, temperature sensitivity in these mutants entirely disappears, and they do not differ from their parents.

It is of considerable interest in this connection to investigate temperature sensitivity of glycolysis in the respiratory-deficient yeast. The Table presents data on aerobic glycolysis in the parent culture *S. cerevisiae* S and 2 of its mutants, c and d, grown under aerobic and anaerobic conditions. It is characteristic for mutants with the impaired respiration that their aerobic glycolysis is enhanced in relation to parent values. For the study of temperature sensitivity of glycolysis, we investigated the effect of short hyperthermy of yeast suspensions at 50 °C upon subsequent metabolism at 28 °C. In these experiments suspensions of yeast cells were heated at 50 °C for 30 min, and their aerobic glycolysis was afterwards measured at 28 °C. Figure 2 clearly indicates selective inhibition by elevated temperature of the glycolysis in mutants grown under aerobic conditions. Hyperthermy inhibits the glycolysis in the parent culture by 41%, but in the respiratory-deficient mutants inhibition of glycolysis attains 72–75%. In distinction from this, temperature sensitivity of glycolysis in mutants grown anaerobically is identical with that of their parents.

It is therefore possible to conclude that temperature sensitivity of the respiratory-deficient mutants of yeast, expressed by the survival values at elevated temperatures, as well as by the effect of hyperthermy upon aerobic glycolysis, can be observed only when yeast cells are grown aerobically, and entirely disappears under anaerobic cultivation. It is well known that anaerobic yeasts do not contain functional mitochondria, and it is remarkable that the loss of temperature sensitivity is coincident with the loss of active mitochondria in the respiratory-deficient mutants of yeast. NEIFAKH et al.[5] suggested that mitochondria may play an important part in the control of glycolysis, and it is also well known that the structure of mitochondria in the respiratory-deficient mutants of yeast is defective[6] and alterations in base composition of mitochondrial DNA in these mutants have been recorded[7].

Temperature sensitivity in the respiratory-deficient mutants of yeast grown under aerobic conditions, and the disappearance of this sensitivity under anaerobic growth, is consistent with the hypothesis that it results from some defects in the organization of mitochondria in the respiratory-deficient mutants of yeast, which may be responsible for the thermosensitivity of their enzymes.

Выводы. Температурная чувствительность мутантов с дефектом дыхания у дрожжей *Saccharomyces cerevisiae* наблюдается лишь при культивировании в аэробных условиях и исчезает при анаэробном росте. Повидимому, температурная чувствительность при аэробном росте связана с дефектной организацией митохондрий у мутантов дрожжей.

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