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Genetica. — *Mutants for respiratory adaptation in* Saccaromyces cerevisiae (*). Nota di GIORGIO MORPURGO, GIOVANNI SERLUPI–CRESCENZI, GAETANO TREZZA e DOMENICA VENETTACCI, presentata (**) dal Socio G. MONTALENTI.

It is well known that yeast grown anaerobically does not respire because it lacks cytochromes and other respiratory enzymes. Incubation of anaerobic cells with oxygen in the presence of low concentrations of glucose, reestablish respiration to the level of aerobic cells, with the contemporaneous appearance of the missing enzymes [1].

This phenomenon has been called respiratory adaptation and it has been shown that it is much faster in cells harvested during the stationary phase than in cells harvested during the exponential phase of growth [2].

The aim of this work was to find mutants constitutive for the respiratory enzymes and therefore capable of immediately respiring even when harvested after cultivation under anaerobic conditions. Although these mutants have not been found, strains with a mutation involving the control of the respiratory adaptation have been selected by the method described in this paper.

The method which has been used consisted in growing yeast anaerobically [3], harvesting the cells during the exponential phase of growth and plating them on a Czapek-Dox minimal medium containing a non-fermentable carbon source. Lactate (2M) was the best substrate and was used throughout the work.

Strain FF53, threonine deficient, kindly supplied by prof. G. Magni, was used for this work. Successive analysis by prof. Magni has shown that this strain is probably aneuploid (1).

In these conditions cells of the original strain, being grown anaerobically and therefore lacking respiratory enzymes, can not utilize lactate. In addition, being harvested in the exponential phase, they have a very low adaptation rate. It was therefore hoped that only constitutive cells eventually present would have grown.

As a matter of fact while the plating efficiency for aerobic cells or anaerobic cells harvested in the stationary phase was nearly 100%, for anaerobic

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(**) Nella seduta del 13 novembre 1965.

(1) Note added in proof: This research has been repeated by Miss. L. Volterra in our laboratory on 50 million cells of the haploid strain EV. 20 *trypt* surviving UV irradiation (10% survival). No constitutive mutants have been found, thus strongly confirming the final conclusion that constitutive respiratory mutants cannot be isolated as the result of a single mutation.

cells, harvested in the exponential phase, it was much lower. It was also soon discovered that plating efficiency for the last type of cells was strongly dependent on pH.

This is shown in the last three columns of Table I.

TABLE I.

Plating efficiency and respiratory adaptation on lactate at different pH *of yeast grown in different conditions.*

Type of cells	Respiration μl/10^7 cells/hr (*)			Plating efficiency %		
	Initial	After 24 hr incubation with lactate and oxygen				
		at pH 3.8	at pH 5	pH 3.3	pH 3.8	pH 5
Aerobic	30	30	30	36	68	100
Anaerobic stationary . . .	0	21	30	45	70	100
Anaerobic exponential . . .	0	0	18	0.006 (**)	0.019 (**)	0.6 (**)

(*) Suspensions containing $3 \cdot 10^9$ cells/ml were vigorously aerated in 0.05 M phosphate–0,2 M lactate buffer at the pH indicated. 0.1 ml samples were withdrawn and added to a cell containing 0.9 ml of the same buffer. The oxygen consumption was measured polarographically [4].

(**) These data derive from one experiment in which the cells were harvested near the end of the exponential phase. At a different stage plating efficiency at pH 3.8 can be lowered down to 0.0001 %.

From the first three columns of the same table one can also see that the reason for this is that respiratory adaptation for exponential phase cells strongly depends on pH when lactate is used as substrate.

At pH 3.5, after 24 h of incubation in oxygen, the respiration of a sample, harvested in the exponential phase, was zero, while at pH 5 adaptation is considerable.

No influence of pH an aerobic growth rate could instead be detected in our conditions, so that growth is only influenced by the rate of adaptation to oxygen. Mutants were therefore isolated at pH 3.8.

All the colonies isolated by this method from the plating of 7.5×10^6 non irradiated cells and 18×10^6 cells surviving UV irradiation have been cultivated in anaerobiosis and their respiration has been measured with the polarographic method [4]. It was found that in some colonies anaerobic respiration, although lower than the aerobic one, was from two to three times higher than that of the anaerobic control strain. These colonies, when grown anaerobically and harvested in the exponential phase, could grow on a medium at pH 3.8 containing lactate as carbon source, thus showing that true mutants had been selected.

One of these strains was isolated and cultivated for a more detailed study.

As shown in fig. 1 the rate of adaptation of this strain is higher than that of the original strain. This is particularly dramatic when cells were harvested in the exponential phase.

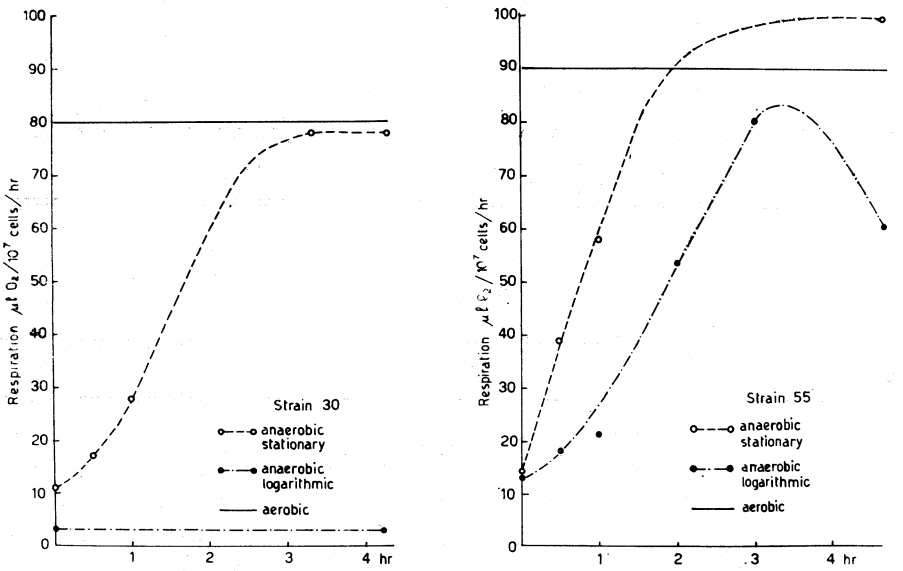

Fig. 1. – Respiratory adaptation on glucose of the original strain (30) and of the mutant strain (55) harvested from different growth conditions.

For details of the techniques see under Table I.

The rate of growth in anaerobiosis is instead about one half of that of the control as shown in fig. 2.

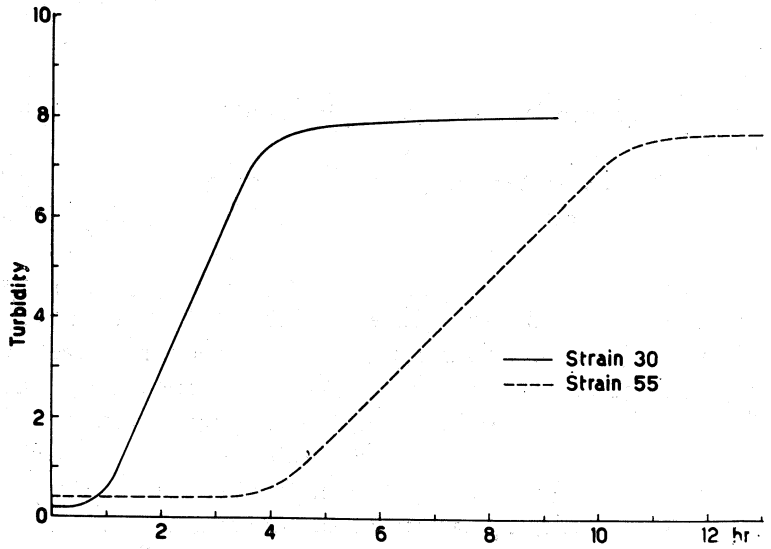

Fig. 2. – Growth curves of the original strain (30) and of the mutant strain (55) in anaerobiosis.

Curves were drawn with a biophotometer constructed in this laboratory.

The level of succinic dehydrogenase was measured by following with a very sensitive double beam spectrophotometer [5] the reduction of ferricyanide. Alcohol dehydrogenase, NAD– and NADP– dependent malic dehydrogenase were measured by following the reduction of the relative coenzymes in the presence of the appropriate substrates, using the same instrument to increase sensitivity. No significant difference was found for any of these enzymes between the mutant and the control strain either cultivated in aerobiosis or in anaerobiosis.

From the results obtained it can be concluded that the method which has been described could select constitutive mutants but it seems very probable that these mutants do not exist at least as the result of a single mutation. However the strains which have been isolated display a mutation involving the control of the respiratory adaptation.

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RIASSUNTO. — È noto che il lievito è un organismo capace di vivere e moltiplicarsi sia in aerobiosi che in anaerobiosi. Le cellule di lievito cresciute in anaerobiosi sono prive di enzimi respiratori, enzimi che vengono tuttavia rapidamente sintetizzati una volta che le cellule siano messe a contatto con l'ossigeno; tale fenomeno è chiamato adattamento respiratorio.

Nella presente ricerca si descrive un metodo che può portare alla selezione di mutamenti per il meccanismo di controllo della respirazione nel lievito.

Le cellule di lievito coltivate in anaerobiosi e prelevate in fase esponenziale di crescita presentano un adattamento respiratorio estremamente lento. A pH 3,8 abbiamo visto che il lievito prelevato in fase esponenziale è incapace di adattarsi nonostante che la sua vitalità sia sostanzialmente inalterata. Cellule di lievito anaerobiche e prelevate in fase esponenziale sono state seminate su di un terreno in cui la fonte di carbonio era costituita dal lattato, substrato non fermentabile. Su tale terreno possono crescere soltanto eventuali mutamenti costitutivi o capaci di adattarsi rapidamente. L'esame di 25×10^6 cellule non ha portato all'isolamento di alcun mutante costitutivo. Sono stati invece isolati numerosi mutamenti capaci di adattarsi altrettanto bene in fase esponenziale che in fase stazionaria. Tali mutamenti presentano inoltre una respirazione iniziale più elevata del controllo e diminuita velocità di crescita in anaerobiosi. Si discutono le cause possibile di tale comportamento.