champignons. Tous les milieux ci-dessus sont répartis à raison de 20 ml par boîte de Pétri de 10 cm de diamètre.

Résultats et discussion. La poursuite de nos recherches sur la protéolyse des viandes nécessitait la mise au point de milieux plus adaptés à ce substrat. L'arrangement particulier et la composition des protéines de la fibre musculaire laisse en effet supposer des modalités d'attaque par les microorganismes différentes de celles de la gélatine et de la caséine.

Le tableau ci-joint contient les résultats obtenus avec les trois substrats utilisés. Il nous montre que l'hydrolyse d'un substrat n'entraîne pas nécessairement celle des deux autres. D'autre part, tous les microorganismes actifs sur la poudre de muscle attaquent également la gélatine, cette dernière propriété étant très répandue, mais l'inverse n'est pas vrai. Cette observation n'est pas surprenante, la poudre de muscle étant un substrat plus complexe que la gélatine.

La recherche de milieux adaptés pour la mise en évidence des propriétés protéolytiques de microorganismes vis à vis de substrats naturels particuliers a été peu développée. Cependant, GROSSBARD et HALL[3] ont proposé un milieu à base de protéines de plantes et, plus récemment, TOM et CRISAN[4] un milieu à l'extrait de poisson. L'utilisation de ce type de milieux pour la mise en évidence et la numération des microorganismes impliqués dans la détérioration de protéines de différentes origines semble préférable à celle des milieux classiques afin d'utiliser le substrat en accord avec celui rencontré par les microorganismes dans les conditions naturelles.

Deux avantages principaux peuvent donc être accordés aux milieux que nous proposons. Ils permettent la numération des microorganismes protéolytiques tout en ayant la possibilité de récupérer ceux-ci. Ceci n'est pas possible avec les milieux classiques à base de gélatine, étant donné que dans ce cas, le révélateur de la gélatinolyse tue les microorganismes. En second lieu, ils permettent la mise en évidence des microorganismes possédant à eux seuls l'équippement enzymatique nécessaire à la dégradation du substrat complexe qu'est la poudre de muscle.

L'emploi de la poudre de viande nous permet de préciser les possibilités enzymatiques des différentes souches testées et par conséquent leur aptitude à dégrader les protéines musculaires.

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Heterozygous Effects on Cell Yield and Generation Time in *Saccharomyces cerevisiae*

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Summary. The data obtained analyzing generation time, cell yield and their variability in different culture media in diploid strains of *Saccharomyces cerevisiae* demonstrate the existence of a biochemically determined heterotic effect, that could be of some relevance for the study of yeast population genetics, as well as for the improvement of microbial fermentation processes.

In *Saccharomyces cerevisiae*, generation time and cell yield depend on its genetic complement and on the chemical composition of the culture media; the variability of these parameters increases with heterosis at least under strong selection pressure[1,2].

The experimental condition in which heterosis has been observed does not permit an interpretation of the effect at a biochemical level, since the genes put in heterozygous condition are unidentified in terms of the molecular nature of the mutant allele and of the gene product involved.

In order to analyze heterosis in yeast at a biochemical level, we have started to study generation time, cell yield, and their variability in different culture media, of a series of isogenic strains of *Saccharomyces cerevisiae*, which are made heterozygous for a single biochemical marker whose gene product and molecular origin (insertion/deletion; nonsense and missense mutation) are known.

In this note we report the data obtained analyzing generation time, cell yield and their variability in diploid strains of *Saccharomyces cerevisiae* which carry different combinations of the gene TRP_5, coding for the enzyme tryptophan synthetase (L-serine hydro-lyase, adding indole, EC 4.2.1.20) and of its allele trp_{5-2}, which appears to be due to a nonsense mutation[3,4].

Materials and methods. The media employed were 1. Medium Max (yeast extract, 1%; peptone, 2%; glucose, 2% w/v)[5] and 2 'stress media': 2. medium Max with glucose reduced to 0.02%, and 3. medium Max with 5% ethanol[2].

Medium	Strains	Generation time[a]	F[b]	Cell yield[a]	F[b]
1. Max	TRP_5/TRP_5	2 h 18′		1.0×10^8	
	TRP_5/trp_{5-2}	1 h 48′		5.9×10^8	
	trp_{5-2}/trp_{5-2}	1 h 54′	6.2*	2.4×10^8	81.5**
	TRP_5/trp_{5-2}	1 h 48′		5.9×10^8	
	TRP_5/trp_{5-2} SR-I	1 h 44′	0.1	3.9×10^8	4.1*
	TRP_5/TRP_5	2 h 18′		1.0×10^8	
	TRP_5/trp_{5-2} SR-I	1 h 44′		3.9×10^8	
	trp_{5-2}/trp_{5-2}	1 h 54′	6.0*	2.4×10^8	65.0**
2. Max	TRP_5/TRP_5	1 h 59′		2.64×10^7	
	TRP_5/trp_{5-2}	1 h 47′		4.18×10^7	
	trp_{5-2}/trp_{5-2}	2 h	22.3**	3.38×10^7	91.4**

[a] Generation time and cell yield are the mean values obtained from six independent cultures. [b] Significance of F at the 5% and 1% level is indicated by * and **, respectively.

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The strains used were *Saccharomyces cerevisiae* 5323/A a/αTRP$_5$/TRP$_5$, 5423/B a/αTRP$_5$/trp$_{5-2}$ and 5423/C a/α trp$_{5-2}$/trp$_{5-2}$ which are highly isogenic for the genetic complement around the tryptophan-synthetase coding gene.

Results and discussion. 1. Generation time. In each culture medium, generation time differs significantly among the strains tested. As the table shows, the heterozygous strain TRP$_5$/trp$_{5-2}$ possesses, in both medium 1 and medium 2, a generation time significantly lower than that of the two homozygous strains trp$_{5-2}$/trp$_{5-2}$ and TRP$_5$/TRP$_5$.

2. Cell yields. Comparable results were obtained for cell yield. As shown in the table, this parameter differs significantly, for a given culture medium, in the strains tested. A significant difference is also observed, for a given strain, in the culture media tested; the heterozygous strain TRP$_5$/trp$_{5-2}$ always possesses the highest cell yield values.

3. Variability. The variability in generation time and in cell yield vary significantly, for a given TRP: trp gene combination, among the culture media. In particular, the strain which shows the maximum of variability in function of the culture medium, is the heterozygous strain TRP$_5$/trp$_{5-2}$, whereas the variability of the two homozygous strains TRP$_5$/TRP$_5$ and trp$_{5-2}$/trp$_{5-2}$ is not statistically significant.

The data we have obtained are confirmed by the analysis of the growth parameters of the heterozygous strain TRP$_5$/trp$_{5-2}$ SR–I which was selected as a spontaneous revertant from the recessive homozygous strain trp$_{5-2}$/trp$_{5-2}$. The reversion is not due to suppressor mutation (G. Signifredi, personal communication).

As shown in the table, TRP$_5$/trp$_{5-2}$ SR–I, which is in the highest possible condition of isogeny of the genetic complement around the heterozygous marker in comparison with the homozygous strain from which it is derived, possesses a lower generation time and a higher cell yield than the control strains confirming the effect of the heterozygous condition on these growth parameters in yeast and thus the existence of biochemically determined heterosis in this organism.

It was observed that the heterotic effect of the gene combination TRP$_5$/trp$_{5-2}$ is higher in medium 1 than in the other culture media.

This observation and the fact that the natural 'habitat' for yeast is probably similar to the one of the 'stress' media, suggest that there exists a significant probability of optimizing, in function of the different culture media, generation time as well as cell yield.

Further studies now in progress will help in establishing whether heterosis in yeast is gene-specific or allele specific, i.e., if it depends on a specific biochemical block in a given biosynthetic pathway, or on the molecular nature of the mutation, independently of the gene in which it has occurred (Puglisi et al., in preparation).

Bactericidal Activity in Conventional or Decontaminated Mice Undergoing GVHD or Radiation-Induced Injury

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Summary. Oral antibiotic prophylaxis of mice, particularly those with radiation-induced injury or those undergoing GVHD, alters bactericidal activity of the host.

Sepsis contributes to mortality in animals with a severely compromised immunologic system such as that found in irradiated mice and in those undergoing graft-versus-host disease (GVHD). To obviate this difficulty, numerous investigators have used oral antibiotic prophylaxis to reduce gut flora, an important source of bacterial infection[1–3]. However, decontamination may also alter host resistance to infection if microbial agents are reintroduced. This hypothesis is supported by the observation that intracellular digestion is impaired in germfree animals even though phagocytic rates are normal[4–6].

The possible relationship between enteric flora and host resistance after irradiation and during GVHD led to the present investigation of bacterial uptake and killing in conventional and decontaminated mice. More specifically, we wished to determine whether the reticuloendothelial (RE) system of antibiotic decontaminated animals had altered capabilities to eliminate challenge doses of bacterial organisms.

Male B6CBF$_1$ mice were irradiated with 850 rads delivered at 40 rads/min by a 300 kVp General Electric Maxitron X-ray[7]. This dose is 100 rads greater than the LD$_{99}$. B6CBF$_1$ animals destined to undergo GVHD received i.v. injections containing 5×10^6 allogeneic CBA spleen cells within 4 h after irradiation[7,8]. Irradiated mice die around day 14 while mice with GVHD only survive 7 days. Mice to be decontaminated were placed in a laminar air flow environment with sterile cages, food, bedding and given bacitracin and neomycin in acidified (pH 4) drinking water[7,9]. Fecal pellets were cultured in Thioglycollate broth to insure that decontamination was successful.

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