Nevertheless, the diplotene chromosomes show many chiasmata, which would necessarily lead to an introgression if the chromosome sets of the 2 parental species were involved.

2. In the *esculenta* oocytes, only the *ridibunda* allozymes could be detected, whereas transcriptional activity taking place on all chromosomes is indicated by the presence of well developed loops. If the 2 genomes were present, the allozymes of both species should also be present. This demonstrates that the *lessonae* genome has been eliminated before meiosis. The *ridibunda* genome must have undergone a supplementary duplication, which restored the diploid number of chromosomes. Bivalent chromosome partners are thus not only homologous, but sister-strand derived identical chromosomes. The ensuing meiotic division segregates then only identical chromosomes. Consequently, all gynogenetic progeny of 1 female form a 'clone'. In fact, the electrophoretic analysis reveals no difference between tadpoles derived from 1 single female. Differences exist however between the 2 females and their respective progeny.

In contrast to the gynogenetic offspring of other amphibians[7,10,11], the viability of the *esculenta* gynogenetics is dramatically reduced. No tadpole was able to survive longer than 20 days or enter into the feeding stage. This might be due to an accumulation of deleterious mutations in the hybrid's *ridibunda* genome, which is clonally reproduced in the mixed *lessonae-esculenta* populations. In *R. esculenta,* these deleterious mutations would be hidden by the *lessonae* genes, whereas in the homozygous gynogenetic progeny they are unrestricted and become lethal. This hypothesis, already suggested by Berger[12], could also explain the frequent occurrence of larval mortality in the offspring of the cross *esculenta* × *esculenta.*

1 Acknowledgments. Prof. M. Fischberg, Genève, and Prof. P. Vogel, Lausanne, provided laboratory facilities. This research was supported by the Swiss National Science Foundation, grants 3.221-0.73 and 3.644-0.75.
2 R.J. Schultz, Am. Nat. *103,* 605 (1969).
3 M.C. Cimino, Evolution *26,* 294 (1972).
4 H.G. Tunner, Z. zool. Syst. Evolutionsforsch. *11,* 219 (1973).
5 T. Uzzel and L. Berger, Proc. Acad. nat. Sci. Philad. *127,* 13 (1975).
6 J.D. Graf, F. Karch and M.C. Moreillon, Experientia *33,* 1582 (1977).
7 W.P. Müller, C.H. Thiébaud, L. Ricard and M. Fischberg, Revue suisse Zool. *85,* 20 (1978), and unpublished data.
8 H.R. Kobel and L. Du Pasquier, Immunogenetics *2,* 87 (1975).
9 W.P. Müller, Chromosoma (Berl.) *59,* 273 (1977).
10 J. Rostand, C. r. Soc. Biol. *141,* 563 (1947).
11 R. Tompkins, J. exp. Zool. *203,* 251 (1978).
12 L. Berger, Ann. Zool. Warszawa *33,* 201 (1976).

Selective enrichment technique for isolation of methanol-utilizing yeasts

R.J. Mehta[1,2]

M.G. Science Institute, Ahmedabad (Gujarat, India), 1 March 1979

Summary. A simple, selective enrichment technique was developed for isolation of methanol-utilizing seasts by supplementing gentamicin or tetracycline in the medium.

In recent years there has been considerable interest in studies concerned with the utilization of methanol as an important raw material for the production of single-cell protein (SCP). The ability of bacteria to assimilate methanol has been known for many years[3], but the first yeast that utilized methanol was isolated as late as 1969[4]. Since yeasts as SCP surpass bacteria in some of their properties, e.g. lower nucleic-acid content and higher density, interest has been focused on the isolation of methanol-utilizing yeasts. This report describes simple, selective enrichment techniques for the isolation of methanol-utilizing yeasts.

Experiments and results. A 1 g soil sample (local) was suspended in 10 ml of sterile distilled water and 1 ml of this suspension was inoculated into 50 ml of half-strength Sabouraud's broth (Difco), pH 5.0, containing 1% (v/v) methanol and various (100–300 µg/ml) concentrations of gentamicin sulfate or tetracycline hydrochloride. These flasks were incubated on a rotary shaker at 25 °C for 5 days. A 1 ml portion of the turbid suspension was then transferred to a mineral-salts-methanol medium[5] supplemented with either gentamicin or tetracycline (100–300 µg/ml) and incubated on a rotary shaker for 1 week. This process was repeated 4 times with fresh medium, and, following the final incubation, a loopful of the suspension was streaked from each flask onto a solid mineral-salts-methanol medium[5]. These plates were incubated for 1 week at 25 °C, followed by microscopic examination of each colony from the plate. The results are summarized in the table. Inspection of the above table indicates that gentamicin and tetracycline were highly effective antibiotics for isolation of methanol-utilizing yeasts.

In conclusion, methanol-utilizing bacteria are generally gram-negative rods and susceptible to broad spectrum antibiotics. In contrast, yeasts are insensitive to these antibiotics, hence they will survive and multiply in the presence of such antibiotics if they can utilize the carbon source provided.

Enrichment for methanol-utilizing yeasts

Antibiotic	Concentration (µg/ml)	No. of yeast colonies	No. of bacteria colonies
None (control)	None	1	35
Gentamicin	100	30	3
	200	20	2
	300	28	0
Tetracycline	100	15	5
	500	25	3

1 Present address: Department of Microbiology, Smith Kline & French Laboratories, 1500 Spring Garden Street, Philadelphia (Pennsylvania 19101, USA).
2 Acknowledgments. The author wishes to thank Prof. J.J. Trivedi for his encouragement and interest during this work and Mr L.R. Fare, Dr D.J. Newman and Dr Y.K. Oh for reviewing this manuscript.
3 M. Dworkin and J. Foster: J. Bact. *72,* 646 (1956).
4 K. Ogata, H. Nishikawa and M. Ohsugi: Agr. Biol. Chem. *33,* 15 (1969).
5 R.J. Mehta: J. Bact. *124,* 1165 (1975).