We are unaware of any work showing dehydroxylation of caffeic acid by pure cultures of micro-organisms.

We have isolated a strain of *Pseudomonas* sp. from rat faeces which has the ability to dehydroxylate caffeic acid yielding both *m*-hydroxy-phenyl-propionic and *m*-coumaric acids, as well as some other products which were not identified. Enriched cultures of this bacterium can be obtained by inoculating nutrient broth with a suspension of fresh normal rat faeces, and then incubating in an atmosphere of carbon dioxide at 37° C for 48 h. The bacterium was isolated from this culture by plating in nutrient agar.

Dehydroxylation of caffeic acid occurs when heavy washed cell suspensions of this bacterium grown on nutrient broth are incubated for 48–72 h in the presence of a solution of caffeic acid in phosphate buffer (*p*H = 7).

At present we are working on the isolation of micro-organisms from various sources capable of removing hydroxyl groups from different phenolic substrates.

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Number of Cytoplasmic Factors in Yeast Cells

IT is well known that the self-reproducing genetic factors (ρ), required for the development of mitochondria, are present in the cytoplasm of yeast cell. When the cells are allowed to grow in a medium containing acriflavine or related dyes, large numbers of mutant cells with deficient respiration are produced, which tend to dominate the entire cell population after several generations[1].

A possible explanation of this finding is that acriflavine may inhibit the replication of ρ factors but not of nuclear chromosomes and that, as a result, the respiration-deficient mutant may be produced because of lack of ρ factors by a process of dilution through successive cell divisions. If we assume that the initially existing ρ factors in a cell are distributed to daughter cells at random under conditions in which the multiplication of ρ factors is completely inhibited, we can calculate, using the method of polynomial expansion, the incidence of cells in any generation which lack ρ factor. The percentage incidences of the respiration-deficient mutant at the given generations are calculated assuming that the number of ρ factors is 4, 5, 6, 7 and 8, and the results are shown in Table 1.

Saccharomyces cerevisiae, *C*-1 (ref. 1) was inoculated at the initial concentration of 2×10^4 cells per ml. in the media containing glucose (2 per cent), 'Difco' proteose-peptone (1 per cent) and 'Difco' yeast extract (1 per cent) added with acriflavine at 2·5 μg per ml., which had almost no effect on the growth rate of this microbe. Incubation was carried out aerobically with reciprocal shaking at 30° C. The cell number was counted at intervals of 1 h and the percentage of the respiration-deficient mutant was determined by the triphenyltetrazolium chloride overlaying technique of Ogur, John and Nagai[3]. The percentage of the respiration-deficient mutant was plotted against the number of cells per ml. By interpolation from this curve, the percentage of the respiration-deficient mutant at a given generation was obtained. The values thus obtained for various generations are shown in Table 2. Comparison of the experimental values with those calculated up to the fourth generation led us to conclude that the number of ρ factors in a cell of this strain is between 6 and 7. At the fifth generation the value obtained experimentally is between those calculated, assuming that the number of ρ factors is 7 or 8. After

Table 1. CALCULATED FREQUENCY OF RESPIRATION DEFICIENT MUTANTS (AS PERCENTAGE)

No. of factors	Number of generation 1	2	3	4	5	6	7	8
4	0·0	6·3	31·6	58·6	77·2	88·1	93·9	96·9
5	0·0	3·1	23·7	51·3	72·4	85·3	92·4	96·2
6	0·0	1·6	17·8	44·9	67·9	82·7	91·0	95·4
7	0·0	0·8	13·4	39·3	63·7	80·1	89·6	94·7
8	0·0	0·4	10·0	34·4	59·5	77·6	88·2	93·9

Table 2. FREQUENCY OF RESPIRATION DEFICIENT MUTANTS (AS PERCENTAGE) OBTAINED FROM EXPERIMENTS

Experimental number	Number of generation 1	2	3	4	5	6	7	8
1	0·0	0·0	15	37	55	70	85	–
2	0·0	0·0	14	46	69	84	91	95
3	0·5	3	18	45	63	75	83	86
Average	0·2	1	16	43	62	76	86	91

the sixth generation, the values obtained experimentally are a little lower than those calculated on the assumption that the number of ρ factors is 8. This is probably because of the slower growth rate of respiration-deficient mutant cells compared with normal cells.

We conclude that there are few ρ factors in each cell and so all mitochondria in aerobically cultured cells may not necessarily contain ρ factors although ρ factors are required for the development of mitochondria.

It is as well to point out that this conclusion is based on rather general arguments and is open to error. In the cell division of yeast, the cytoplasmic volume does not divide equally into two daughter cells, which suggests that there may be an uneven distribution of ρ factors into the two daughter cells. In the cell population of this strain used in the experiments described here, about 90 per cent of the cells are individually separated, and the rest of the cells are in clusters consisting of two or more cells. If one of the cells in a cluster is a respiration-deficient mutant and the rest are normal, the colony raised from such a cluster would consist of normal cells, as the growth of normal cells overcomes that of the respiration-deficient mutant cells on the agar plate. This factor could lead to under-estimation of the frequency of the respiration-deficient cells and overestimation of the number of ρ factors in a cell. The same would be true in the case of incomplete inhibition of the replication of ρ factors by acriflavine or lesser growth rate of respiration-deficient mutant than of wild cells. The latter possibility has already been pointed out. In any case overestimation of the number of ρ factors is possible, but underestimation is not. Clearly, then, there are only a small number of ρ factors in a normal cell. As the cells of strain *C*-1 are known to be tetraploid, investigations of the number of ρ factors in diploid or haploid cells are now in progress. In these cells, the existence of clusters of many cells in the population makes the experiment difficult.

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BIOLOGY

Nuclear Histones and Early Embryogenesis of the Chick

THE biological processes of differentiation and development must involve a differential expression of the genome. The mechanisms for control of such expression are as yet unknown. A genetic regulatory function has been postu-