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DISMUTASE ACTIVITY YET ABLE TO GROW EFFICIENTLY ON MINIMAL,
OXYGENATED MEDIUM

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FIRST DESCRIPTION OF A VARIANT OF *E.coli* LACKING SUPEROXIDE
DISMUTASE ACTIVITY YET ABLE TO GROW EFFICIENTLY ON MINIMAL,
OXYGENATED MEDIUM

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INTRODUCTION

E. coli contains two loci coding for proteins which catalyze
superoxide dismutation

sodA lies near 87 min. on the chromosome and codes for a Mn-
requiring enzyme; the gene has been cloned¹ and sequenced². sodB
lies near 37 min on the chromosome and codes for the Fe-requiring
enzyme; this gene has also been cloned^{3,4} and sequenced⁵. The
three dimensional structure of the Fe-protein is known at ~3 Å
resolution⁶, and recent work suggests that the Fe- and Mn-
proteins probably have identical three dimensional structures⁵.
Considerable work has been done on the mechanism of their
superoxide dismutase activity⁷.

Under normal laboratory growth conditions, the two proteins
are synthesized in approximately equal amounts, but their
biosynthesis depends, in part, on the concentration of the
individual metal ions in the culture medium⁸. Thus, increasing
the iron concentration causes more Fe-protein to be synthesized
while increasing the Mn²⁺ concentration causes more of the Mn-
protein to be synthesized. The expression of the Mn-protein
usually depends on the presence of air and is enhanced by a
higher oxygen concentration or by treatment with redox active
agents such as methylviologen (cf Ref. 9 and references therein).
Some workers have suggested that this is due to a O₂⁻ mediated
induction of the protein (cf Ref. 3). However, this cannot be
true as Moody and Hassan¹⁰ have shown that the Mn-protein is
readily synthesized under anaerobic conditions when the amount of
Fe in the culture medium is selectively diminished. By contrast,
the amount of Fe-protein synthesized generally decreases with
increased oxygenation of the culture medium³. A satisfactory
interpretation of these observations has not been formulated.

It is widely assumed that superoxide dismutases protect organisms from oxygen toxicity. Fridovich¹¹ has maintained that superoxide dismutases are necessary for aerobic life because they reduce the concentration of toxic O_2^- by promoting Reaction [1]. Fee¹² has objected to this interpretation, pointing out that O_2^- is an extremely unreactive species, and has argued that the true biological function of these proteins is not known. The tools of molecular biology, as applied to *E. coli* provide a logical approach to the solution of this controversy.

In 1986, Carlloz and Touati¹³ described a double mutant of *E. coli* in which the sodA gene was inactivated by insertion of a Mu transposon, MudIIPR13, carrying chloramphenicol resistance and sodB was inactivated by a Mu transposon, MudIIPR3, carrying kanamycin resistance. This strain (QC774) is completely devoid of superoxide dismutase activity. It can thus be used to examine the biological function of the sodAB gene products.

The properties of strain QC774 are listed as follows:

- ability to grow normally on oxygenated, rich medium (cf Ref. 14)
- high sensitivity to pro-oxidants (H_2O_2 , and methylviologen)
- high, spontaneous rate of mutation (xth and oxygen dependent)
- unable to grow aerobically in minimal glucose/salts medium

We describe here a variant strain of sodAB (QC774-LA) which is able to grow efficiently on oxygenated, minimal glucose/salts media. In March of 1985, we received from Dr. Touati strains GC4468 (parent, deleted for the lactose operon) and QC774 (sodAB). The latter was shown to carry resistance to both chloramphenicol and kanamycin and to be deficient in Fe- and Mn-dismutases (Fig. 1). The cytosol of these cells is completely deficient in superoxide dismutase activity. In contrast to the observations of Carlloz and Touati¹³, however, this double mutant grew remarkably well on glucose minimal media under normal conditions of aeration (Fig. 2A). Receipt of a new culture of QC774 from the Touati laboratory showed the prescribed properties and suggested that we had, by good fortune, received a variant strain in the first shipment.

We have examined the effects of diverse perturbations on the new strain, its parent, and the new strain into which we introduced the plasmid pHS1-4 which carries the sodB gene. Some of the phenotypes of QC774-LA are:

- able to grow on oxygenated, minimal glucose/salts medium(Fig. 2B)
- high sensitivity to methylviologen
- relatively low sensitivity to H_2O_2 and able to develop resistance by pre-treatment with $50 \mu M H_2O_2$
- growth completely inhibited by low concentrations of diethylenetriaminepentaacetic acid (DETAPAC)
- high sensitivity to Co^{2+}
- does not spontaneously transform to Co^{2+} resistance (cf Ref. 15 and references therein)
- unable to grow on glycerol

The latter four phenotypes are distinct from the parent strain (GC4468), and they are not transformable with pHS1-4. We therefore presume that this strain (QC774-LA) has suffered an additional, spontaneous mutation which complements the absence of the *sodAB* gene products. We have recently shown that it is possible to isolate strains similar to QC774-LA after mutagenesis with nitrosoguanidine, and we are now attempting to identify the modified loci.

Fig. 1. Stain for superoxide dismutase activity in electrophoretograms of *E. coli* strains: GC4468 and QC774-LA (*sod2*). The staining was carried out according to the method of Beauchamp and Fridovich.

Fig. 2. Growth curves for GC4468, QC774-LA, and QC774-LA:pHS1-4 on MOPS minimal medium with normal aeration(A) and sparging with pure oxygen(B).

CONCLUSIONS

A strain of *E. coli* has been obtained which is completely lacking in superoxide dismutase activity and is able to grow efficiently on glucose minimal media being sparged with pure oxygen. While its genotype is unknown, it appears to have arisen from mutation(s) which complements the absence of *sodAB* gene products without introducing superoxide dismutase activity.

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REFERENCES

1. D. Touati, Isolation of the Mn-superoxide dismutase gene (*sodA*) from *E. coli* K12, *J. Bacteriol.* 155:1078-1085 (1983).

2. Y. Takeda and H. Avila, Structure and expression of the *E. coli* Mn-superoxide dismutase gene, Nucleic. Acids Res. 14: 4577-4589 (1986).
3. C. Nettleton, C. Bull, and J. A. Fee, Isolation of the *E. coli* iron superoxide dismutase gene, Proc. Nat'l. Acad. Sci. 81:4970-4973 (1984).
4. H. Sakemoto and D. Touati, Cloning of the iron superoxide dismutase gene (*sodB*) in *E. coli* K12, J. Bacteriol. 159:418-420 (1984).
5. A. Carlíoz, M. L. Ludwig, W. C. Stallings, J. A. Fee, H. M. Steinman, and D. Touati, Iron superoxide dismutase: nucleotide sequence of the gene from *E. Coli* and correlations with crystal structure, Submitted for publication.
6. W. C. Stallings, T. B. Powers, K. A. Pattridge, J. A. Fee, and M. L. Ludwig, Iron superoxide dismutase from *E. coli* at 3.1 Å, Proc. Nat'l. Acad. Sci. 80:3884-3888 (1983).
7. C. Bull and J. A. Fee, Steady-state kinetic studies of superoxide dismutases, J. Am. Chem. Soc. 107:3295-3304 (1985).
8. S. Y. R. Pugh and I. Fridovich, Inductions of superoxide dismutases in *E. coli* by manganese and iron, J. Bacteriol. 160:137-140 (1984).
9. S. Y. R. Pugh and I. Fridovich, Induction of superoxide dismutases in *E. coli* by metal chelators, J. Bacteriol. 162:196-202 (1985).
10. C. S. Moody and H. M. Hassan, Anaerobic biosynthesis of Mn-containing superoxide dismutase in *E. coli*, J. Biol. Chem. 259:12821-12825 (1984).
11. I. Fridovich, The biology of superoxide and of superoxide dismutases - in brief, in "Oxygen and Oxy-Radicals in Chemistry and Biology," M. A. J. Rodgers and E. L. Powers, Academic Press, New York, page 197ff (1981)
12. J. A. Fee, Is superoxide important in oxygen poisoning?, Trends in Biochemical Sciences 7:84-86 (1982).
13. A. Carlíoz and D. Touati, Isolation of superoxide dismutase mutants in *E. coli*, EMBO J. 5:623-630 (1986).
14. E. Chan and B. Weiss, Endonuclease IV of *E. coli* is induced by paraquat, Proc. Nat'l. Acad. Sci. 84:3189-3193 (1987).
15. S. P. Hmiel, M. D. Snavely, C. G. Miller, and M. E. Maguire, Magnesium transport in *S. typhimurium*, J. Bacteriol. 168:1444-1450 (1986)