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# EVIDENCE FOR SUPEROXIDE DISMUTASE AND CATALASE IN MOLLICUTES AND RELEASE OF REACTIVE OXYGEN SPECIES

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The presence of superoxide dismutase was demonstrated in 21 strains of mollicutes, including achuloplasmas, mycoplasmas and ureaplasmas. Additionally, catalase activities were demonstrated in nearly 50% of the cell lysates, whereas no peroxide activities were detectable. The production of $O_2^-$ and $H_2O_2$ with glucose as substrate was demonstrated for 8 strains of 10 strains tested. Anaerobic mycoplasmas showed the highest amount of radical production, whereas superoxide dismutase and catalase activities were in the range of activities estimated for aerobic mollicutes. Some pathogenic strains additionally released compounds into the culture medium, which stimulated $O_2^-$ production by PMNs.

KEY WORDS: Superoxide dismutase, mollicutes, mycoplasma, catalase, superoxide.

ABBREVIATIONS: SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; PBS, phosphate buffered saline; A., Achuloplasma; IMT, Institut für Mikrobiologie und Tierseuchen, Hannover, FRG; NIH, National Institute of Health, Bethesda, USA; M., Mycoplasma; IRC, Int. Reference Center for Animal Mycopl., Arhus, DK; Laber, Prof. G. Laber, Sandoz Researche Inst., Vienna, Austria; U. Ureaplasma

## INTRODUCTION

Only three cases of aerobic living bacteria consuming oxygen but totally lacking SOD have been reported: some strains of lactic acid bacteria,[1,2] some strains of *Neisseria gonorrhoeae*[3,4] and some strains of mollicutes (mycoplasmas and ureaplasmas).[5-7] Mollicutes are the smallest procaryotic organisms capable of *in vitro* cultivation. Unlike bacteria, they lack a cell wall and often parasitize mammalian cells by attacking the plasma membrane. Some of them are the causative agents of infectious diseases like pleuropneumonia, urogenitalinfections, and arthritis. The present investigation gives evidence that several mycoplasma possess SOD, release reactive oxygen species and release substances into the culture medium, which additionally stimulate PMNs to release reactive oxygen species.

## METHODS

The culture of the mollicutes is described.[8,9] PMNs were isolated from human blood by separation in a Percoll-gradient. SOD was assayed and the units were defined by

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the xanthine oxidase-cytochrome *c* assay,[10] CAT was determined by following the $O_2^-$ evolution of $H_2O_2$ polarographically, POD activity was tested by catalysing the oxidation of o-dianisidine[11] or scopoletine.[12] Activity staining for SOD was performed according to[13] $O_2^-$ release by the mollicutes or activated PMNs was assayed with cytochrome *c*[10]-SOD inhibited part- or by chemiluminescence.[13] $H_2O_2$ release was determined by the POD mediated oxidation of scopoletin[12] or by chemiluminescence.[13] The nature of the primary radical released was assayed by ESR-spin-trapping.

## RESULTS

The presence of SOD was demonstrated in 21 strains of mollicutes, including achuloplasmas, mycoplasmas and ureaplasmas (Table I). No SOD activities or only traces were detectable in fresh prepared cell lysates, whereas activities were evident after dialysis of the cell lysates. A further increase of SOD activities were observed after heating the cell lysates to 65° C for 30 s. This might be due to the destruction of enzymatic reactions interfering with the activity tests. Additionally, CAT activities

TABLE I
Activity of Superoxide Dismutase and Catalase in several mollicutes

| Species | [1]SOD [3]fresh | [1]SOD [4]dialysed | [1]SOD [5]old | [1]SOD [6]heated | [2]CAT [4]dialysed |
|---|---|---|---|---|---|
| *M. agalactiae* | n.t. | n.t. | 0 ± 0 | 18.0 ± 0.9 | n.t. |
| *M. arthritidis* | 0 | 10.1 ± 2.1 | 18.4 ± 1.4 | 26.7 ± 1.3 | 31.9 ± 9.3 |
| *M. bovigenitalium* | 0 | 8.3 ± 1.9 | 11.7 ± 1.0 | 25.8 ± 1.3 | 0 ± 0.2 |
| *M. bovis* | n.t. | n.t. | 20.8 ± 2.1 | 22.4 ± 1.2 | 0 ± 0.2 |
| *M. canis* | n.t. | n.t. | 0 ± 0.2 | 17.0 ± 0.8 | n.t. |
| *M. capricolum* | traces | 15.4 ± 2.3 | 23.4 ± 1.9 | 27.0 ± 1.4 | 0 ± 0 |
| *M. columbinum* | 0 | 9.7 ± 2.1 | 0 ± 0.2 | 25.3 ± 1.2 | n.t. |
| *M. equigenitalium* | n.t. | n.t. | 0 ± 0 | 33.4 ± 1.7 | 0 ± 0 |
| *M. felis* | traces | 17.2 ± 3.1 | 35.7 ± 2.4 | 35.1 ± 1.7 | 0 ± 0 |
| *M. hyorhinis* (BTS7) | n.t. | n.t. | 19.0 ± 1.1 | 22.0 ± 1.1 | n.t. |
| *M. hyorhinis* | n.t. | n.t. | 15.0 ± 0.9 | 21.4 ± 1.1 | n.t. |
| *M. ovipneumoniae* | n.t. | n.t. | 0 ± 0.4 | 13.8 ± 0.8 | n.t. |
| *M. pulmonis* | traces | 14.8 ± 1.9 | 13.8 ± 1.0 | 23.1 ± 1.2 | 1.4 ± 0.5 |
| *M. testudinis* | 0 | 9.4 ± 2.3 | 0 ± 0.1 | 24.9 ± 1.3 | n.t. |
| *anaerobe M.* sp.a | 0 | 12.5 ± 1.4 | 27.0 ± 1.7 | 28.7 ± 1.6 | 0 ± 0.0 |
| *anaerobe M.* sp.b | 0 | 10.1 ± 1.7 | 25.9 ± 1.7 | 26.2 ± 1.5 | 11.7 ± 2.7 |
| *anaerobe M.* sp.b | 0 | 9.3 ± 2.7 | 17.1 ± 1.3 | 24.8 ± 1.4 | 0 ± 0.0 |
| *A. equifetale* | n.t. | n.t. | 42.0 ± 2.2 | 40.1 ± 1.9 | 14.4 ± 1.1 |
| *A. hippikon* | n.t. | n.t. | 18.6 ± 1.2 | 23.6 ± 1.3 | 15.2 0.7 |
| *A. laidlawii* | traces | 25.4 ± 2.9 | 14.8 ± 0.9 | 36.8 ± 1.8 | 8.4 ± 1.5 |
| U. sp.a | n.t. | n.t. | 14.6 ± 1.0 | 14.3 ± 0.9 | n.t. |
| U. sp.b | n.t. | n.t. | 13.0 ± 0.9 | 17.2 ± 1.0 | n.t. |
| Erysipelothrix rhusiopathiae | 6.2 ± 1.3 | 14.6 ± 1.9 | 12.1 ± 1.2 | 19.7 ± 1.5 | 22.9 ± 1.7 |

[1]SOD was determined with cytochrome *c* (units/mg) (27); [2]CAT was determied polarographically with $H_2O_2$ (nkat); [3]freshly prepared cell lysates were used for the determination; [4]freshly prepared cell lysates were dialysed twice 12 hours against 1000 volumes PBS at 4° C; [5]old cell lysates which were stored between 1 and 3 years at −20° C were used for the determination; [6]cell lysates (freshly prepared and old) in PBS were heated to 60°C for 30 s; n.t., not tested. The mean values and standard deviations of 6 different determinations are presented.

TABLE II
Determination of the $O_2^-$ and $H_2O_2$ production by mollicutes.

| species | $^1O_2^-$ | $^2H_2O_2$ |
|---|---|---|
| *M. arthritidis* | 1860 ± 15.6 | 283 ± 9.0 |
| *M. bovigenitalum* | 3733 ± 47.3 | 930 ± 22.1 |
| *M. capricolum* | 19 ± 0.5 | 0 ± 0.0 |
| *M. columbinum* | 3 ± 0.0 | 0 ± 0.0 |
| *M. felis* | 0 ± 0.0 | 0 ± 0.0 |
| *M. pulmonis* | 261 ± 13.0 | 39 ± 1.2 |
| *M. testudinis* | 5740 ± 43.0 | 1185 ± 18.3 |
| *anaerobe M.* | 18110 ± 67.0 | 3750 ± 51.0 |
| *anaerobe M.* | 10030 ± 59.0 | 2369 ± 69.0 |
| *A. laidlawwi* | 5268 ± 23.0 | 1317 ± 34.0 |

$^1O_2^-$ production was determined photometrically at 550 nm by the reduction of acetylated cytochrome *c* (32) over a period of 30 min at 37° C (nmol/30 min × mg protein), $^2H_2O_2$ production was determined fluorimetrically with scopoletin (excitation 381 nm, emission 436 nm) (29) over a period of 30 min at room temperature, 22° C (nmol/30 min × mg protein) The mean values and standard deviations of 6 different determinations are presented.

TABLE III
Release of $O_2^-$ by PMNs upon Stimulation with culture supernatants of mycoplasmas

| culture supernarant | (pathogenity) | nmol $O_2^-$ /15 $min^{-1}$ × $10^6$ PMNs |
|---|---|---|
| *M. pneumoniae* | (pathogen) | 35.5 |
| *M. arthritidis* ISR-1 | (pathogen) | 33.1 |
| *M. pulmonis* | (pathogen) | 30.5 |
| *M. equifetale* | (apathogen) | 1.8 |
| *M. mobile* | (apathogen) | 1.6 |
| *M. columbinum* | (unknown) | 1.5 |
| *M. arginini* | (apathogen) | 1.1 |
| *M. testudinis* | (unknown) | 0.3 |

$O_2^-$ release was followed by the reduction of cytochrome *c* (SOD inhibited part) (10).

were demonstrated in nearly 50% of the cell lysates, whereas no POD activities were detectable (Table I). The production of $O_2^-$ and $H_2O_2$ with glucose as substrate was demonstrated for 8 strains of 10 strains tested (Table II). No correlation to the pathogenicities of the strains were indicated. Anaerobic mycoplasmas showed the highest amount of radical production, whereas SOD and CAT activities were in the range of activities estimated for aerobic mollicutes.

The primary radical released was $O_2^-$ as shown by ESR-spin-trapping.

Some strains (mainly pathogenic) additionally released compounds into the culture medium, which stimulated PMNs to release reactive oxygen species (Table III).

## DISCUSSION

Mollicutes, including mycoplasma and ureaplasma possess SOD activity. These results are in accordance with the theory that aerobic organisms consuming oxygen require SOD as protection from the noxious effects of reactive oxygen species. CAT activity was present in some strains, too. Although a weak correlation between CAT

activity and release of reactive oxygen species was observable, there was one exception, the anaerobic mycoplasma strains sp.a and sp.b. While the release of reactive oxygen species was twice as high opposed to the aerobic strains, they either lacked CAT or the activity was comparable to the aerobic strains. SOD activities were also comparable to the aerobic mollicutes tested.

It might be speculated that "anaerobiosis" is rather due to an immoderate liberation of reactive oxygen species causing a *"relative"* deficiency of protective enzymes.

A correlation between the amount of reactive oxygen species released by the mollicutes and the pathogenicity was not observable, whereas all the pathogenic strains tested release substances into the culture medium, stimulating PMNs to release reactive oxygen species.

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*References*

1. F.S. Archibald and I. Fridovich (1981) *Journal of Bacteriology*, **145**, 442–451.
2. F.S. Archibald and I. Fridovich (1981) *Journal of Bacteriology*, **146**, 928–936.
3. E.P. Norrod and S.A. Morse (1979) *Biochemical and Biophysical Research Communications*, **90**, 1287–1294.
4. F.S. Archibald and M.N. Duong (1986) *Infection and Immunity*, **51**, 631–641.
5. R.E. Lynch and B.C. Cole (1980) *Biochemical and Biophysical Research Communication*, **96**, 98–105.
6. S.J. O'Brien, J.M. Simonson, M.W. Grabowski, and M.F. Barile (1981) *Journal of Bacteriology*, **146**, 222–232.
7. G.Y. Lee and G.E. Kenny (1984) *International Journal of Systematic Bacterology*, **34**, 74–76.
8. F. Friis (1975) *Nordic Veternary Medicine*, **27**, 337–339.
9. R.M. Chanock, L. Hayflick and M.F. Barile (1962) *Proceedings of the National Academy of Sciences USA*, **48**, 41–49.
10. J.M. McCord and I. Fridovich, (1969) *Journal of Biological Chemistry*, **244**, 6049–6055.
11. G. Guidotti, J.P. Colombo and P.P. Foa (1961) *Analitical Chemistry*, **33**, 151–153.
12. R.K. Root, J. Metcalf, N. Oshino and B. Chance (1975) *Journal of Clinical Investigation*, **55**, 945–955.
13. C. Beauchamp and I. Fridovich (1971) *Analitical Biochemistry*, **44**, 276–287.
14. R.C. Allen (1981) In *Bioluminescence and Cheiluminescence* (M.A. DeLuca and W.D. McElroy, Ed.) 63–73, Academic Press, New York.
15. E. Finkelstein, G.M. Rosen and E.J. Rauckman, (1980) *Archives of Biochemistry and Biophysics*, **200**, 1–16.

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