Response of *Pseudomonas aeruginosa* PAO following exposure to hydrogen peroxide

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Abstract

Hydrogen peroxide (H_2O_2) is used in various applications to prevent, control, or decrease bacterial activity in e.g. cooling water, hospitals, recreational waters and the food industry. The aim of the work reported here was to investigate the response of *P. aeruginosa* following exposure to H_2O_2 during both logarithmic and stationary phases of growth. The catalase levels were determined following exposure to H_2O_2, and the general cellular response was investigated by pulse-labelling using ^{35}S methionine. Stationary phase cells did not demonstrate a stress response to H_2O_2. Where *de hovo* protein synthesis was inhibited, cells were less susceptible to growth inhibition, indicating an inverse stress response to H_2O_2 in *P. aeruginosa*. The addition of H_2O_2 to cultures in logarithmic growth phase resulted in the induction of a short lag phase. The growth rate following a return to logarithmic growth phase was lower than before addition of $H2O_2$, and was inversely related to the concentration of H_2O_2 added. Oxidising stress elicited *de novo* synthesis of four proteins within 5 min following exposure to stress. Cellular catalase levels doubled from 16 U-mg-^{1} protein to over 30 U-mg-' protein within 10 min following exposure to oxidising stress but' no new catalase,isozymes were induced. H_2O_3 was demonstrated to interrupt cell division as well as to decrease the ensuing rate of division in *'P. aeruginosa*, and the culture did not exhibit an effective stress response to $H2O_2$.

Introduction

Hydrogen peroxide (H_2O_2) is used in various applications to prevent, control, or decrease bacterial activity. It is used amongst others in industrial water systems to control biofouling, in swimming pools and for the disinfection of surfaces and pipelines in food and other industries (Baldry and Fraser, 1988; Characklis, 1990; Cloete et al., 1992). Surfaces exposed to water are often colonised by bacteria which grow to form biofilms (Characklis, 1990; Cloete et al., 1992). These biofilms are composed largely of gram-negative aerobic rods. *Pseudomonas aeruginosa* is one of the dominant members of biofilm communities and is often used as a model organism in the investigation of biofouling processes and control strategies (Characklis, 1990; Cloete et al., 1992).

Hydrogen peroxide is formed as a by-product during aerobic metabolism along with superoxide (Fridovich, 1978), and reacts with a wide array of biological macromolecules such as DNA, proteins and membrane lipids (Tao et al., 1989). For example, H_2O_2 penetrates cells, causing site-directed damage especially of DNA due to metal-dependant OH formation (Storz et al., 1990).

Many bacteria have been reported to respond to a wide range of environmental stresses including heat, cold, salt, UV radiation and oxidising stresses (e.g. exposure to a sub-inhibitory concentration of H_2O_2). Such stress responses result, generally, in the synthesis of small sets of stress proteins which lead to tolerance of the cells to further exposure to otherwise lethal levels of the same stress (Völker et al., 1992; Watson, 1990). A variety of bacteria, all facultative anaerobes, exhibit oxidising stress response by producing oxidant-degrading as well as damage-repair enzymes, provided that the initial concentration of H_2O_2 is not lethal (Storz et al., 1990). These include *Escherichia coli, Salmonella typhimurium* (Storz et al., 1990), and *Bacillus subtilis* (Hartford and Dowds, 1992). A variety of defence genes to naturally occurring oxidising agents have been characterised in *Escherichia coli*. These defence genes encode various superoxide dismutases, catalases, alkyl hydroperoxide reductases and glutathione reductases, as well as DNA repair enzymes (Ahem, 1993; Storz et al., 1990). Few reports have focused on oxidising stress response in strict aerobes, and none have addressed the response of *P. aeruginosa* upon exposure to H_2O_2.

The aim of the work reported here was to investigate the possible inhibitory activity of H_2O_2 towards *P. aeruginosa* in various stages of growth, as well as the response of *P. aeruginosa* following exposure to H_2O_2 in order to shed more light on the suitability of H_2O_2 as a biofouling control agent.

Materials and methods

Bacterial strains and chemicals

Pseudomonas aeruginosa PAO1 was obtained from the Deutsche Sammlung von Mikroorganismen. The culture was maintained on R2Aagarslants(ReasonerandGeldreich, 1985)with l%glycerol, and was subcultured monthly. Casamino acids (Difco), soluble starch and glucose (BDH chemicals), peptone, yeast extract and bacteriological grade agar (Biolab), Na pyruvate, K_2HPO_4 and $MgSO_4$ (Merck) and chloramphenicol (Sigma) were used throughout this study. H_2O_2 (8.8 mol-l') was obtained from Saarchem.

Reaction during 24 h following exposure to sub-inhibitory oxidising stress

Pseudomonas aeruginosa PAO1 was cultured for 24 h in R2A broth (Reasoner and Geldreich, 1985), but omitting sodium pyruvate,

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