

Adaptation of bacterial cultures to non-oxidising water treatment bactericides

VS Brözel*, B Pietersen and TE Cloete

Environmental Biotechnology Laboratory, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0001, South Africa

Abstract

Bacterial communities in water-cooling systems treated with bactericides often become resistant to these bactericides. This has been ascribed to selection for resistant cells. Certain bacteria, having a high inherent susceptibility to water treatment bactericides, become dominant in systems after bactericide treatment. We investigated the idea that bacterial isolates adapt to growth in the presence of bactericides. Pure cultures of *Pseudomonas stutzeri* and *Bacillus cereus* were cultured repeatedly in the presence of sub-inhibitory concentrations of 2,2'-methylenebis(4-chlorophenol), sodium dimethyldithiocarbamate and isothiazolone. Both isolates adapted to growth in the presence of increasing concentrations of the bactericides. *P. stutzeri* adapted from 22 µg·ml⁻¹ 2,2'-methylenebis(4-chlorophenol) to 80 µg·ml⁻¹ from 12 µg·ml⁻¹ Na dimethyldithiocarbamate to 310 µg·ml⁻¹ and from 50 µl l⁻¹ isothiazolone to 250 µl l⁻¹. *B. cereus* adapted from 20 µg ml⁻¹ 2,2'-methylenebis(4-chlorophenol) to 75 µg·ml⁻¹, from 6 µg·ml⁻¹ Na dimethyldithiocarbamate to 132 (µg·ml⁻¹), and from 50 µl l⁻¹ isothiazolone to 300 µl l⁻¹. The phenomenon of resistance to water treatment bactericides can be ascribed not only to selection but also to adaptation.

Introduction

Surfaces in industrial water systems (e.g. cooling-water systems in power plants and mines) are prone to colonisation by bacteria. The resulting biofilms cause biofouling, leading to a decrease in system efficiency and life expectancy. The nature and mechanisms of biofouling have been reviewed extensively (Cloete et al., 1992; Ford and Mitchell, 1990). Many systems are treated with bactericides to eliminate or reduce biofouling. The various bacteria present differ in their susceptibility to the various bactericides available (Brözel and Cloete, 1991a). Some have a low degree of resistance under pure culture conditions, but play an important role in the microbial communities in cooling waters. Examples are *Pseudomonas stutzeri* and *Bacillus cereus* which often attain a dominant position in communities after bactericide treatment (Brözel and Cloete, 1992b). This indicates some form of adaptation to the bactericide over time (Jones et al., 1989). These bacteria appear to adapt to grow in the presence of otherwise inhibitory concentrations of certain bactericides. We have previously established that bacteria isolated from cooling-water systems do develop an increased level of resistance to bactericides, and grow in the presence of otherwise inhibitory concentrations (Brözel and Cloete, 1991b). Resistance can be defined in two ways, meaning either the ability of an organism and its progeny to multiply or to remain viable under conditions that would usually bring about the opposite (Gilbert and Wright, 1987). In this paper the first definition will hold as biofouling control aims at the prevention (i.e. inhibition) of bacterial growth and metabolic activity in water systems. The phenomenon of bacterial resistance to bactericides can be due to one of three reasons. Certain bacteria, notably gram-negative ones, have a high degree of inherent resistance to many bactericides due to the barrier nature of their cell envelope (Gilbert and Wright, 1987). Secondly, certain resistance mechanisms are genetically encoded and can be acquired by the

contraction of an R plasmid (Franklin and Snow, 1981). Thirdly bacteria have, in certain cases, been reported to adapt to a more resistant physiological state (Jones et al., 1989). Decreased susceptibility of bacteria in cooling-water systems to non-oxidising bactericides after periods of treatment has been ascribed to the selection for less sensitive species (Characklis, 1990). Organisms less susceptible to the bactericide survive treatment and become dominant in the system, rendering the community more resistant to the following treatment.

Many authors have reported that bacteria acquire resistance to antiseptics such as quaternary ammonium compounds (QACs) (Heinzel, 1989; Jones et al., 1989; Sakagami et al., 1989) and biguanides (Heinzel, 1989; Jones et al., 1989). Development of resistance to aldehyde-releasing bactericides (e.g. hexahydro-1,3,5-triethyl-s-triazine) is also documented (Eagon and Barnes, 1986). Biofilm bacteria have been reported to be up to 100 times more resistant to ClO₂ than are free-floating ones (LeChevalier et al., 1988). Costerton and Lashen (1983) reported inherent resistance of biofilm bacteria to an isothiazolone-based bactericide due to the impermeability of the extracellular polysaccharide layer surrounding cells to the bactericide.

Water-cooling systems are often treated with isothiazolone, thiocarbamate or chlorinated phenol-based bactericides (Cloete et al., 1992). Isothiazolones are non-oxidising, do not release formaldehyde and are not membrane-active (Collier et al., 1990). They react oxidatively with thiols to form disulphides. Chlorinated phenols uncouple oxidative phosphorylation from respiration (Gilbert and Brown, 1978; WallhauBer, 1988). The antimicrobial mechanism of thiocarbamates has not been reported to date.

The objective of this study was to investigate the rate at which pure cultures of bacteria, isolated from water-cooling systems, increase their tolerance to 3 selected bactericides, i.e. dichlorophenol, thiocarbamate and isothiazolone during growth in the presence of sub-inhibitory concentrations of these bactericides. For this purpose we chose one gram-positive and one gram-negative isolate found to survive bactericide treatment in cooling-water systems, i.e. *Pseudomonas stutzeri* and *Bacillus cereus*.

*To whom all correspondence should be addressed.

Received 9 October 1992; accepted in revised form 27 January 1993.