



In vitro* mineral transformations by the human pathogens *Staphylococcus aureus* and *Candida albicans

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Abstract

The ability of the pathogenic bacterium *Staphylococcus aureus* and the pathogenic yeast *Candida albicans* to nitrify, solubilize insoluble phosphate and silicic acid and oxidise elemental sulphur was determined *in vitro*. Both organisms oxidised ammonium to nitrate, with *C. albicans* being particularly active; similarly, both organisms oxidised elemental sulphur and solubilized silicic acid. In contrast, while *C. albicans* solubilized insoluble phosphate, *S. aureus* was incapable of such solubilization; since *Staphylococcus pasteurii* was shown to be capable of solubilizing phosphate the lack of ability, in this respect seen in *S. aureus*, is not characteristic of all species of *Staphylococcus*. The results are discussed in relation to the possible role of human pathogenic microorganisms in mineral cycling in soil.

Key words: Pathogenic bacteria, pathogenic yeasts, nitrification, S-oxidation, P-solubilization, silicic acid solubilization, environmental microbiology.

Introduction

Microorganisms are involved in a number of transformations of elements including (a) solubilization of inorganic insoluble phosphorus compounds, (b) mineralization of organic phosphorus compounds to inorganic phosphate, (c) immobilization of inorganic phosphate into cell compounds and (d) oxidation and reduction of inorganic phosphorus compounds¹⁴⁻¹⁶. The ability of bacteria and fungi, including yeasts, to oxidise and reduce forms of elements is well recognised. Such studies have, however, been restricted to saprophytic microorganisms that grow as non-pathogens in the environment, including soils and waters^{1,2,4,7-12,14-16}. Surprisingly, there have been few studies on the ability of pathogenic microorganisms to participate in processes like the oxidation and reduction of elements such as nitrogen and sulphur. This no doubt reflects the fact that such organisms are considered to be restricted to the role they play as pathogens in man and animals¹. However, many pathogens also spend some of their time living in the environment, for example in sewage waters and polluted soil; here, they are exposed to reduced and oxidised forms of elements. As a result, it is of interest to determine if such organisms can contribute to nutrient transformations in the environment. The aim of the study was therefore to determine if two common pathogens, the bacterium *Staphylococcus aureus* and the human pathogenic yeast *Candida albicans* can mediate the transformation of nitrogen (ammonium oxidation) and sulphur (S-oxidation) and the solubilization of insoluble phosphate and silicic acid.

Materials and Methods

Both *Candida albicans* and *Staphylococcus aureus* were grown in triplicate in liquid medium (25 ml) in Erlenmeyer flasks (250 ml),

using Sabouraud liquid medium (Oxoid) and LB medium (Oxoid), respectively. The flasks were incubated with shaking for 4 weeks at 37°C. The two media were amended with either elemental sulphur (1.0% w/v), calcium phosphate (1.0% w/v), silicic acid (1.0% w/v) or ammonium sulphate (100 µg ml⁻¹). The production of ions was measured colorimetrically after filtration of the medium through Whatman No.1 filter paper. Nitrate and sulphate were measured as described by Wainwright and Grayston¹⁴, phosphate as described by Wainwright and Sowden¹⁶ and silicon as detailed by Wainwright *et al.*¹⁵. Non-inoculated flasks were included to account for any non-biological oxidation and reduction; the results are expressed as the difference between values for inoculated and non-inoculated controls.

Results and Discussion

The results are shown in Table 1. *Staphylococcus aureus* produced only small amounts of ammonium to nitrate over the course of the incubation periods; this contrasts with the relatively high rates of net nitrate production by *C. albicans* (Table 1a). Both organisms were active in oxidizing elemental sulphur to sulphate, in this case higher rates were achieved by the bacterium compared to the yeast (Table 1b). Growth of *Candida albicans*, unlike that of *S. aureus*, led to the production of excess phosphate in the medium over the entire incubation period, no such excess phosphate production was, however, seen in medium supporting the growth of *S. aureus* (Table 1c). Finally, both *S. aureus* and *C. albicans* solubilized silicic acid, releasing soluble silicon into the medium; after the first week sampling point, the amounts of soluble silicon produced by *C. albicans* were generally twice as great as those produced by *S. aureus* (Table 1d). In summary, both organisms

Table 1. Nitrification, S-oxidation, P-solubilization and silicic acid solubilization by *Staphylococcus aureus* and *Candida albicans*.

Species	Time (weeks)			
	1	2	3	4
a) Nitrification (net nitrate production) NO ₃ ⁻ µg ml ⁻¹				
<i>S. aureus</i>	0	2.0 ± 0.1	1.4 ± 0.1	1.0 ± 0.08
<i>C. albicans</i>	20.1 ± 0.2	29.7 ± 0.3	50.0 ± 0.3	57.0 ± 0.5
b) Elemental sulphur oxidation SO ₄ ²⁻ µg ml ⁻¹				
<i>S. aureus</i>	50 ± 0.4	125 ± 0.3	137 ± 10.0	201 ± 16.0
<i>C. albicans</i>	10.0 ± 0.1	25.0 ± 2.6	37.2 ± 3.1	42.2 ± 4.0
c) Phosphate solubilization PO ₄ ⁻ µg ml ⁻¹				
<i>S. aureus</i>	0	0	0	0
<i>C. albicans</i>	40.5 ± 4.1	57.2 ± 6.2	57.2 ± 5.1	98.4 ± 4.0
<i>S. pasteurii</i>	42.1 ± 4.0	110.0 ± 15.0	130.2 ± 12.0	105.0 ± 12.0
d) Solubilization of silicic acid Si ⁻ µg ml ⁻¹				
<i>S. aureus</i>	21.2 ± 0.7	20.7 ± 2.2	30.1 ± 0.7	32 ± 5.2
<i>C. albicans</i>	58.0 ± 5.2	54.4 ± 4.2	58.2 ± 6.0	72.4 ± 6.3

Means of triplicates ± standard deviation. Values expressed as increase in ion concentration over uninoculated control.

oxidized ammonium and elemental sulphur and solubilized insoluble silicon; *S. aureus*, in contrast did not produce any excess phosphate in the medium. With the exception of S-oxidation, *C. albicans* was generally more active at participating in the mineral transformations studied than *S. aureus*.

In the past, pathogenic bacteria have been regarded as exceptional organisms which live only in association with humans and animals and do not have the ability for long-sustained periods of existence in the environment. This is clearly not the case, as it is well-recognised that some pathogenic bacteria can live in soils and waters, and there survive the rigours of the environment. This fact clearly goes against the commonly held view that pathogens are weak fastidious organisms that are adapted to growth in their hosts and, as a result, cannot cope with the rigours of life in the environment. Many pathogens can survive in untreated or partially treated wastes which are applied to soils as fertilizers, from where they can act as reservoirs of disease. It is often assumed that pathogenic bacteria and yeasts merely lie dormant in such environments, although it is likely that under certain conditions they are active and can contribute to the environmental cycling of elements. The biochemistry of pathogenic bacteria and yeasts, although different in some respects from their non-pathogenic relatives (due to adaptation to pathogenicity) is likely to be sufficiently similar, such that major metabolic transformations, including nitrification and sulphur oxidation, will be found in these bacteria independent of their ability to act as pathogens. This assumption is borne out by the findings described here, where both the pathogenic bacterium *S. aureus* and the human pathogenic yeast *C. albicans* were found to participate in certain *in vitro* oxidation reactions involved in the cycling of nitrogen and sulphur. While both organisms were found to be able to solubilize insoluble silicon, only *C. albicans* solubilized insoluble calcium phosphate. Are all species of *Staphylococcus* then incapable of solubilizing insoluble phosphate? The results (Table 1c) show that another species of *Staphylococcus*, *S. pasteurii* (a bacterium commonly found in soils), in contrast to *S. aureus* can solubilize insoluble phosphate, even more effectively than *C. albicans*; clearly not all *Staphylococci* are incapable of

phosphate solubilization. It may turn out that this ability can be used to differentiate between pathogenic and saprophytic, environmentally-adapted species of *Staphylococcus*.

A wide range of soil yeasts have been shown to be involved in nitrification *in vitro*^{2,3}, as have filamentous fungi like *Aspergillus flavus*¹⁴; the ability of saprophytic bacteria to participate in the *in vitro* transformation of sulphur and nitrogen has similarly been widely reported¹⁴.

The fundamental point arising from these studies is that pathogenic bacteria are likely to perform many of the biochemical transformations seen in their non-pathogenic relatives. As a result, there is no reason why pathogens should not participate in nutrient cycling when they reach the environment, for example in partially treated or untreated sewage effluents which inadvertently reach rivers or are purposely applied to agricultural soils as fertilizers. In such environments, when they are capable of surviving and growing, the pathogens will act as saprophytes and play a role in mineral cycling. Such oxidation and reduction reactions, carried out by pathogens when living in the environment, may play a major role in their survival. It has been suggested for example, that some heterotrophic bacteria and fungi may gain additional energy by oxidizing reduced forms of nitrogen and sulphur¹⁴. Such energy gains, even when small, may enable the pathogens to survive in nutrient-limited environments. Oxidation of reduced forms of nitrogen and sulphur may allow bacteria and yeasts to avoid the toxic effects of certain reduced forms of these elements. The participation in such oxidation reduction reactions by pathogens may be extremely important in allowing these organisms to form survival foci from where they can infect their animal and human hosts.

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