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Effect of silicic acid and other silicon compounds on fungal growth in oligotrophic and nutrient-rich media

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Mycelium grew from a spore-mycelial inoculum of *Aspergillus oryzae* added to ultra-pure water (upw) containing silicon compounds, but did not grow in upw alone. Growth of other fungi also occurred in upw only when silicon compounds were added. Increased growth of *A. oryzae*, and other fungi, also followed the addition of silicic acid and other silicon compounds to Czapek Dox. *Aspergillus oryzae* solubilized silicon compounds in both upw and nutrient-rich media. Although interactions between microorganisms and silicon have been generally neglected, the results show that silicon compounds can increase fungal growth under both oligotrophic and nutrient-rich conditions.

Except for studies on silicon accumulation by diatoms, the microbial metabolism of silicon has been largely ignored. Fungi and bacteria can solubilize insoluble silicates, a process which may be important in the biological weathering of rocks (Duff & Webley, 1963). Silicon compounds also increase bacterial growth and have been implicated in aggravating tubercular infections of the lung in patients suffering from silicosis (Price, 1932). It has also been suggested, although not conclusively proven, that bacteria use silicon-based autotrophy as a source of energy to support CO_2 fixation (Bigger & Nelson, 1943; Chakrabarty *et al*., 1988; Das *et al*., 1992).

The ability of fungi to grow on nutrient-free silica gel is now well-established (Wainwright, 1993). While CO_2 fixation has been implicated in growth under these conditions, it is generally believed that fungi grow oligotrophically by using nutrients adsorbed by the silica gel from the atmosphere (Parkinson *et al*., 1990, 1991). It is possible, however, that silica gel itself increases hyphal growth or stimulates fungal spore germination.

The aim of the work reported here was to determine the effect of silicon compounds on fungi when growing in: (i) upw under conditions in which stringent efforts were made to exclude nutrients and (ii) nutrient-rich Czapek Dox media. While the effects of silicic acid on the growth of *A. oryzae* is emphasized, the effect of a range of silicon compounds on the growth of other fungi was also investigated under both oligotrophic and nutrient-rich conditions.

MATERIALS AND METHODS

Effect of silicic acid on growth of A. oryzae *under oligotrophic growth conditions*

Aspergillus oryzae (Ahlb.) Cohn was obtained from the Sheffield University Animal and Plant Sciences Culture Collection. The fungus was subcultured onto Czapek Dox agar (Oxoid), and incubated at 25 °C for 7 d. Mycelium plus spores were carefully removed using a sterile inoculating needle to avoid the transfer of any of the underlying nutrient-rich medium. Small amounts of this inoculum were then transferred to ultra-pure water (upw, 30 ml) in plastic Petri dishes. The upw was obtained using a Millipore RO-4 water filter system and was sterilized by autoclaving at 120° for 20 min. Although the inoculum size was not strictly controlled, approximately equal amounts of mycelium were transferred on each occasion. Inoculated plates were then amended with silicic acid (0·3 g; Sigma Chemical Company, St Louis). Control plates were not supplemented. Three control plates and three plates containing silicic acid were inoculated and the experiment was repeated four times, resulting in a total of 12 replicates. All plates were incubated at 25° for 14 d.

In a separate set of experiments the above protocol was repeated using silicic acid which had been heated in a muffle furnace to remove organic contaminants. Silicic acid was placed in aluminium containers loosely covered with aluminium foil. The cases were then heated at 400° for 2·5 h and allowed to cool to room temperature inside the furnace before transfer to upw.

The above experiments were also repeated using silicic acid which had been heat-treated and then acid-washed to remove inorganic contaminants. After heat treatment, the powder was washed three times with upw for 30 min, followed by two washes with HCl (0·1 M) for 1 h. The powder was then finally washed three times for 30 min in upw and then transferred to upw in Petri dishes.

Effect of silicic acid on growth of various fungi under oligotrophic conditions

The above experiments were repeated using the following

fungi (obtained from the same collection as *A. oryzae*): *Aspergillus repens* de Bary, *Aspergillus niger* Tiegh., *Fusarium oxysporum* Schltdl., *Penicillium chrysogenum* Thom, *Penicillium janthinellum* Biourge. An asporogenous mutant of *Neurospora crassa* Shear & B. O. Dodge, was also included (culture number 3263, Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City).

Effect of silicon compounds on growth of A. oryzae *and other fungi under oligotrophic conditions*

The above experiments were repeated with *A. oryzae* and the fungi listed in the previous section. The following silicon compounds (Sigma) were added to the medium (0·3 g): calcium silicate, colloidal silica, silicon nitride, sodium silicate and hydrated magnesium silicate (perfume-free talc, from Boots Pharmaceuticals, Nottingham).

Effect of silicic acid on growth of A. oryzae *in nutrient-rich growth conditions*

Aspergillus oryzae was grown on Czapek Dox agar (Oxoid) for 10 d at 25°. Discs (4 mm) were then cut from the leading edge of the colonies using a flame-sterilized cork borer. These were transferred (1 disc per flask) to unbuffered Czapek Dox liquid medium (Oxoid, 100 ml in a 250 ml Erlenmeyer flask), amended with 0·5, 1·0, 1·5 and or 2·0 g of silicic acid. Controls lacking silicic acid, and containing silicic acid but not fungus, were also included. All treatments were incubated for 7 d in triplicate on an orbital shaker (100 throws min^{-1}) at 25°. The contents of the flasks were then filtered through Whatman No. 1 filter paper and the dry weight of the mycelium was determined after drying to constant weight at 40°. Filtrate pH was determined immediately after filtration using a glass electrode. The soluble silicon content of the medium was determined colorimetrically by adding the following to 1 ml of the filtrate: ammonium molybdate (2 ml, 10% v/v); ascorbic acid (2 ml, 5% w/v); oxalic acid (1 ml, 10% w/v); and HCl (5 ml, 1:1 dilution of conc. HCl). After 15 min at room temperature, without shaking, the absorption of the blue colour was measured spectrophotometrically at 600 nm. The concentration of soluble silicon (as SiO_2) in the filtrate was then determined by reference to a standard curve prepared using EIL standard silicon solution (BDH Chemicals, Poole, Dorset). When acid-washed silicic acid was added to nutrient-rich media, the washing procedure described for the oligotrophy experiments was used. The results were analysed for standard error, and significant difference using Student's *t*-test.

Effect of silicic acid on the growth of fungi in Czapek Dox medium

Unbuffered Czapek Dox medium was directly amended with untreated silicic acid (1·5% w/v) and inoculated with one of the following fungi: *Aspergillus niger*, *A. oryzae*, *A. repens*, *P. janthinellum* and *F. oxysporum*. Flasks were set up in triplicate and incubated as above.

Effect of silicon compounds on growth of A. oryzae *in Czapek Dox medium*

Unbuffered Czapek Dox medium was directly amended with one of the silicon compounds listed previously (1·5% w/v) and inoculated with *A. oryzae*. The silicon compounds were not heat-treated or washed. Flasks were set up in triplicate and incubated as above.

Effect of silicic acid contained in dialysis tubing on growth of A. oryzae *under nutrient rich conditions*

The experiment described above in the section growth in nutrient rich conditions was repeated except that the silicic acid was added to unbuffered Czapek Dox medium in a dialysis tubing envelope. This was made by cutting dialysis tubing (3 × 6 cm), inserting the individual silica compounds, and then folding and sealing the ends of the parcel using metal staples. Dialysis tubing envelopes lacking silica compounds were incubated without fungal inoculum as controls. Medium pH, fungal dry weight and soluble silicon concentration were determined as described above.

RESULTS AND DISCUSSION

Effect of silicic acid on growth of A. oryzae *under oligotrophic conditions*

Aspergillus oryzae consistently failed to grow from the myceliaspore inoculum when added to upw. However, visible mycelial growth occurred in the presence of silicic acid (Fig. 1). Growth occurred in all twelve plates containing silicic acid, but in none of the control plates containing only upw. Particles of silicic acid could clearly be seen attached to the surface of *A. oryzae*. Spot tests, using the reagents for colorimetric determination of silicon, also showed that *A. oryzae* solubilized silicic acid in upw. Growth also occurred when acid washed silicic acid was used, showing that the effect was not due to contamination by inorganic nutrients.

Due to limitations of analytical equipment available to us, the biomass, or protein content, of the small amounts of mycelium produced could not be determined, so our results are limited to observation.

Effect of silicic acid on growth of various fungi under oligotrophic conditions

While *Aspergillus repens*, *A. niger*, *F. oxysporum*, and *P. janthinellum*, did not grow in upw alone, all grew (12 replicates of each fungus) when silicic acid (untreated, heat-treated, and heat-treated plus acid-washed) was added to upw.

Effect of silicon compounds on growth of A. oryzae *under oligotrophic conditions*

While *A. oryzae* and the fungi listed in oligotrophic conditions failed to grow in upw alone, all grew in upw (all 12 replicates in each case) after the addition of one of the following silicon compounds: calcium silicate; colloidal silica; talc (hydrated

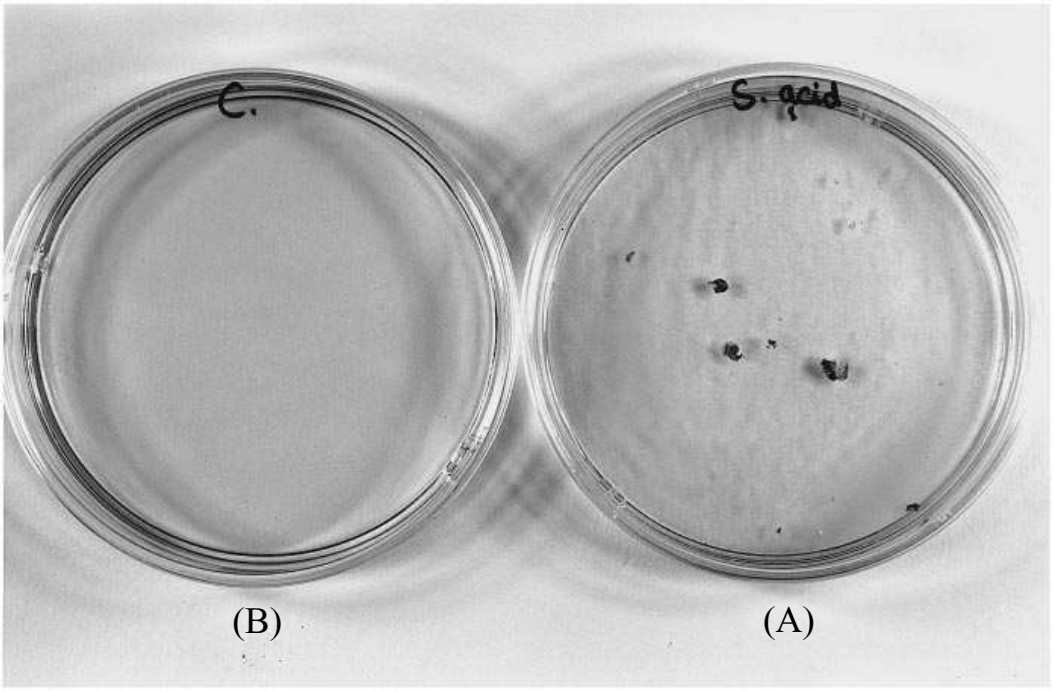

Fig. 1. Growth of *A. oryzae* in upw containing silicic acid (A), and absence of growth in upw alone (B).

magnesium silicate) and rock potash (untreated, heat-treated, and heat-treated plus acid-washed).

The above observations show that fungi which cannot grow from a spore-mycelium inoculum in upw, grow following the addition of silicic acid and other silicon compounds. These observations can be explained as follows. First, by overriding some fungistatic agent, the silicon compounds may have stimulated fungal spore germination. However, when mycelium of an asporogenous mutant of *N. crassa* was used, growth also occurred in upw containing silicic acid (but not in its absence). This shows that silicic acid can promote mycelial growth alone.

A second possibility is that, during storage, silicic acid adsorbed nutrients which then provide the fungus with the necessary growth substrates absent from upw. This is unlikely where heat-treated silicic acid was used, since all organic substrates would have been removed following heating to 400°. Fungal growth continued to occur in the presence of silicic acid and the other silicon compounds following heat treatment and acid-washing. Therefore, the effect of silicon compounds on fungal growth was not due to the presence of contaminating nutrients, including trace elements.

A third possibility is that silicon compounds, which are efficient at adsorbing gases and volatiles, removed combined carbon and nitrogen from the atmosphere, which then acted as nutrient sources for fungal growth.

Finally, the fungi may have used the silicon compounds as an energy source, enabling them to fix CO_2 from the atmosphere. The possibility that fungi can grow autotrophically under oligotrophic conditions (using energy obtained from hydrogen oxidation) was suggested by Mirocha & Devay (1971). Bigger & Nelson (1941, 1943) also suggested that silicon compounds might adsorb ammonia and CO_2 from the atmosphere, thereby allowing bacteria to fix CO_2, using energy obtained from the oxidation of ammonium. Chakrabarty *et al.* (1988) have similarly suggested, although not conclusively proved, that certain bacteria can grow as silicon autotrophs. Although it is generally thought that silicon compounds are biologically unreactive, Allison (1968) stated that there is no theoretical reason why the reaction of Si-Si-Si with oxygen or oxygen compounds could not act as an energy-yielding reaction. However, the possibility that fungi and other microorganisms might use silicon-based autotrophy clearly remains speculative.

Whatever the mechanism involved, it is clear that silicic acid and other silicon-containing compounds, promote fungal growth under oligotrophic conditions, a fact which helps explain the ability of fungi to grow on nutrient-free silica gel. Silicon is not, however, essential for such oligotrophic growth since fungi will also grow on nutrient-free pluronic polyol, a gelling agent which lacks silicon (Wainwright & Grayston, 1988).

Effect of silicic acid on growth of A. oryzae *in nutrient-rich media*

The addition of silicic acid to Czapek Dox liquid medium led to an increase in growth (biomass) of *A. oryzae* over the 7 d incubation period (Fig. 2*a*); with biomass production increasing with increasing amounts of added silicic acid. The biomass increase was associated with increases in the concentration of soluble silicon, which also increased with increasing weight of added silicic acid (Fig. 2*b*). Negligible amounts of silicic acid were solubilised in the absence of fungal inoculum. Since, in these experiments, some of the silicic acid was adsorbed onto the surface of the growing mycelium, the measured biomass was larger than the real biomass. However, not all of the silicic acid was removed from

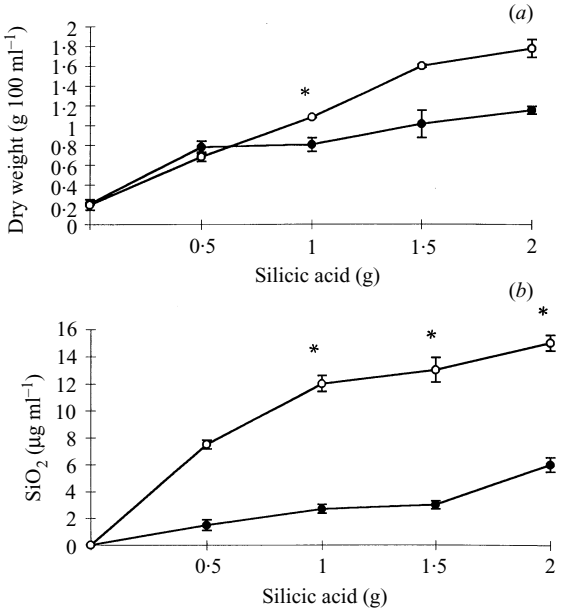

Fig. 2. (*a*) Effect of silicic acid on growth of *A. oryzae* in Czapek Dox liquid medium; (*b*) release of soluble silicon from silicic acid by *A. oryzae*, ○——○ silicic acid added directly to medium; ●——● silicic acid added to medium in dialysis tube. Means of triplicates, ± standard error. * Significant difference, $P < 0{\cdot}05$.

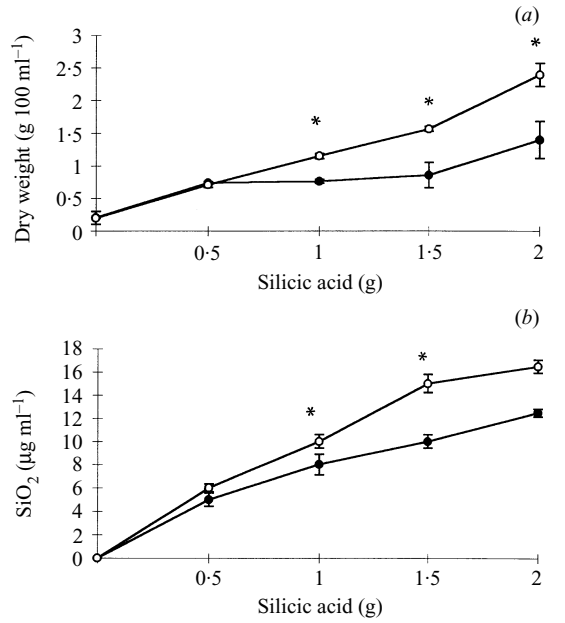

Fig. 3. (*a*) Effect of silicic acid on growth of *A. oryzae* in Czapek Dox medium buffered to pH 6·8; (*b*) release of soluble silicon from silicic acid by *A. oryzae*. Symbols as for Fig. 2.

solution by the *A. oryzae*, even when the lowest amount of silicic acid (0·5% w/v) was added. This suggests that the biomass increase resulting from the addition of increasing amounts of added silicic acid was not solely due to adsorption of silicic acid to the mycelium.

In order to determine if direct contact between the silicic acid and fungus was necessary for the observed growth increase, and increase in solubilization of silicon, silicic acid was added to the flasks in sealed dialysis tubing. Fig. 2 shows that an increase in biomass and soluble silicon continued to

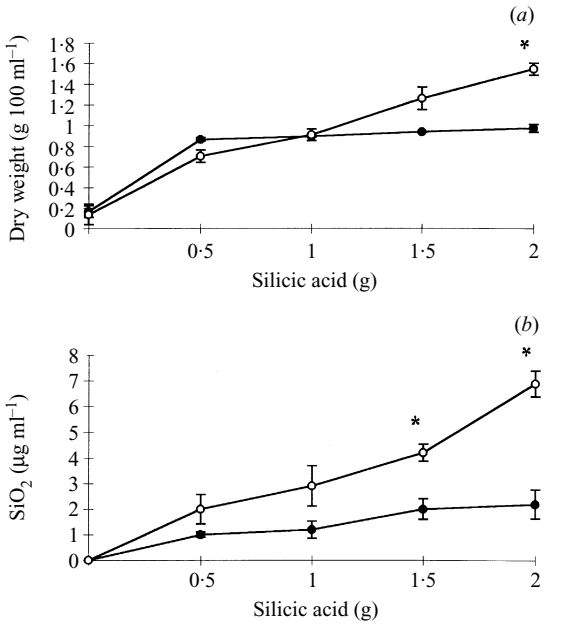

Fig. 4. (*a*) Effect of HCl-washed silicic acid on growth of *A. oryzae* in buffered Czapek Dox medium (pH 6·8); (*b*) release of soluble silicon from acid washed silicic acid in buffered medium. Symbols as for Fig. 2.

occur under these conditions, although in both cases the effect was reduced in comparison to the treatment in which silicic acid was added directly to the medium. Again, only negligible amounts of silicon solubilization occurred in the absence of added fungus. These results show that while *A. oryzae* can solubilize silicic acid, maximum solubilization requires direct contact between the fungus and the particles of silicic acid. These results also re-emphasise that the amount of soluble silicon present in the medium is related to the amount of biomass produced.

It could be argued that the increased biomass produced with increasing silicic acid concentration was due to pH effects resulting from the addition of differing amounts of silicic acid. However, when the medium was buffered to pH 6·8 (using Sørensen's buffer) the same trends seen above were observed (Fig. 3), showing that variations in the pH of the medium, resulting from the addition of silicic acid, were not responsible for the observed growth increases. A similar increase in growth of *A. oryzae* and the release of soluble silicon occurred in buffered medium (pH 6·8), when HCl-washed silicic acid was added to the medium (Fig. 4). This shows that the observed increases in growth and silicon release were not due to the growth stimulating effects of trace elements present in the silicic acid.

A range of silicon compounds stimulated the growth of *A. oryzae* when added to Czapek Dox medium (Fig. 5). However, in the case of sodium silicate, the observed increases were small and statistically insignificant. Why sodium silicate should be exceptional in this respect is not clear. However, like the other compounds it was not completely soluble in the medium, so the lack of growth stimulation was not due to the absence of insoluble particulates.

Silicic acid (untreated, added directly to the medium) also increased the growth of various fungi when growing in un-

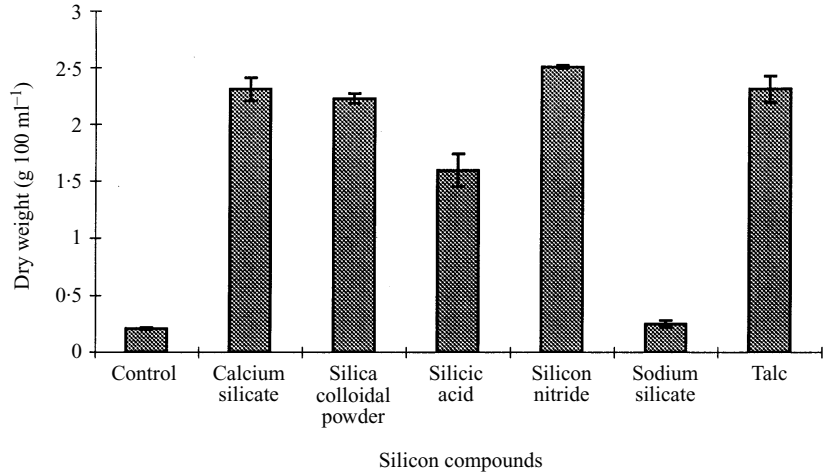

Fig. 5. Effect of various silicon compounds on growth of *A. oryzae* in unbuffered Czapek Dox medium. (All increases, except for sodium silicate, were significant, $P < 0{\cdot}05$).

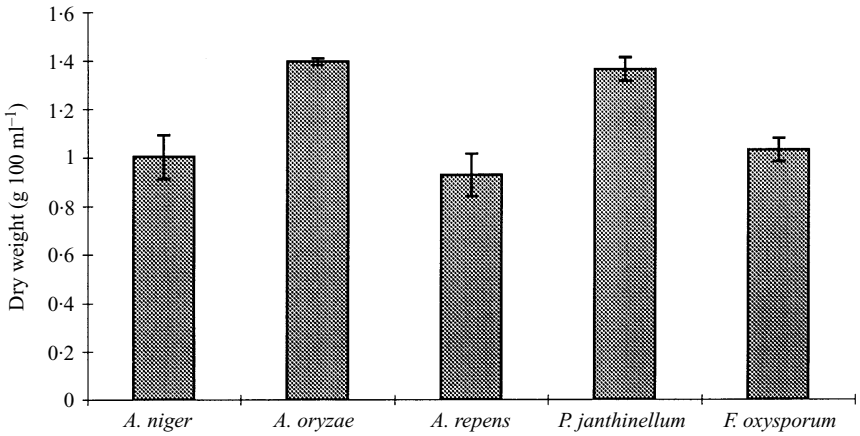

Fig. 6. Effect of silicic acid on growth of fungi in unbuffered Czapek Dox medium, expressed as increase in dry weight after subtraction of the weight of the control for each individual fungus. (All increases were significant at $P < 0{\cdot}05$).

buffered medium, (Fig. 6) so the stimulatory effect was not restricted to *A. oryzae*. (Note that some of the apparent increase in biomass shown in Figs 5 and 6 resulted from adsorption of silicon compounds by the fungi).

While the ability of silicon compounds to stimulate bacterial growth has been reported previously, this appears to be the first report on the ability of silicic acid and other silicon compounds to promote fungal growth under oligotrophic conditions, and increase growth in nutrient-rich media.

Silicon-containing compounds clearly have a stimulatory effect on fungal growth, under both oligotrophic and nutrient-rich conditions. At present we are unable to explain this growth stimulation. One possibility is that the effects are non-specific and merely due to the silicon compounds acting as a solid contact surface; similar growth increases might have resulted from the addition of any, non-silicon containing, particulate material. However, in some of the experiments where nutrient-rich media were employed, increases in the growth of *A. oryzae* occurred in Czapek Dox medium containing silicic acid when dialysis tubing was used to prevent direct contact between silicic acid and the fungus. This suggests that the observed effects on growth were due to some specific biochemical, rather than purely physical, interaction between the particles of silicic acid and the fungus.

While chemo-autotrophic growth, using energy gained from silicon metabolism, may be involved in growth under oligotrophic conditions, it cannot explain why silicon compounds increase both bacterial (Das *et al.*, 1992) and fungal growth in nutrient-rich media.

Silicon is the second most common element on Earth after oxygen and is abundant in soils. Despite the fact that silicon is generally thought to be biologically inert, it is possible that soil microorganisms will have evolved some means of metabolising this element. The presence of silicon in clays and other soil minerals may help explain why soil microorganisms, including fungi, can grow in such an apparently nutrient-poor environment by: (i) using nutrients adsorbed by silicon compounds from the soil atmosphere, or (ii) by using energy derived from silicon metabolism to fix CO_2 chemoautotrophically. *Aspergillus oryzae* is not generally regarded as a soil fungus, but we have also shown that silicon compounds can stimulate the growth of a range of fungi, including common, soil-inhabiting species like *F. oxysporum*.

Further studies are now in progress to determine the

mechanisms involved in the stimulatory effect of silicon compounds on fungal growth.

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