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Short Communication

In vitro solubilization of insoluble fluorides by selected fungi

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The ability of three species of *Penicillium*, isolated from soil, and *Fusarium solani* to solubilize insoluble fluorides was studied. All three *Penicillium* species and *F. solani* were capable of solubilizing calcium fluoride and fluorite. A single species of *Penicillium* and *F. solani* also solubilized a range of insoluble fluoride compounds. The ability of *F. solani* to solubilize calcium fluoride varied with the type of carbon source used and increased with an increasing amount of added fluoride up to 2% (w/v). No growth occurred above this amount. The addition of 1.5% (w/v) calcium fluoride also inhibited the formation of the black spores that are typically produced by *Aspergillus niger*, although sporulation returned when the fungus was transferred to medium lacking calcium fluoride.

Key words: Fluoride solubilization, fungi, *Penicillium*, *Fusarium solani*, environmental mycology.

INTRODUCTION

Fungi are known to be able to participate in the major mineral cycles in soils and the environment in general. They can, for example, both oxidize and reduce nitrogen and sulphur. In addition, many fungi can solubilize insoluble phosphates and they may also play a role in the cycling of silicon (Wainwright, 1981). However, little is known about the ability of fungi to soluble insoluble fluoride compounds, as evidenced by a dearth of literature on this subject over the last three decades. This may be related to the fact that the fluoride (F^-) ion is not regarded as an essential element for plant growth and, as a result, few studies have been performed regarding the role of micro-organisms in mobilizing this element in the environment. However, insoluble fluorides do occur, both naturally and as pollutants from a variety of industrial processes (Marier, 1977; Camargo, 2003). Consequently, it is of interest to determine if fungi can solubilize such insoluble fluoride compounds. The aim of the work reported here was therefore to study the ability of *Penicillium* isolates and *Fusarium solani* to solubilize a range of insoluble fluoride compounds *in vitro*.

MATERIALS AND METHODS

Fungal isolates

Fungal isolates having the ability to solubilize fluoride were isolated from an agricultural loam (previous crop wheat) from the Sheffield area. The following method was used to isolate presumptive fluoride solubilizers, as developed in this laboratory. A soil suspension was made by adding 1 g of soil and 100 ml of sterile Ringer's solution. Serial dilutions of the soil suspension were then made and the dilutions (0.1 ml) were spread onto the surface of Czapek Dox medium containing either cryolite (Na_3AlF_6) or calcium fluoride (CaF_2). Fungal colonies growing on the surface of the agar, which produced clear haloes, were considered to be presumptive fluoride solubilizers. The colonies were picked and *Penicillium* species were sub-cultured, which were designated *Penicillium* 1 through 3. An isolate of *F. solani* was obtained from the departmental culture collection (FS1/2006) and included in the study.

Solubilization of insoluble fluorides *in vitro*

The fungi were grown in Czapek Dox liquid medium (50 ml in 250 ml Erlenmeyer flasks) amended with various amounts of a range of fluoride compounds, as indicated in Table 2. The flasks were incubated with shaking at 25°C for 7 days. The amount of soluble fluoride in the medium was then determined by using a fluoride specific electrode (Philips IS550, plus an inert reference electrode, Phillips R44/2-D/1 connected to a Phillips PW9414 digital pH/pX meter). The medium was inoculated with a 3 cm disc, cut from the leading edge of a fungal colony grown for 7 days at 25°C. Uninoculated controls were included to account for any non-biological solubilization, and the amount of solubilization was determined by subtracting the values from inoculated flasks from their corresponding controls. The pH of the medium was determined using a glass electrode (connected to the above mentioned pH meter).

Table 1. Solubilization of calcium fluoride and cryolite by three species of *Penicillium.*

Penicillium	CaF_2	Na_3AlF_6
1	2.4 ± 0.3	33.0 ± 0.6
2	1.9 ± 0.2	14.0 ± 0.6
3	2.0 ± 0.4	65 ± 5.0

Means of triplicates ± standard deviation.

Table 2. Solubilization of a range of insoluble fluorides by *Penicillium* 1.

Penicillium 1	Insoluble fluoride (1% [w/v])	F^- µg.ml^{-1}
	AlF_3	11.0 ± 0.4
	CaF_3	8.0 ± 0.7
	Na_3Al_6	25.0 ± 0.4
	MgF_2	20.0 ± 0.2
	LiF_2	No growth
	MnF_2	No growth

Means of triplicates ± standard deviation.

Table 3. Solubilization of a range of insoluble fluorides (1% [w/v]) by *F. solani*.

F. solani	Insoluble fluoride	Solubilization
	AlF_3	4.0 ± 0.5.
	CaF_3	5.0 ± 0.3
	$Na3Al_6$	10.0 ± 1.0
	MgF_2	4.8 ± 0.3
	LiF_2	No growth
	MnF_2	No growth

Means of triplicates ± standard deviation.

Table 4. Effect of carbon source on solubilization of CaF_2 (15% [w/v]) by *F. solani*.

Carbon source (0.3% [w/v])	Solubilization
Sucrose	7.9 ± 0.6
Glucose	14.0 ± 0.3
Malt	17.5 ± 0.2

Means of triplicates ± standard deviation

Detection of 2- and 5-ketogluconic acids

The filtrates, in which fungi had grown, in the presence of insoluble fluorides, were evaporated *in vacuo* at 25 - 30°C to approximately a quarter of their original volume. Aliquots (0.2 ml) were then spotted onto Whatman No.1 chromatography paper. The chromatograms were run at room temperature in *n*-butanol:acetic acid:water in a ratio of 4:1:5 by volume (Duff et al., 1963). Ketogluconic acid was detected by spraying with aniline phthalate (0.93 g of aniline and 1.6 g of phthalic anhydride in 100 ml of water), thus detecting both 2- and 5-ketogluconic acids. Spots of the former are red in colour, while the latter are yellow in colour. Standard solutions of the two ketogluconic acids were run alongside the filtrates.

RESULTS AND DISCUSSION

The results presented in Table 1 show that the three *Penicillium* isolates were able to solubilize both calcium fluoride and cryolite. Similar small amounts of calcium fluoride were solubilized by all three *Penicillium* species. All of the *Penicillium* species showed a much greater ability to solubilize cryolite than calcium fluoride, with *Penicillium* 3 being particularly effective, producing almost twice the amount of solubilization shown by *Penicillium* 1, which in turn solubilized more than twice as much of the insoluble fluoride than did *Penicillium* 2 (Table 1).

Since sodium fluoride has been shown to be toxic to plant pathogenic fungi, any fungus that is able to solu-bilize insoluble fluorides, and thereby release fluoride (F-) into solution, must therefore be able to resist the toxic effects of this ion (Treshow, 1965). The ability of *Peni-cillium* 1 to solubilize a range of insoluble fluorides is shown in Table 2. Cryolite and magnesium fluoride were the most readily solubilized insoluble fluoride compounds, with over twice the amount of soluble ion being released compared to to calcium and aluminium fluorides. No growth, and therefore no solubilization, occurred in the presence of lithium and manganese fluorides (Table 2).

F. solani also solubilized the insoluble fluorides, except lithium and manganese fluorides which were toxic, although the amount of solubilization seen was much less than that achieved by *Penicillium* 1 (Table 3).

From the above results, it can be concluded that fungi can solubilize insoluble fluorides, but they differ in their ability to do so, and that the type of fluoride added to the medium markedly influences the degree of solubilization. Table 4 shows that the type of carbon source used can also markedly influence the amount of insoluble fluoride that is solubilized by *F. solani*. Malt is the most favoured carbon source, followed by glucose and then sucrose.

Finally, the effect of amount of calcium fluoride on the degree of solubilization is shown in Table 5. Solubilization was found to increase with an increase in the amount of added calcium fluoride until 5.0% (w/v), when no growth occurred. The optimum amount for solubilization occurred at 2.0% (w/v) and was associated with an increase in the pH of the medium.

During these studies, a plate became contaminated with a white fungus, which had the ability to solubilize calcium fluoride. This contaminant was transferred to fresh Czapek Dox medium and it grew as a black colony, which was subsequently identified as *A. niger*. This finding led to further studies of the growth of this fungus. Figure 1 shows that calcium fluoride (1.0% [w/v]) inhibited the formation of the typical black spores of *A. niger*.

Spore formation was re-imposed, however, when this fungus was transferred to Czapek Dox medium lacking added calcium fluoride. These observations are similar to

Table 5. Effect of range of amounts of CaF_2 on solubilization by *F. solani*

CaF_2 concentration (% w/v)	Solubilization
0.5	6.0 ± 0.2
1.0	9.0 ± 0.3
2.0	28.0 ± 0.2
5.0	No growth
10.0	No growth

Means of replicates ± standard deviation.

Figure 1. Inhibition of black spore formation by *A. niger* in the presence of 1% (w/v) calcium fluoride (CaF_2). The Petri dish at the bottom right shows the re-establishment of conidia

those reported by Kahlon and Vyas (1980), who found that sodium fluoride inhibited enolase activity in *A. niger* and that it suppressed conidiation, but not vegetative growth.

In conclusion, the results presented here show that common soil fungi clearly have the ability to solubilize insoluble fluorides. It is important to note, however, that micro-organisms can both adsorb (Gaugler et al., 1981) and release fluoride. The results therefore show the difference between fluoride accumulation and solubilization.

Insoluble phosphates can undergo similar solubilization by fungi (Agnihotri, 1970) and other micro-organisms (Duff et al., 1963). Unlike fluoride solubilization, however, this process is usually associated with the production of both mineral and organic acids. Phosphate solubilization is often associated with the production of both 2-and 5-ketogluconic acids. However, no evidence was found for the production of these acids when the fungi employed here solubilized insoluble fluorides. As a result, the solubilization of insoluble fluorides by fungi must be achieved by mechanisms different from those involved in the solubilization of insoluble phosphates.

Supharungson (1982) showed that microbial fluoride solubilization also occurs in autoclaved soils. Thus, the process is likely to occur in the environment, as well as *in vitro* provided that sufficient carbon substrates are available to maintain fungal growth. As a result, the solubilization of insoluble fluorides, by both bacteria and fungi, in the environment may have a major environmental impact leading to the release of potentially toxic fluoride ion.

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