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Evaluation of aluminium mobilization from its soil mineral pools by simultaneous effect of *Aspergillus* strains' acidic and chelating exometabolites

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ABSTRACT

This contribution investigates aluminium mobilization from main aluminium pools in soils, phyllosilicates and oxyhydroxides, by acidic and chelating exometabolites of common soil fungi *Aspergillus niger* and *A. clavatus*. Their exometabolites' acidity as well as their ability to extract aluminium from solid mineral phases differed significantly during incubation. While both strains are able to mobilize aluminium from boehmite and aluminium oxide mixture to some extent, *A. clavatus* struggles to mobilize any aluminium from gibbsite. Furthermore, passive and active fungal uptake of aluminium enhances its mobilization from boehmite, especially in later growth phase, with strong linear correlation between aluminium bioaccumulated fraction and increasing culture medium pH. We also provide data on concentrations of oxalate, citrate and gluconate which are synthesized by *A. niger* and contribute to aluminium mobilization. Compared to boehmite-free treatment, fungus reduces oxalate production significantly in boehmite presence to restrict aluminium extraction efficiency. However, in presence of high phyllosilicates' dosages, aluminium is released to an extent that acetate and citrate is overproduced by fungus. Our results also highlight fungal capability to significantly enhance iron and silicon mobility as these elements are extracted from mineral lattice of phyllosilicates by fungal exometabolites alongside aluminium.

1. Introduction

Recent studies on aluminium interaction with fungi are primarily focused on microbial treatment of waste by-product from bauxite processing [1,2] and spent refinery processing catalyst [3,4] as well as direct processing of low-grade or other secondary aluminium ores [5,6]. The most common and efficient bio-hydrometallurgical technique is controlled one-step or two-step bioextraction which utilizes natural fungal ability to produce acidic and metal-chelating exometabolites from available organic nutrients [7,8]. However, this process does not occur only under well-regulated laboratory and industrial conditions, but also in natural fungal habitat, in soils, where occurrence of low molecular mass organic acids [9] as well as some other active chelators is partially attributed to fungal activity [10].

Industrial as well as natural processes utilize microbial exometabolites which induce extraction of metals from solid phases via mechanistically complex chemical reactions of acidolysis, compelxolysis and redoxolysis [11]. Thus, besides well studied bio-hydrometallurgical processes of aluminium extraction from secondary ores which might apply adapted or engineered microorganisms [12], we strongly suggest that scientific interest should focus more on influence of native soil microorganisms on stability of dominant aluminium pools, such as phyllosilicates, and aluminium oxides and hydroxides [13]. The necessity of this research is highlighted by the lack of understanding of complex biogeochemical behavior of aluminium in soils and its transport to higher plant [14] which is undoubtedly influenced by rhizosphere microorganisms' organic acids production [15]. This inspired us to study fungal exometabolites in presence of common aluminium bearing mineral phases as their activity influences mobility of aluminium even when in stable mineral form. These are aluminium oxides and hydroxides, boehmite and gibbsite, as well as naturally occurring phyllosilicates. As biological agent, two strains of genus *Aspergillus* were selected. Both, *Aspergillus niger* and *Aspergillus clavatus* are ubiquitous and frequent in soil environment [16–18].

2. Material and methods

2.1. Microbial cultures

Strains of *Aspergillus niger* CBS 140837 and *A. clavatus* G-119, used

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in this study, are deposited in fungal collection of the Department of Mycology and Physiology, Institute of Botany at Slovak Academy of Sciences in Bratislava (Slovakia).

2.2. Aluminium bearing substrates

First two aluminium bearing mineral samples were deposited at institute of Laboratory Research on Geomaterials at Comenius University in Bratislava (Slovakia), and later characterized as gibbsite mineral phase and boehmite and aluminium oxide mixture. Third sample of naturally occurring phyllosilicates was collected from Lieskovec (Slovakia) bentonite deposit [19–21]. Bentonite is clay industrial rock with more than 50% of smectite (montmorillonite is the most common mineral from smectite group). A size fraction below 15 μm was used for all experimental treatments as well as X-ray powder diffraction and elemental analysis. Elemental analysis was performed by inductively coupled plasma-mass spectrometer. Total concentrations of studied elements (Al, Fe, Si, K, Na, Ca and Mn) in samples were determined after their decomposition by acid mixture of HF + HNO_3 + $HClO_4$ + H_2O_2 in open system at 200 °C.

2.3. Heterotrophic leaching of aluminium bearing substrates

Series of 50 ml culture Sabouraud Dextrose Broth media (HiMedia, India) autoclaved at 121 °C for 15 min were supplemented with 0.05 to 3 g of dry weight of aluminium bearing solid substrate in polyethylene cultivation flasks. The culture media supplemented with solid phase were left to settle for 8 h and subsequently inoculated with fungal spores. The 5 ml spore suspensions prepared from 7-day old *Aspergillus niger* and *A. clavatus* cultures diluted to approximately 10^6 ml^{-1} were transferred to growth medium under aseptic conditions. This was followed by static 19-day incubation at 25 °C in the dark. On various days during cultivation, the compact fungal biomass was carefully mechanically separated using sterilized pincer, then washed with distilled water and dried at 50 °C. Prior analytical determination of bioextracted aluminium, silicon and iron, the resultant culture medium was collected by filtering the medium through 0.45 μm MCE membrane filter. Dry biomass was weighted, and culture medium pH was determined (HI 8424; Hanna, Italy) before further analysis. Samples of collected culture media were also used for determination of organic acids concentration by isotachophoresis. Control experiments were carried out without either aluminium bearing solid phase or fungal inoculum following the same protocol as described previously. Arithmetic means of aluminium, silicon, iron and organic acid concentrations and their respective standard deviations from triplicate parallel experiments conducted for each experimental condition were recorded.

2.4. Analytical methods

Aluminium and other selected metals were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) by ICP spectrometer Jobin Yvon 70 Plus (France) equipped with concentric nebulizer (Meinhard, USA) and cyclonic spray chamber. Aluminium was determined using ICP-OES at line Al I 396.152 nm. Plasma power: 1000 W.

The limit of quantification (LOQ) of the method used for aluminium determination was 100 $\mu g \cdot L^{-1}$.

Isotachophoretic separation of organic acids in culture medium was performed using a ZKI 01 isotachophoretic analyser (Villa Labeco, Spišská Nová Ves, Slovak Republic) operated in the itp-itp mode. The isotachopherograms were evaluated by a software supplied with the analyser [22].

Samples X-ray characteristics were established by X-ray powder diffraction (XRD) analyses on difractometer (Bruker D8) Advance in Bragg-Brentano geometry (theta-2theta). The XRD patterns were collected using Cu Kα1 (λ Kα1 = 1.5406 Å) radiation in the 5–65 2θ range with 0.01 step size and a counting time of 1 s per step [23].

Table 1
Elemental composition of aluminium bearing substrates.

	Concentration of major components in sample ($mg \cdot g^{-1}$)						
	Si	Al	Fe	K	Ca	Na	Mn
Boehmite		465.0					
Gibbsite		269.8					
Bentonite	275.6	99.6	50.3	14.1	9.9	3.9	0.5

3. Results and discussion

Three types of natural aluminium bearing solid phases, with aluminium content ranging from 99.6 to 465 $mg.g^{-1}$ (Table 1), were characterized by X-ray diffraction analysis. The diffraction spectra (see Appendix 1) indicate that these phases consist of (i) a mixture of alumina (Al_2O_3) and boehmite (AlO(OH)) (from now on referred to as boehmite) and (ii) gibbsite mineral ($Al(OH)_3$). Sample referred from now on as bentonite (iii) is composed of quartz (SiO_2), albite (Na($AlSi_3O_8$)), opal-CT ($SiO_2 \cdot nH_2O$) and naturally occurring phyllosilicate (clay minerals) mixture consisting of montmorillonite ((Ca,-Na,K)$_x$($Al_{2.61}Fe_{1.01}Mg_{0.38}$)($Si_{7.44}Al_{0.56}$)$O_{20}$(OH).$nH_2O$) [21], illite ($K_{0.89}$($Al_{3.7}Fe_{0.1}Mg_{0.2}$)($Si_{6.4}Al_{1.6}$)$O_{20}$$(OH)_4$) and kaolinite ($Al_4Si_8O_{10}(OH)_8$).

Microbially induced extraction of aluminium from boehmite calculated from aluminium concentration in culture medium after 19-day cultivation, highlighted in Fig. 1, significantly differs in presence of *Aspergillus niger* and *A. clavatus* fungal exometabolites. While *A. clavatus* extracted lesser, up to 1.1% aluminium from boehmite phase, aluminium extraction efficiency by *A. niger* exometabolites was at least three-times higher with relatively constant efficiency value around 3.4%. Strain specific efficiency of biologically driven aluminium release from low soluble (k_{sp} 3×10^{-34}) and chemically rigid gibbsite crystal lattice was even more significant. While aluminium concentration in culture medium in treatments with *A. clavatus* was below detection limit,

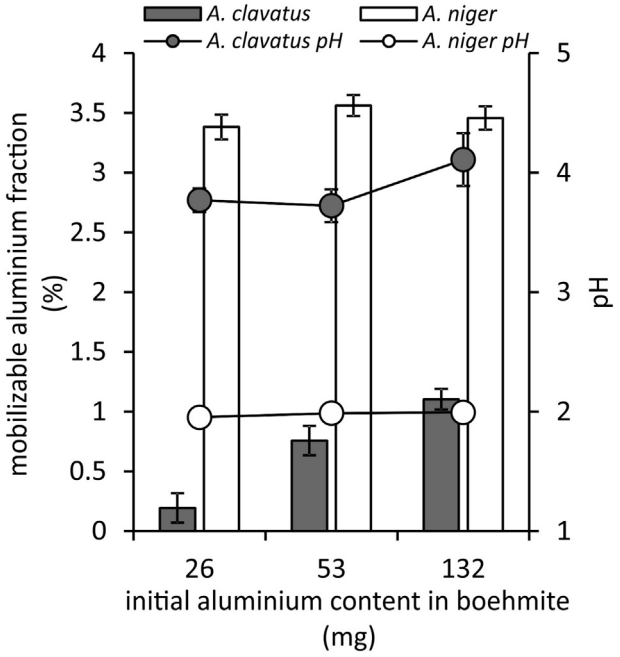

Fig. 1. Comparison of aluminium mobilization efficiency of *Aspergillus clavatus* and *A. niger* which resulted from 19-day static fungal cultivation in presence of various initial amount of boehmite.