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SHORT COMMUNICATIONS

Phosphorus Compensates Aluminium-Induced Effects on *Arthrobacter* Cells*

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At 8.13%, aluminium is the most abundant metal in the earth's crust and a key constituent in many common rocks. Nevertheless, in addition to Sr, Pb and Sb, it is one of the very few elements not essential for biological functions, which is due to its chemical properties [1] and its very strong toxicity for all forms of life [2, 3]. At a cellular level, a number of toxic effects of Al has been established [3] and interactions of Al with P and Mg often seem to be involved [4–6]. Besides pH, other physicochemical parameters like temperature, content of organic matter, and concentrations of possibly interfering constituents like Fe, Ca, Mg or phosphates also influence Al availability and thus toxicity [2, 4, 7].

Within the present investigation we investigated whether the effects of Al on growth and cell size of a typical soil-borne bacterium are caused by nutritional reasons (lack of P or Mg) and if these effects could be compensated by the addition of these two elements.

Bacteria of the genus *Arthrobacter* are gram-positive, strictly aerobic, coryneform rod with a distinct rod–coccus cycle used media (soil extract medium, SEM, at pH 7.6), culture conditions, and inoculation with *Arthrobacter* sp. PI/1-95, a typical member of the genus and analysis of bacterial cells using a Coulter® Multisizer II (Beckmann Coulter Inc., Fullerton, USA) are described elsewhere [8, 9]. Total concentrations of several elements in SEM were determined using ICP analysis (amounts given in μmol L^{-1}): Al 1.1 ± 0.01, Ca 39.6 ± 0.47, Fe 1.3 ± 0.03, Mg 0.02 ± 0.01 and P 31.8 ± 1.6. Filter sterile solutions of $AlCl_3 \cdot 6H_2O$, KH_2PO_4 and $MgCl_2$ were added to the autoclaved SEM to reach the desired final concentrations of 30, 60, and 90 μM Ai, P and Mg respectively. The phosphorus already present in SEM (see above) was taken into account so that application of 60 μmol P L^{-1} guaranteed a concentration of 90 μM total P. All experiments were conducted in three replicates. Results were analyzed with ANOVA, and LSD-tests were performed within the post hoc analysis to investigate the significance of individual groups.

Growth Inhibition through Al. The application of P to Al-free control samples had no significant effects on growth, thus indicating a sufficient P supply of *Arthrobacter* sp. PI/1-95 in SEM. At Al concentrations well within the range of those usually found in soil solutions [10], a distinct inhibition of microbial growth was established (Fig. 1), which corresponds to earlier results [9]. Al concentrations of 90 μM were high enough to keep the number of microbial cells constant at the level of the initial inoculum (5×10^4 cells ml^{-1}), thus inhibiting any detectable growth (table). Results concerning cell concentrations were confirmed by the determination of the generation times of *Arthrobacter* sp. PI/1-95 during exponential growth. Significant effects of 60 μM Al were detected, leading to a shift of t_{gen} from 2.2 h to 6.9 h (table).

Starvation. In parallel to the increasing age of the culture, all Al-free samples showed a distinct reduction in cell size, pointing to starvation reactions at the stationary phase (Fig. 2). Koch also clearly outlined that

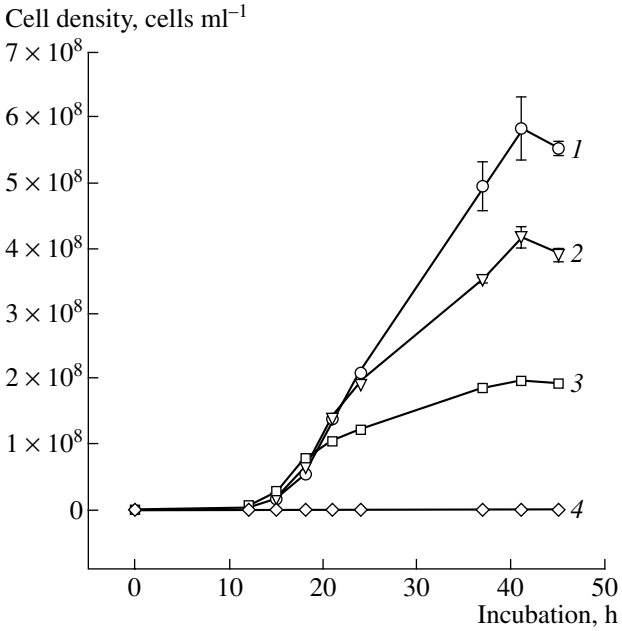

Fig. 1. Effect of different concentrations of aluminium (0, 30, 60, and 90 μM) on growth of *Arthrobacter* sp. PI/1-95 in soil extract medium (SEM) without additional P application. Symbols represent means of triplicates; whiskers give standard deviations. Inoculum, 5×10^4 cells/ml.

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Cell concentrations (CC) and generation times (t_{gen} given in hours) of *Arthrobacter* sp. PI/1-95 grown in SEM as influenced by Al (0, 30, 60 and 90 μM) and P (30, 60 and 90 μM)

Al	0	0	0	30	30	30	60	60	60	90	90	90
P-addition	0	30	60	0	30	60	0	30	60	0	30	60
P-total	30	60	90	30	60	90	30	60	90	30	60	90
CC	5.5×10^8	6.0×10^8	5.6×10^8	3.9×10^8	4.9×10^8	4.7×10^8	1.9×10^8	3.2×10^8	3.2×10^8	5.0×10^4	1.6×10^8	2.2×10^8
s.d.	1.13×10^7	3.30×10^7	4.13×10^6	1.05×10^7	3.42×10^6	5.76×10^7	2.32×10^6	2.12×10^7	3.53×10^7	3.00×10^7	1.26×10^7	1.70×10^7
h. group	ab	a	ab	de	bc	cd	g	ef	f	h	g	g
t_{gen}	2.2	2.5	2.6	2.6	2.6	2.9	6.9	3.9	3.8	n.d.*	4.2	4.4
s.d.	0.32	0.21	0.03	0.04	0.25	0.25	0.12	0.08	0.14	n.d.*	0.00	0.70
h. group	a	ab	ab	ab	ab	b	e	cd	c		cd	d

Note: Cell concentrations were measured at $t = 48$ h (stationary phase), and generation times, at $t = 18$ h (exponential phase). Values represent averages ± standard deviation; different letters indicate assignment to different homogeneous groups ($P < 0.05$).

* not determined as no growth was detectable.

bacterial cells reduce their size when nutrients become rare, a phenomenon which was attributed to a greater surface-to-volume ratio and thus to an enhanced ability for nutrient uptake [11]. This universally valid mechanism was confirmed by the Al-free samples and should be intensified if Al works as a nutritional blocker. However, cell sizes increased significantly after the application of Al (Fig. 2), which indicates that another reason for Al toxicity not connected with nutrition or starvation seems to be involved. Especially in less recent publications concerning Al effects on plants, Al toxicity was sometimes presumed to be caused by reduced phosphorus uptake and thus by a lack of P instead of a direct effect of Al per se. This could obviously be disproved for *Arthrobacter* sp. PI/1-95 with the present data.

Mg–Al interactions: Several studies established involvement of Mg in Al toxicity [5, 12]. Contrary to that, we could not find any Al-Mg interaction within the present investigation. Irrespective of the presence or lack of Al, the application of Mg had no significant effect on growth or cell size. However, Al sensitivity was shown to be a species-dependent phenomenon [13, 14] and thus the present results should be verified with various other organsims.

P–Al interactions. Addition of phosphorus did not alter cell sizes and generation times in Al-free solutions, but P had significant effects when applied together with Al (table). All effects of Al on cell numbers, generation times, and cell sizes were (at least partially) compensated by the addition of equimolar amounts of phosphorus (Fig. 2, table). Even the complete growth inhibition at 90 μM Al was nearly entirely compensated, resulting yet again in high cell numbers and low t_{gen} (table). However, differences between 0 and 90 μM Al remained significant at the highest P application rate with equimolar amounts of Al and P (90 μM each), and still the surplus P supply had no additional effect, neither on cell number nor on generation time. Similar to microbial growth, Al effects on cell size were mainly compensated via the addition of P (Fig. 2). No significant difference was detectable between samples where 30 and 60 μM P were added together with 60 μM Al, which might be due to the phosphorus already present in SEM. Again, a surplus P supply had no effect on cell size (Fig. 2), nor did complete compensation of the effects of 60 μM Al occur. Although Al–P antagonism is widely accepted, its causal interrelationship is still unclear [6]. The formation of $AlPO_4$ is sometimes held responsible for a surprisingly high Al tolerance, but, as described by Martin [1] and others, PO_4^{3-} is the dominant species at a pH higher than 11.6, whereas Al^{3+} dominates at a pH lower than 5.5. Thus, it is impossible for the two ions to meet in appreciable amounts in solutions, whereas the formation of $Al(OH)_2 \cdot H_2PO_4$ is probable at physiological pH levels (around 6). Despite the better solubility of this complex, it might also result in a reduction of Al availability and toxicity in solution [1, 15]. However, two details of our results point to the danger of underrating the complexity of Al–P interactions: on the one hand, no complete compensation of Al effects was detected, even when the concentration of P exceeded that of Al (e.g., 60 μM Al and 90 μM total P, see table and Fig. 2), and, on the other hand, no complete complexation of P occurred via the application of surplus Al (e.g., 60 μM

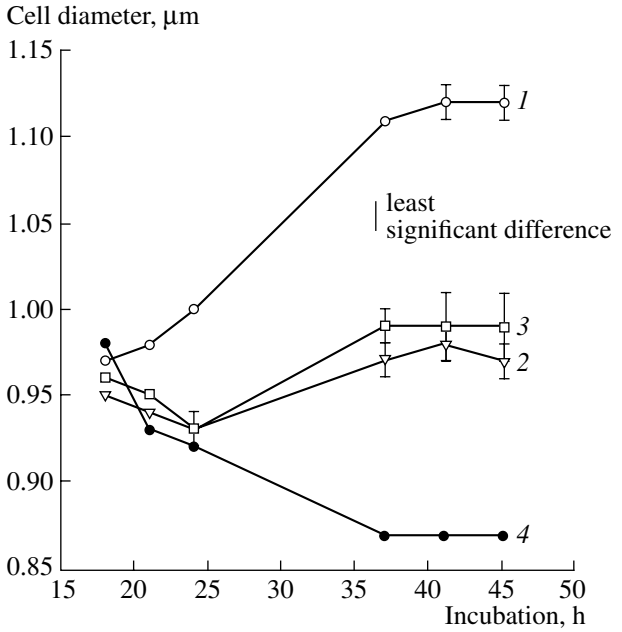

Fig. 2. Effects of aluminium (60 μM Al) and phosphorus (30, 60 and 90 μM total P) concentrations on cell size of *Arthrobacter* sp. PI/1-95 during growth. SEM: medium without Al or P addition. Symbols represent means of triplicates; whiskers give standard deviations. Further explanations see text.

Al without additional P), as growth decreased under these conditions but remained evident (Fig. 1).

CONCLUSIONS

In a prior investigation, indirect effects of Al via reduced pH (Al^{3+} acts as a cationic acid) could be outlined and a distinct influence of Al on cell size was detected [9]. This effect was attributed to a malfunction of the cell membrane caused by osmoregulative disorders, a failure which led to an increase of intracellular water [9]. As it was possible to exclude nutritional reasons for the Al-induced effects within the present investigation, a reduced bioavailability of Al (and possibly Fe) towards the plasma membrane of *Arthrobacter* sp. PI/1-95 remains the most probable reason for reduced Al toxicity in P-applied samples. An Al-induced disturbance of the membrane function was also proposed by various other authors, who found an altered composition, structure, permeability and fluidity of the membrane [14, 16]. Zatta and colleagues showed that Al accelerates iron-mediated peroxidation of membrane lipids, thus increasing oxidative damages [6]. As phosphorus forms stable complexes with both Fe and Al the oxidative activity of Al and possible interaction with iron should receive more attention in further investigations [17].

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