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Treatment of soil with L-alanine and inosine as germinants may drop the number of living cells of *Bacillus anthracis* below an infectious dose level

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Introduction: Spores of *B. anthracis* are highly resistant to adverse environmental conditions and chemical disinfectants. Reducing the soil spore count below a certain risk level can be highly desirable. The synergistic effect of spore germinants and chemical disinfectants have shown to significantly reduce spore counts in soil in laboratory based soil microcosm experiments. Here we investigated the effect of germinants alone on spore reduction.

Methods: 200 g of commercial potting soil (Universal potting mix) and Natural soil (Mature compost from Hohenheim garden wastes) was spiked with 200 ml containing spores of the Sterne strain with and without a GFP label, concentrated at $1.6x10^6$ spores/ml, and dried overnight at 40°C. 42 g of the spiked soil was treated with 20 ml of 100 mM L-Alanine and 5 mM inosine. Another 42 g of the spiked soil was treated with 20 ml of sterile deionised water as a control. Samples were incubated both at 37°C and at 4°C. 2 g from each soil type were monitored in triplicate after 1, 2, 6 hours and 1, 2, 5 and 14 days, with and without heat treatment at 65°C for 30 min.

Results: 90% reduction of heat resistant spores was found after 1 hour at 37°C incubation temperature, while the total cell count remained stable. After 1 day at 37°C, both total cell and heat resistant spore counts were reduced by ≥99.9%. At 4°C the total cell counts and heat resistance spores reduced by >1 log10 and ≥2 log10 respectively only after 14 days. In the control the number of spores remained steadily at spike level during the entire period. Microscopic observations of gfp labeled spores revealed a complete germination of a great majority of spores with single, non- replicating vegetative cells at 37°C after 24 hours and at 4°C after 14 days.

Conclusion: The results indicate that the use of germinants alone can significantly reduce the number of living *B. anthracis* in soils independently on the soil type. However, the role of indigenous soil microflora on the fate of the germinating spores and vegetative cells in these soils needs further to be investigated.

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