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# THE ENVIRONMENTALLY FRIENDLY DECONTAMINATION OF *BACILLUS ANTHRACIS* SPORES USING BACTERIOPHAGES

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*B. anthracis* is endemic in the Caucasus Infected animals are simply buried with no treatment. Result is *B. anthracis* spore contaminated land.

Biocides used for the environmental decontamination of *B. anthracis* spores:

4 hectare *B. anthracis* spore contaminated area of Gruinard Island decontaminated with 2,000,000 litres of 5% formaldehyde in sea water- estimated cost £1.8 million.

The principal chemical decontaminant used to clean up US sites contaminated with *B. anthracis* in 2001 was chlorine dioxide gas- estimated cost $1 billion.

The *B. anthracis* spore release in the Scottish borders was decontaminated using chlorine dioxide gas- estimated cost unknown.

Chemicals used for bacterial spore decontamination are toxic.

Developing an environmentally friendly approach based on bacterial spore germinants and lytic bacteriophages. *Bacillus anthracis* is a bacteria which under the right conditions converts from a chemically resistant, inert spore into its active form which is more susceptible to killing.

Inert Spores Survival in soil for years resistant to chemicals. Vegetative bacteria Have to be in this form to cause disease Susceptible to chemicals. The addition of germinants (alanine and inosine) converts chemical resistant spores into there chemical sensitive vegetative form. A comparison of the germination efficacy of alanine and inosine purchased from different sources for *B. anthracis* spores.

Identified a new, cheaper sources of L-alanine and inosine: L-alanine from Sigma (costs £0,544 per gram.), L-alanine from Bulk powders (Amazon) (costs £0,0794 per gram.), Inosine purchased from Sigma (costs £2,55 per gram.), Inosine purchased from (Amazon) (costs £0,36 per gram.).

Spore germinants enhance the activity of biocides against *B. anthracis.*

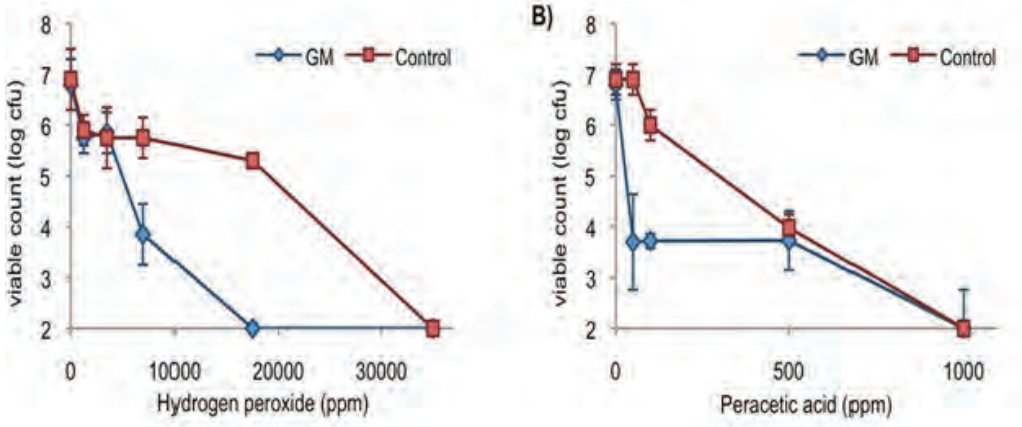


**Figure 1.** The efficacy of A) liquid hydrogen peroxide and B) per acetic acid against anthrax spores in the presence or absence of germinant mixture (GM) following a 30 minute exposure at room temperature

The ability of the spore germinants Alanine and inosine to enhance biocidal activity was determined using the EN1276 Suspension test. Conditions tested were 30 min contact time under clean conditions and at room temperature (20 $^{o}$C). Concentrations of PAA above 1000 ppm showed ≥5 log$_{10}$ reduction within 30 min.

Kinetics of *B. anthracis* recovery from Turkish soil supplemented with spores of the Sterne strain of *B. anthracis* after treatment with A( sterile PBS, B) Germinants. Microcosms were treated with germinant on Day 0 (300 mM L-alanine, 15 mM inosine) and Day +2 (500 mM L-alanine, 25 mM inosine). Solid lines represent the total count and dashed lines indicate spore counts ± SD.

The addition of germinants and Peracetic acid to *B. anthracis* spore contaminated soil reduces the spore load

Soil decontamination means are developed and tested in frames of EU funded AEDnet project. The combination of germinants with *B. anthracis* specific lytic bacteriophages as an environmentally friendly decontamination approach.

One of the project's activities is Isolation of the bacteriophages from the soil

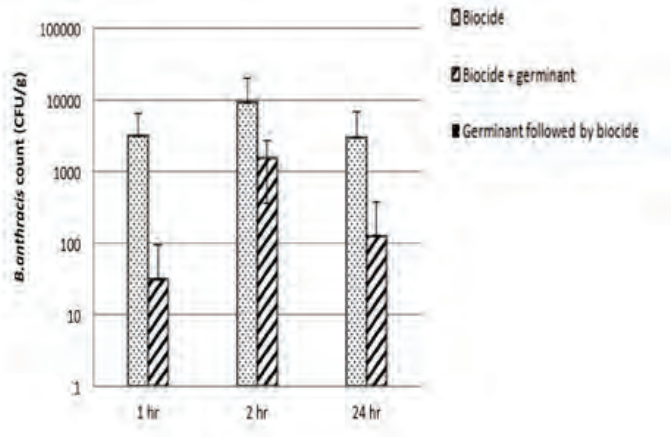


**Figure 2.** The effect of germinants on the biocidal activity of 5000 ppm PAA againts spores of the Sterne strain of *B. anthracis* suspended in soil collected from test site B. The bar indicates the number of B. anthracis remaining following treatment. Data are the mean of two replicates ±SD. The lower limit of detection was 200 cfu/g of soil per assay

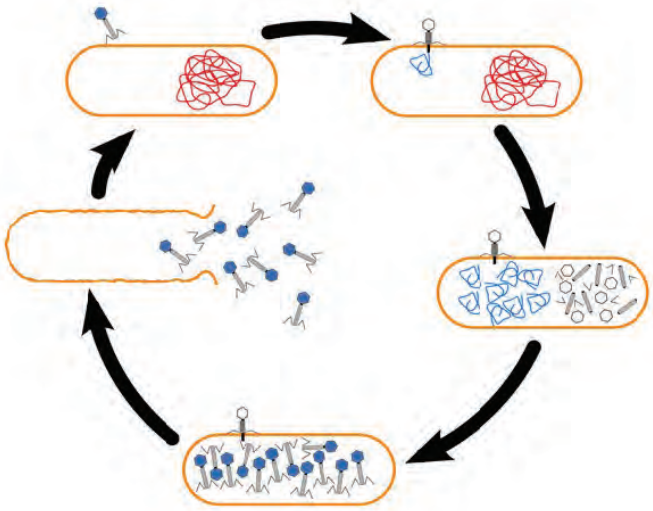

**Figure 3.** Bacteriophage life cycle Groups involved

Phage LC1H911 is able to infect all *B. anthracis* strains tested including *B. anthracis* LSU463 – a gamma phage resistant isolate/ The delivery of bacteriophages using a commercial spray device *B. anthracis.* Sterne untreated and *B. anthracis* Sterne treated with phage new compased (Fig. 4). Bacteriophages can survive delivery and retain there biological activity following delivery using a commercial spray device.

| Participant Number | Participant name | Participant short name | Country |
|---|---|---|---|
| Beneficiary 1 (coordinator) | Cardiff University | CU | UK |
| Beneficiary 2 | Istituto Zooprofilattico Sperimentale of Puglia and Basilicata | IZSPB | ITALY |
| Beneficiary 3 | Kafkas University | KAU | TURKEY |
| Beneficiary 4 | University of Hohenheim | UHOH | GERMANY |
| Beneficiary 5 | Military Institute of Hygiene and Epidemiology | WIHiE | POLAND |
| Partner 6 | National Center for Disease Control | NCDC | GEORGIA |
| Partner 7 | Ilia State University | ISU | GEORGIA |
| Partner 8 | G. Eliava Institute of BacteriophageMicrobiology and Virology | EIG | GEORGIA |
| Partner 9 | National Scientific Center Institute of Experimental and Clinical Veterinary Medicine | NSC IECVM | UKRAINE |

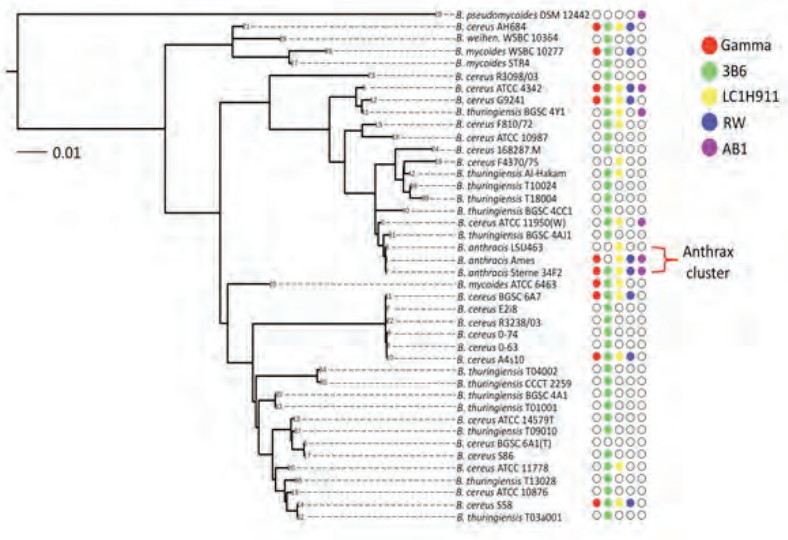


**Figure 4.** Host range specificity of bacteriophages

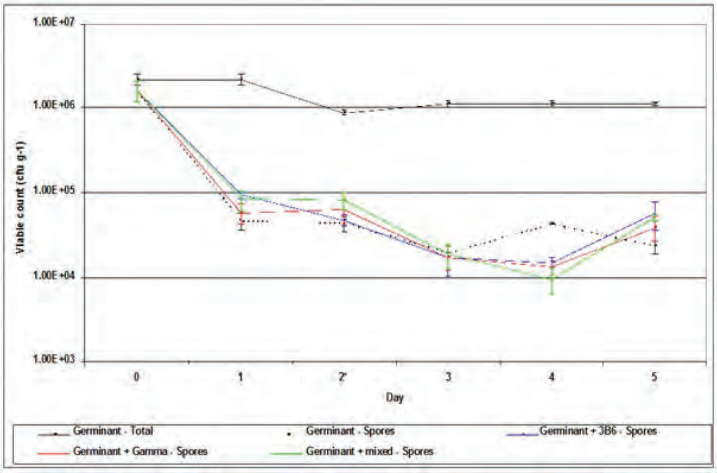


**Figure 5.** The ability of a mixture of germinants and bacteriophages to reduce the *B. anthracis* spore load of contaminated soil

Future plans including the Undertaking field decontamination studies in Turkey, Georgia, Ukraine using pre-tested mixtures of the germinants and bacteriophages.

## ЕКОЛОГІЧНО ЧИСТИЙ МЕТОД ЗНЕЗАРАЖЕННЯ СПОР *BACILLUS ANTHRACIS* З ВИКОРИСТАННЯМ БАКТЕРІОФАГІВ

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*Сибірка є ендемічним захворюванням тварин на Кавказі, що є наслідком великої кількості незнезаражених місць захована інфікованих загиблих тварин. Як результат, значні території контаміновані спорами B. anthracis. Хімічний метод знезараження є токсичним та негативно впливає на навколишнє середовище. Тому розробка екологічно чистого підходу, заснованому на застосуванні літичних бактеріофагів, є наразі дуже актуальним питанням. У статті наведені результати випробування препарату, виготовленого на основі фага LC1H911. Препарат здатний знезаражувати B. anthracis, включаючи штам LSU463, які є стійким до гамма-фага. Розроблений метод знезараження полягає у розпиленні попередньо накопичених бактеріофагів у вакцинному штамі Stern за допомогою спеціального устаткування на території скотомогильників. Встановлено, що бактеріофаги зберігають свою біологічну активність після їх потрапляння до ґрунту. У рамках міжнародного проекту планується проведення досліджень щодо знезаражування скотомогильників на території Туреччини, Грузії та України з використанням препаратів на основі бактеріофагів.*