RADIORESISTANCE OF BIODEGRADATION FUNGI AND ITS IMPORTANCE IN ESTABLISHING THE DECONTAMINATION DOSE

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The decontamination and preservation of artifacts from natural materials like paper, parchment, leather, textile, etc. is a continuous struggle against their colonization with bacteria, fungi or insects. Decontamination of cultural heritage objects by gamma radiation is a better alternative to chemical disinfection. Optimal decontamination dose selection is challenging. For this, the nature of the objects, the bioburden and the radioresistance of the contaminant microbial communities should be considered. Also, when establishing the radioresistance of a microorganism, some physical factors (irradiation support, storage temperature before irradiation) should be considered. These factors, especially the water content, influence the radioresistance, expressed as D10 value. Studies on Aspergillus niger and Trichoderma viride, which are common moulds that colonize and attack a wide range of artifacts, were carried out. The range of D10 value, influenced by the factors mentioned above, was studied. The focus of the study was the isolation of microorganisms from cultural heritage artifacts and their characterization regarding the radioresistance which further represents one of the preliminary steps in preservation/ restoration of cultural heritage artifacts.

Keywords: D10 value, radioresistance, fungi

INTRODUCTION

Fungi have a great impact on the deteriorating of the cultural heritage, due to their ability to grow in different environmental condition. They can produce decaying of very wide types of cultural heritage artifacts (paintings, textiles, paper, parchment, leather).

Remediating the mould growth in a storage place for cultural heritage objects needs to consider the following aspects: remove the mould attacked items, clean the air spaces, control the physical conditions like temperature and humidity. Appropriate conservation measures and restoration treatment to deal with fungal infections include mechanical, chemical and physical methods, which entail effects on the object itself and health hazard for human.

Several techniques have been developed for book and document conservation reducing the threat of biodeteriorating agents, such as fungi. Some of these techniques involve the use of very toxic chemicals, including ethylene oxide, which has carcinogenic properties and is banned in a number of countries, besides being expensive (Flieder *et al.*, 1994; Adamo *et al.*, 2001; Gonzales *et al.*, 2002, da Silva *et al.*, 2006). An alternative is the use of gamma radiation, a promising treatment in the preservation field (da Silva *et al.*, 2006). Studies demonstrated that the damage in mechanical-physical properties caused by gamma rays on paper was not significant. The doses of 10 kGy did not cause any negative effect on the mechanical-physical properties of the paper, even after an accelerate ageing of 12 days (Adamo *et al.*, 1998, 2001; Gonzales *et al.*, 2002).

Fungi have been successfully inactivated from different materials, such as paper, wood and soil with radiation dose ranging from 6 to 15 kGy (Hanus, 1985; Jörg *et al.*, 1992; Pointing *et al.*, 1998; McNamara *et al.*, 2003). In a Brazilian study some fungi from books could not be completely eliminated after irradiation with doses of 20kGy (Tomazello and Wiendl, 1995).

However, it is not necessary or practical to remove 100% of all mould spores from all items. Even if this goal is achieved, the result would only be temporary as new mould spores will continuously settle on items (www.moldservicesgroup.com).

The killing effect of radiation in microorganisms is generally expressed by the decimal reduction dose or D10 value (Thornley, 1963). The D10 value is the reciprocal of the slope of the exponential part of a survival curve. This value may also be obtained from the following equation:

$$D10 = \text{Radiation dose} / \log10 (X0-X) \quad (1)$$

where X0 is the initial number of organisms, and X is the number of organisms surviving the radiation dose.

Consequent treatment of artifacts, at a certain dose can be applied, using D10 value, to provide the desired reduction of microorganisms. The bioburden population is normally reported as the number of colony forming units (or CFU). In order to establish the treatment irradiation dose, aspects like the degree of degradation of the artifacts and the degree of contamination should be taken into account. Establishing the optimum dose is real challenge. The irradiation dose can be established starting from the radiation resistance of the contaminants.

The study aims to establish how the environmental condition like humidity (common condition in the storage place) influence the D10 value and as a consequence, the irradiation dose.

MATERIALS AND METHODS

The present study was focused on the determination of the D10 value of Aspergillus niger and Trichoderma viride, which are common agents of biodegradation of the cultural heritage objects (paper and cellulose textiles) (Mesquita *et al.*, 2009; Meier and Petersen, 2006; Blyskal, 2009; Pangallo *et al.*, 2009; Sterflinger, 2010, Chirila *et al.*, 2014). The influence of humidity on D10 value was than analyzed.

Spores from a biodegradated Orthodox religious book (Fig. 1), from early 19th century “Strastnic”, Blaj, Romania were harvested using wet swabbing method.

Figure 1. Orthodox religious book Strastnic, Blaj, 1817

The spores were directly transferred on Sabourand agar in Petri dish, incubated at 220C and purified by further subcultures on the same agar medium.

Two types of mould were identified based on the morphological features as Aspergillus niger (Fig. 2) and Trichoderma viride (Fig. 3), using microscopy techniques (Zeiss Axio Imager D1m). Trichoderma viride was also characterized by Scanning Electron Microscopy (SEM) (Quanta 200-FEI) (Fig. 4).

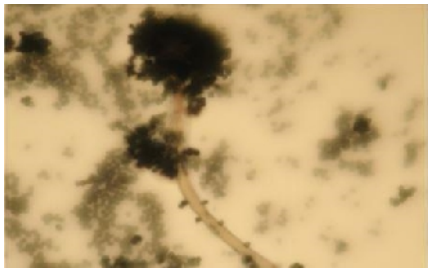

Figure 2. Aspergillus niger (400X)

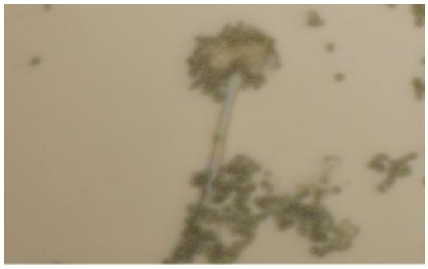

Figure 3. Trichoderma viride (400X)

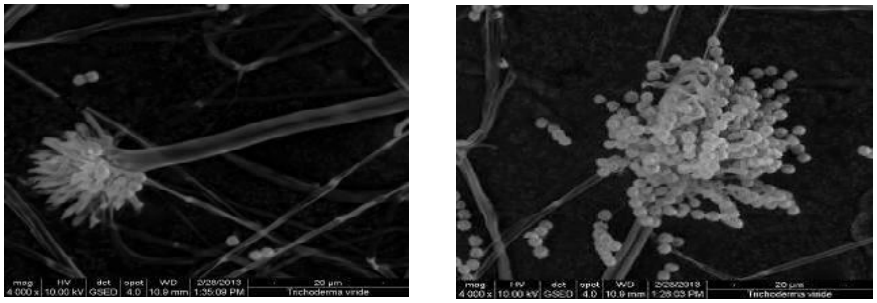

Figure 4. Trichoderma viride: conidiophores with phialides

For both species, pure cultures were made on Sabourand agar, in Erlemeyer flasks and incubated at 22.5°C for 7 days until abundant sporulation was obtained. After sporulation, the spores were harvested by flooding the flasks with 10 ml of Sodium Chloride Peptone Broth with 0.3% (w/v) Tween 80 (APS 0.3%Tw) and by shaking with glass beads. The washings procedure was repeated 3 times. The collected suspension containing the spores was than centrifuged 10 minutes at 3500 rpm, discarding the supernatant each time. At the end the spores were resuspended in APS 0.3%Tw and subjected to vortex in 3 session of 30 seconds each, for a better dispersion. The degree of dispersion of the spores was checked by phase contrast microscopy (optical microscope Zeiss Axio Imager D1m). The concentration of the spore suspension was confirmed by plating dilutions. The final concentration of the suspension was 8x108 CFU/ ml in case of A. niger and 5x108 CFU/ ml in case of T. viride.

The gamma irradiation was made on paper, in order to simulate the natural support of the cultural heritage artifact.

Sterile pieces of paper of 1 cm^2 were evenly contaminated with 0.1 ml of suspension. Aliquots of 0.02 ml were distributed in 5 points of the paper, in order to prevent clustering of spores.

The pieces of paper were then dried in sterile conditions and put into plastic cap tubes. Three tubes were irradiated for each of the following doses 0.25 kGy; 0.5 kGy; 1 kGy; 1.5 kGy; 2 kGy; 3 kGy. The spores were removed by shaking each piece of paper in APS 0.3% Tw diluent, in a stomacher (Bag Mixer), at 300 rpm, for 5 minutes, after 10 minutes of soaking for each. The recovery percent from total matrices mass was not quantified but it was assumed to be equal among all replicates and dilutions. The decimal reduction was calculated by comparison with the recovery from the non-irradiated sample. The surviving microorganisms were enumerated by serial dilutions immediately after irradiation, using Sabourand agar. The regression slope and the D10 value were calculated.

The same experiment was performed two weeks later, for other two sets of identical samples. During this interval, one set of paper was kept in environmental humidity, and the other was kept in humid atmosphere (> 85%) at 28°C, in order to simulate the humid storage conditions for an artifact.

RESULTS AND DISCUSSION

The D10 values obtained in different experimental conditions of time and humidity were compared in case of both fungi. The two fungi have a very different behavior in similar conditions (humidity).

As seen in the table and figures below (Table 1 and Fig. 5 - 6), in case of A. niger, D10 value decreased after two weeks of storage in humidity conditions from 0.86 kGy to 0.51 kGy. Also, after two weeks of storage at environmental humidity, the D10 decrease to 0.66 kGy.

The decreasing trend of D10 value mantained also for Trichoderma viride although the diferences were not so remarcable. In fortheen days of storage, the radioresistance ranged between 0.50 kGy and 0.45 kGy.

Comparing the radioresistance of the two fungi, it can be concluded that A. niger is more resistant to gamma radiation than T. viride. Also, time and humidity have a greater downward influence of the D10 value for A. niger, while decreasing is almost insignificant for D10 value in case of T. viride.

Table 1. D10 value for A. niger and T. viride

Microorganism / Conditions	Paper, 28°C, 2 days	Paper, 28°C, 14 days	Paper, 28°C, 14 days, humidity
Aspergillus niger	0.86 kGy	0.67 kGy	0.51 kGy
Trichoderma viride	0.50 kGy	0.47 kGy	0.45 kGy

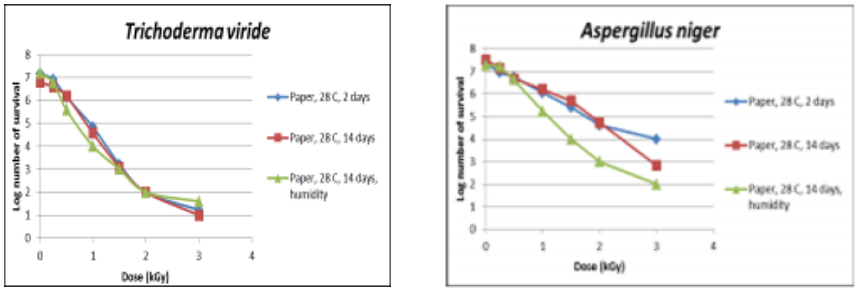

Figure 5. Survival of T. viride and A. niger after gamma irradiation

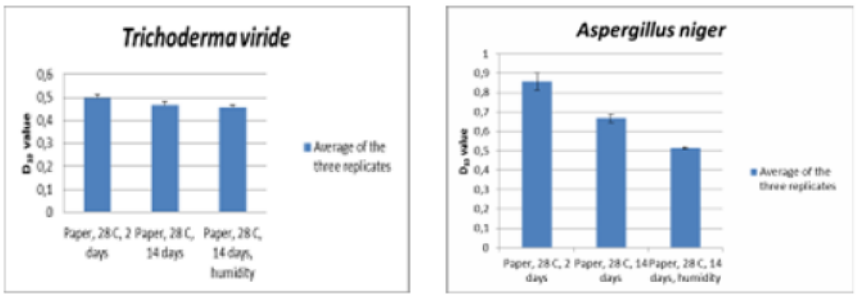

Figure 6. D10 values for T.viride and A. niger after gamma irradiation treatment

CONCLUSION

Taking into account the decreasing of D10 in time and in humidity conditions, we can draw the conclusion that these factors do not influence the radioresistance in a way that the increasing of the decontamination dose should be considered. We emphasize that the decreasing was obtained within two weeks of exposing the spores at high humidity condition and at environmental humidity.

Further investigations are needed to study the degradation and the behavior of different fungi species recovered from the cultural heritage artifacts under gamma irradiation exposure.

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