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Short Communication

Radiosensitivity of fungi isolated from waterlogged archaeological wood

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Waterlogged archaeological timbers of the *Mary Rose* were shown to support a range of fungi, including marine soft rot fungi. These isolates, and other wood degrading fungi, were inactivated by gamma irradiation at doses of 3.1–15.0 kGy. No clear pattern of variation in radioresistance appeared between the Ascomycota, Basidiomycota and Deuteromycota. Terrestrial fungi were generally more resistant than marine fungi. Little variation in radioresistance was observed between vegetative hyphae and sporulating cultures/fruiting bodies. Sublethal doses resulted in reduced viability in some species. Gamma irradiation is suggested as a possible means of controlling fungal colonisation of waterlogged archaeological wood.

Key Words——archaeological wood; fungal radiosensitivity; passive conservation.

Many fungi with soft rot and white rot capability have been isolated from aerobic marine environments (Hyde et al., 1987; Jones, 1987). Such organisms, together with marine borers and bacteria can cause significant decay to archaeologically important timbers exposed in these environments (Blanchette et al., 1990). Despite this many well preserved wooden wrecks such as the *Wasa* (Barkman, 1975); Bremen Cog (Hoffmann, 1981); *Mary Rose* (Rule, 1983); and the Kinneret Boat (Cohen, 1991) have been excavated from marine environments. Their preservation is attributed to burial in anaerobic sediments unable to support aggressive wood decay organisms. However excavation of such timbers and re-exposure to aerobic conditions on the seabed and in storage has been shown to result in significant biodeterioration, with marine soft rot fungi most frequently encountered (Mouzouras et al., 1986). This probably reflects the waterlogged nature of such timbers which are unable to support terrestrial brown rot and white rot fungi.

The storage or 'passive conservation' phase for excavated timbers is often long term due to delays in the onset of an active conservation treatment to stabilise and dry the wood. This can be due to financial restrictions; the volume of wood recovered; or the lack of an appropriate active conservation method. Commonly used passive conservation methods are: chilled freshwater sprays, storage in water tanks and wrapping in polyethylene; with or without the use of biocides (Dawson et al., 1982).

Excavation of the Tudor warship *Mary Rose* was commenced by divers in 1971 with the recovery of small artefacts including some wood, the main hull section was raised in 1982 (Rule, 1983). Active conservation of the *Mary Rose* hull commenced in 1994 after more than ten years of storage in a chilled freshwater spray system. Some wooden artefacts have been conserved and are now on public display, however many ship timbers remain stored hermetically sealed in volumetric barrier foil (T1-19V, Branopac Ltd, UK) while awaiting active conservation treatment. Here growth of wood degrading marine fungi continues despite the use of biocides (Jones et al.,1986; Mouzouras et al., 1986; Jones and Jones, 1993). The failure of currently used passive conservation systems to prevent microbial growth has also been reported by other authors (Van der Heide, 1972; Barkman, 1975; Baynes-Cope, 1975; Dawson et al., 1982; Jesperssen, 1985).

The widespread use of gamma irradiation to sterilise foodstuffs, stored grain, cosmetics, medical instruments and archival papers has prompted interest in this method for disinfesting waterlogged archaeological wood. Timbers could be wrapped and sterilised, and then be placed in storage without the threat of biodeterioration. The gamma irradiation dose required for medical and cosmetic product sterilisation in the UK is 25 kGy (Anon, 1994), this is required for pathogen inactivation. A review of literature on gamma irradiation in the food industry suggests a dose of 10 kGy will inactivate most terrestrial moulds such as *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Fusarium*, *Mucor* and *Penicillium* species (Pointing, 1995). Doses of up to 20 kGy are required for food spoilage yeasts such as *Candida* and *Saccharomyces* (Silliker, 1980; Frazier, 1988). Data on the radiosensitivity of specific wood decay fungi is limited to gammasterilisa-

Table 1. Fungi isolated from wrapped timbers of the *Mary Rose*.

Marine fungi	Terrestrial fungi
Ceriosporopsis halima	*Chaetomium globosum*
Cirrenalia macrocephala	*Gliomastix murorum*
Corollospora maritima	*Penicillium* sp.
Dictyosporium toruloides	*Stachybotrys chartarum*
Digitatispora marina	*Trichoderma viride*
Trichocladium alopallonellum	
Lulworthia sp.	
Monodictys pelagica	
Neriospora cristata	
Nia vibrissa	
Trichocladium achrasporum	
Zalerion maritimum	

tion of archival papers: selected terrestrial moulds, ascomycetes and vegetative hyphae of basidiomycetes are inactivated by a dose of 5–10 kGy, with the deuteromycetes generally displaying greater radioresistance than ascomycetes and basidiomycetes (PavonFlores, 1975; Urban, 1983; Horakova and Frantisek, 1984; Butterfield, 1987).

Fungi were isolated from wrapped timbers of the *Mary Rose* and axenic cultures grown on agar slopes to encourage sporulation and identification (Table 1). Most isolates from *Mary Rose* timbers displayed slow growth rates with no evidence of sporulation, no attempt was made to identify these isolates. Additional isolates were obtained from the University of Portsmouth Culture Collection. Vegetative and sporulating cultures on agar media (7–21 d at 20°C) and intact fruiting structures (whole ascocarps and basidiocarps) were then irradiated using a ^{60}Co gamma radiation source (Isotron plc, Swindon, UK). Plugs of agar containing irradiated fungal hyphae/spores, and spore suspensions obtained from irradiated fruiting structures (suspended in 1/4 ringers) were transferred to non-irradiated agar media and survival at 20°C recorded over a 21 d period. Survival in 10 replicates per test fungus was recorded by colony formation, since it is suggested that the germination mechanism and young mycelia are more radioresistant than colony formation (Grecz, 1983; Keresztses et al., 1985; Jay, 1986). The minimum gamma irradiation dose for 100% inactivation was then calculated from the dose-response curve of each fungus.

The radiosensitivity of test fungi is presented in Table 2. From this data some observations can be made regarding fungal radiosensitivity. The absorbed dose range necessary to kill all fungal species tested is 3.1–15 kGy. Terrestrial fungi generally displayed a greater radioresistance than marine fungi, although the most resistant organism in this study was the marine fungus *Corollospora fusca* Nakagiri et Tokura. Perithecia of *C. fusca* showed greater radioresistance than vegetative hyphae, however this trend was not common to other test species. No variation in radioresistance was observed between vegetative hyphae and sporulating cultures/fruiting bodies for any other test species. No clear pattern of variations in resistance appears between ascomycetes, basidiomycetes and deuteromycetes. It was observed that sub-lethal doses of gamma radiation resulted in reduced viability in some of the more resistant species tested, notably *Aureobasidium pullulans* (de Bary) Arnaud; *Chaetomium globosum* Kunze: Fries; *C. fusca; Leptographium procerum* (W. B. Kendr.) M. J. Wingf.; *Ophiostoma piliferum* (Fries: Fries) H. et P. Sydow and *Trichoderma viride* Persoon: Fries. These were also the most dematiaceous test organisms used in this study.

This is the first report of radiosensitivity data for marine fungi. None of the isolates originating from waterlogged archaeological timbers survived a gamma irradiation dose greater than 5.05 kGy, this reflects the relatively poor radioresistance of marine fungi compared to terrestrial species. Such a dose would be unlikely to ensure sterility of a wrapped timber, however, since common aerial contaminants alighting on a timber prior to wrapping for irradiation may survive such a low dose. Some

Table 2. Radiosensitivity of selected fungi.

Species	Minimum gamma irradiation dose (kGy) for 100% inactivation	
	Vegetative hyphae	Intact fruiting structures
Marine fungi		
Ceriosporopsis halima	3.1	N/T[a]
Cirrenalia sp.	3.1	N/T
Corollospora fusca	5.0	15.0
Corollospora maritima	3.1	3.1
Dictyosporium toruloides	5.05	N/T
Digitatispora marina	3.1	N/T
Trichocladium alopallonellum	3.1	N/T
Lulworthia sp.	3.1	N/T
Monodictys pelagica	3.1	3.1
Neriospora cristata	3.1	N/T
Nia vibrissa	3.1	3.1[b]
Trichocladium achrasporum	3.1	N/T
Zalerion maritimum	3.1	3.1
Terrestrial fungi		
Aspergillus niger	6.0	6.0
Aureobasidium pullulans	13.05	13.05
Chaetomium globosum	6.0	6.0
Coniophora puteana	3.1	N/T
Gliomastix murorum	3.2	3.2
Lentinus lepideus	2.7	N/T
Leptographium procerum	10.1	10.1
Ophiostoma piliferum	5.6	5.6
Penicillium notatum	6.0	6.0
Stachybotrys chartarum	3.1	3.1
Trichoderma viride	10.1	10.1
Ulocladium sp.	10.1	10.1

a) Not tested; b) Young and mature basidiocarps.

of the test fungi used in this study are known not to colonise waterlogged wood, noteably the terrestrial basidiomycetes *Coniophora puteana* (Schumacher: Fries) Karsten and *Lentinus lepideus* Fries (Findlay, 1975). However, all other test species have the physiological ability to colonise waterlogged timbers, though not all are known wood degraders. These non-lignolytic organisms are still regarded by archaeological conservators as biodeteriogens since fungal growth on the surface of timbers may interfere with the examination and subsequent conservation of the timbers. The dose range required to inactivate axenic cultures of terrestrial fungi capable of waterlogged wood colonisation, and not previously isolated from Mary Rose timbers is 5.6–13.05 kGy.

Although marine fungi generally displayed greater sensitivity to irradiation than terrestrial species, the most resistant organism tested was the marine fungus *C. fusca* (not previously isolated from Mary Rose timbers). Ascospores required a dose of 15 kGy for inactivation and, this high radioresistance displayed by *C. fusca* perithecia was atypical of marine fungi tested in this study. However perithecia of *Corollospora maritima* Werdermann were inactivated at the lowest dose applied of 3.1 kGy. Resistance may be due to melanisation, since a possible role for melanin in microbial radioresistance has been suggested (Durrell, 1964; Somer, 1973; Keresztses et al., 1985). *C. fusca* has been shown to have heavily melanised spores and the thick peridial wall is itself highly melanised and carbonaceous in texture (Mckeown et al., 1996).

Research suggests sorption, strength and chemical properties of angiosperm and gymnosperm wood will be unaffected by gamma irradiation doses necessary to kill fungal biodeteriogens, wrapping materials also appear stable at such doses (Pointing, 1995). Once irradiated, the treated timbers require no specialised storage environment, and are completely safe to handle with no residual radioactivity or toxic residues. Gamma irradiation is therefore of great interest to archaeological conservators as a potential means of passive conservation without the threat of fungal wood decay.

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