Nematodes Rotifers Tardigrades and Diatoms as Vehicles for the Panspermic Transfer of Microbes

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Abstract: Nematodes, rotifers and tardigrades are extremotolerant invertebrates which can survive long periods of stasis brought about by extreme drying and cold. They can also resist the effects of UV radiation, and as result could act as vehicles for the panspermic transfer of microorganisms. Here we show that NRT contain a variety of bacteria and fungi within their bodies in which environment they could be protected from the extremes of the space and released into new cosmic environments. Diatoms were also shown to contain a viable alga and *Escherichia coli* and so could also act as panspermic vehicles for the transfer of these and perhaps other, microbes through space. Although not studied here, NRT, and possibly diatoms, also carry protozoa and viruses within their bodies and could act as vehicles for the panspermic transfer of an even wider range of microbes than shown here.
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1. Introduction

There has been considerable speculation concerning the possibility that microbes might be transferred to space in rocks following Earth impact events (Wainwright *et al*. 2009). Less emphasis however, has been placed on the similar transfer of microorganisms within plants and invertebrates, although Tepfer and Leach (2006) have suggested that plant seeds might act as vectors for the transfer of life though space, a possibility which also applies to microorganisms which seeds are known to internally carry. The possibility also exists that nematodes, rotifers, tardigrades and diatoms (NRTD) contain microorganisms which they could carry into space following an Earth impact event.

Nematodes, rotifers and tardigrades (NRT) are extremotolerant invertebrates which live in a variety of habits on earth including marine and fresh waters, soil, moss, and some extreme environments including hot springs and a submarine vents. All three organisms are able to survive long periods of desiccation and freezing (Crowe and Crowe, 1992, Gladyshev and Meselson, 2008 Jonsson et al., 2005, Lee, 2002, Ruttner-Kolisko, 1974 Treonis and Wall, 2005, Tunnacliffe and Lapinski, 2003, Wharton et al., 2003, Kinchin,1994,). It has also been suggested that tardigrades may be able to survive the extremes of space (Jonsson, 2007,Jonsson et al.2008, Mayer, 2007,Persson et al., 2011), a potential which may also apply to nematodes and rotifers. All three of these organisms feed on microbes including algae, bacteria and viruses and carry microorganism internally and on their surfaces (Benoit et al. 2000, Kinchin, 1994, Lee, 2002,Vanderkerckhove et al. 2000).

It has also been pointed out that diatoms could act as vehicles for the panspermic transfer of the algal protoplast which they contain (Hoover et al., 2000), and since diatoms are also known to contain bacteria (Schmid,2000) they might also act as protective "vehicles" for the transfer of prokaryotes through space. The living algal component of diatoms is protected by a silicon frustule which could act as a hard, protective vehicle for the panspermic transfer of both the protoplast and any other microbes carried inside the diatom shell.

It is likely that individual NRTD (unlike other biological entities) could survive the rigours of many space environments and thereby provide a means of transferring viable microbes from Earth to space. On reaching suitable environments, NRT could be resuscitated from a dormant state and release the microbes they carry within them, either following defecation or cellular degradation. Such protection of microbes could only be effective occur if they exist within NRTD, since those occurring on the surface of the invertebrates would be exposed to sterilizing UV-C radiation.

It has been suggested that, in the distant past, life may have been transferred between planets (Clarke, 2001) most notably from Earth to Mars and or *vice versa, as* well as between other planets (Clarke, 2001). If such life forms included evolutionary developed organism, such as NRTD, then the microorganisms contained within them would have gained an added degree of protection which would have provided them with an advantage in surviving and then colonizing their new-found environments.

The aim of the work reported here use experimental protocol to unequivocally demonstrate that bacteria and fungi exist within the bodies of NRTD and also to determine if algae and bacteria grow from surface-sterilized, crushed diatoms. The results are then discussed in relation to the possibility that NRTD might act as vehicles for the panspermic transfer of microorganism in general.

2. Material and Methods

The organism used in this study were obtained from commercial suppliers; the slug parasitic nematode (*Phasmarhabditis hermaphrodita*) from Nemaslug, Becker Underwood, UK, rotifers (*Brachionus pictalis*) from Reefphyto, UK tardigrades (*Macrobioticus*) obtained from Sciento, UK and a mixed diatom culture also from Sciento Ltd UK. In order to make sure that the organism had the opportunity to consume bacteria and fungi they were then left overnight in a tap water extract of soil (10g soil: 100ml water, filtered through Whatman No1 filter paper). The organisms were then concentrated by filtration through a 0.2 micropore filter (Millipore). A small (undetermined) quantity of the concentrated cells were then transferred to boiling, autoclave -sterilized isolation medium tubes in glass tubes, which contained glass ballatini beads(3mm) and then allowed to cool at room temperature. All experiments were repeated on three occasions.

Incubation and maceration of NRT

The tubes containing the individual surface sterilized organisms in the medium for bacterial or fungal growth respectively were incubated at 37^0C (for bacteria) or 25^0 C (for fungi) for 7days without shaking. An algal growth medium was used for diatoms (K10, Algal Growth Medium, and Sciento, UK) and here, tubes were exposed at 25^oC to white light over a 14 day incubation period. After this period, any tubes in which visible bacterial or fungal growth appeared were discarded. Tubes containing no microbial growth were then aggressively mixed using a whirlimixer, such that the glass ballotini beads macerated the animals. The tubes were then re-incubated at the relevant temperatures for bacterial or fungal growth and samples of the medium contains bacterial or fungal growth after maceration were transferred agar versions of the liquid isolation medium (i.e. Nutrient Agar, Oxoid or Czapek Dox Agar, Oxoid). Any obviously morphologically different bacterial or fungal colonies which subsequently grew were then transferred to fresh solid media and singles colonies of individual isolates were obtained. Single colonies of the isolates were obtained and these were identified; bacteria were identified using a combination of 16SrRNA and classical identification techniques; fungal isolates were identified using classical approaches based on morphology.

Uptake by NRT of the bacterium *Serratia marcescens* from culture medium

Nematodes, rotifers and tardigrades were transferred to tubes containing a small amount of a culture of the bacterium *Serratia marcescens* (an overnight culture grown in nutrient broth at 37^0C and diluted until the culture was no longer turbid). The organisms were then removed from the *Serratia* culture and dried on silica glass coverslip as described above. The dried animals were then exposed to UV-C and then macerated as described above. The presence of *Serratia marcescens* in the medium, following maceration (but not prior to maceration) was confirmed on the basis of the isolate's morphology, red pigment formation on Nutrient Agar, Gram stain(Gram negative) and on its ability to grow on the erythritol-based medium described by Slotnick and Dougherty (1972), which is selective for *Serratia marcescens*.

3. Results and Discussion

The experimental approach used here was designed to isolate bacteria and fungi from the inside of surface sterilized NRTD. A wide range of bacteria were isolated from all of the organisms although; fungi were also isolated from nematodes and rotifers, but not from tardigrades and diatoms (Table 1). An algae, which is presumed to be the parent species (although it could have been a separate species carried within the frustule) was also isolated also from the mixed diatoms. The isolated bacteria include a range of different genera, including species of *Bacillus* (Table1), the spores of which are themselves highly resistant to a variety of environmental extremes, including heat tolerance and resistance to UV and drying which allows them to survive in extreme environments on Earth and potentially also space Nicholson et al., (2000). *Microbacterium halotolerans*, a bacterium which is capable of surviving wide ranges of salt concentrations, provides another example of the ability of rotifers to potentially transport extremophilic bacteria into space. Clearly, NRTD

carry internally a variety of microbes were isolated form, and are therefore present within, NRT and could be therefore carried by these animals from Earth to space following impact events and so protected from the extremes of the space environment, particularly from high levels of UVC. For such transmission from Earth to space, it is essential that NRT occur within rocks or other solid materials which can withstand the initial impact event and subsequent transfer into space. It is noteworthy therefore that nematodes have been isolated from the deep (0.9-3.6 km) fracture water in deep mines in South Africa (Borgoni *et al*., 2011) and it is probable that these organisms (possibly also rotifers and tardigrades) live within hairline rock fissures where they graze the bacterial flora, the lithopanspermic transfer of these organisms should therefore be possible.

Table 1. Bacteria and fungi isolated from the inside of nematodes, rotifers and tardigrades

Invertebrate source	**Bacterial Species**	**Fungal Species**
Nematodes	*Bacillus simplex*	*Penicillium* Sp
	Pseudomonas stutzeri	*Verticillium lateritium*
	Staphylococcus saprophyticus	
Rotifers	*Bacillus amyloliquifaciens*	*Aspergillus oryzae*
	B.cereus	*Mucor racemosus*
	Microbacterium halotolerans	
Tardigrades	*Pseudomonas stutzeri*	None
	Bacillus licheniformis	
Diatoms	*Escherichia coli*	None

The demonstration here of the presence of *Escherichia coli* inside surface –sterilized diatoms agrees with the report by Schmid (2000) who found that diatoms contain unspecified, Gram native bacteria. One possible disadvantage of diatoms as a panspermic vehicle for microbes is the fact that the diatom frustules are made up of silica, a substance which is generally UV–C permeable which could kill any microorganisms carried inside the frustule. The frustules of marine diatoms are however, known to contain UV absorbing, mycosporine-like amino acids (Ingalls *et al*., 2010) which would help reduce UVC penetration. Microbes living inside diatoms at the centre of a clump of these organisms would also be exposed to lower levels of damaging UV radiation and would be completely protected if the individual diatoms or clumps were covered in a UVC-protective layer of soil, mud, silt or slime.

Nematodes, rotifers and tardigrades are extremely resistant animals and can withstand long periods of extreme cold and drying (Kinchin, 1994, Lee, 2002. Ruttner-Kolisko, 1974) and are therefore likely to be particular adept at surviving the rigours of the space environment where, on finding a suitable environment they could be resuscitated and release the microbial content which they carry within them.

Our aim here was not to isolate all of the bacteria and fungi which might be present inside NRT and diatoms (NRTD), but to show that these microbes are present and could be carried by NRTD. Other microorganism, including viruses, are likely to be found within NRTD diatoms depending on the environment in which they grow and the culture conditions used to isolate them; for example NRT may carry anaerobic bacteria which could grow in suitable anaerobic space environments. Nematodes, rotifers and tardigrades growing in extreme environments are also likely to carry chemoautotrophic bacteria and a wide range of extremophilic microbes.

The ability of NRT to acquire bacteria internally by feeding was confirmed by studies using the bacterium *Serratia marcescens*, which was shown to be present inside NRT after they had been exposed to a pure culture of this bacterium. This experiment confirms that internally carried bacteria remain viable and are not destroyed by the gut enzymes of the organisms. Such internally-carried microbes are likely to remain viable when NRTD enter a state of stasis brought about, for example, by extreme cold or drying. As well as bacteria and fungi, algae and protozoa could be carried within NRTD (rotifers are and nematodes are known to carry phytoplankton etc); animal, human and plant pathogens may also be carried internally by NRTD.

There a number of ways in which microorganisms could be carried and protected from the rigours of the space environment following Earth impact events, for example by being carried within other animals and plants, as well as soil; the advantage of NRTD as a panspermic carriers of life is that are relatively small and could therefore be carried long distance and the likelihood of burning up on re-entry into an atmosphere is reduced. On arrival in a suitable space environment, NRTD could be resuscitated, although such resuscitation is not essential as the microorganisms which they carry internally would be released from the dead bodies of NRTD on cellular breakdown. Finally, some of the microbes present within NRTD will be expected to carry genes which are foreign to them and which could also be protected from space extremes and carried to extraterrestrial environments.

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