NO ANTIFREEZE, NO PROBLEM

AN INVERTEBRATE SHOWS CRYOBIOSIS WITHOUT CRYOPROTECTANTS

By

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ABSTRACT

Bdelloid rotifers are asexual, microscopic invertebrates that exhibit the ability to survive the most extreme conditions. Work in our lab showed that bdelloids could survive freezing at -80°C and - 195°C, with the survival rates ranging from 10-94% at -80°C. This poses an interesting phenomenon because current research has been unable to identify any osmoprotectant or cryoprotectant molecules that would aid this process. The goal of the research presented here was to examine any genotypic differences in freezing survival both among and within species of bdelloids. Statistical analyses revealed no significant differences in freezing survival rates in both cases.

INTRODUCTION

Bdelloids rotifers are a remarkable group of freshwater microscopic invertebrates. They are capable of being dessicated, during which their DNA becomes fragmented, but recover fully and repair the DNA damage upon rehydration. Additionally, they represent one of the few classes of invertebrates that can survive freezing. The ability of bdelloids to withstand freezing is interesting because they are found all over the world, including Antarctica (Sohlenius et al. 2004). Previous work in our lab showed that bdelloids could survive freezing at -80°C and -195°C, even if frozen for up to 6 years in some cases. However, no survival was found when frozen at -20°C. The highest survival rates were observed at -80°C and ranged from 10% to 94% depending on the species!

In animals that undergo freeze tolerance, the costs of not being able to suvive these conditions are extremely high. As water freezes, ice crystals can form which can cause cell damage by rupturing cell membranes or other organelles. Additionally, as the extracellular fluid in an organism freezes, it forms a hypertonic solution which pulls water from the cell cytoplasm via osmosis. This can lead to irreversible membrane collapse. Many organisms avoid these events by employing special biomolecules called cryoprotectants. These biomolecules minimize cell shrinkage, lower the freezing point of extracellular fluids and stabilize the cell membrane during freezing (Storey and Storey 2006).

Bdelloids present a unique case because current evidence does not indicate that they utilize of any of the cryoprotectants commonly employed by other microscopic invertebrates. One study presents strong indirect evidence that a protein, late embryogenesis abundant protein (LEA), may be involved in minimizing cell damage during dessication, and similar effects may

be related to freezing (Tunnacliffe et al. 2005). Thus, while cryobiosis has been observed in rotifers, the mechanisms which facilitate the process have not been identified. The focus of the research presented here has been to examine the genotypic effects on cryobiosis in various species of bdelloids.

METHODS AND MATERIALS

Organisms

Bdelloid rotifers of clones WPr1.1 (*Philodina roseola*), Snail 1.2 (*Philodina* sp.), Daly1.5 (*Philodina* sp.), and Adir1.2 (*Adineta ricciae*) were grown in distilled water and fed algae (*Chlamydomonas reinhardtii*) and bacteria (*Escherichia coli*). It should be noted that the Snail 1.2 and Daly 1.5 have not been identified as phenotypic species, but rather have been assigned as evolutionary species and given a preliminary species designation and number.

Freezing

Approximately 1mL of rotifer cultures were pipetted into individual 1.5mL microfuge tubes and frozen at -80°C.

Thawing

The samples were thawed for 1.5 hours in a beaker of water equilibrated in a 37°C incubator. The tubes were then emptied into culture dishes and washed twice with carbonated water to remove any animals that might have clung to the inside of the tube. The total number of individuals and live individuals were counted in each sample. Dilute solutions of bacteria and algae were then added to the culture dishes. Each sample was then monitored for a 3 day period and any additional live animals were counted.

Experiment 1: Among species differences

One sample each of WPr 1.1, Snail 1.2, Daly 1.5 and Adir 1.2 was frozen and thawed as described, then measured for survival.

Experiment 2: Within species differences

12 survivors from a previously frozen and thawed Adir 1.2 sample were isolated into individual culture dishes and measured over a 1-week period for population growth. 6 subclones were selected and frozen based on growth rate: the 2 fastest, the 2 most intermediate and the 2 slowest. These samples were subsequently thawed and measured for survival.

RESULTS

Using these techniques, I was able to obtain live rotifers after freezing at -80°C. This confirms the initial observation that bdelloid rotifers can successfully be reanimated after being frozen. Additionally, I confirmed that the rotifers regained full vitality and reproductive capacity. The two-way table Chi-squared test of significance (P = 0.05) was used to analyze the data for live versus dead individuals in all cases.

Table 1. Survival data for the 4 bdelloid species

Species	Live	Dead
WPr 1.1	9	80
Daly 1.5	6	45
Snail 1.2	3	66
AR 1.2	32	70

Table 2. Survival data for the fast, intermediate and slow growth rate duplicates for Adir 1.2.

Duplicate	Live	Dead
Fast	84	202
Medium	55	151
Slow	300	227

Experiment 1: Among species differences

The Chi-squared analysis of the 4 bdelloid species indicated that there was no significant difference in survival among the 4 species (Table 3).

Table 3. X^2 analysis results for "Among species" differences.

Test	X^2-Value	Degrees of Freedom	P-Value

Among Species	0.000239	3	P > 0.25

Experiment 2: Within species differences

Since 2 samples were used for each growth rate class, these values were also analyzed using the Chi-squared test and were deemed acceptable of being pooled as 1 sample each for the overall data set (Table 4). The data for these trials was then pooled and analyzed using the Chi-squared test to exam differences within a species. The results of the test indicate that there were no significant differences in survival among the 3 duplicates within the single species (Table 5).

Table 4. X^2 test for poolability of growth rate duplicates for "within species" test

Test	X^2-Value	Degrees of Freedom	P-Value
Fast Duplicate	0.001992	1	P > 0.25
Intermediate Duplicate	0.311408	1	P > 0.25
Slow Duplicate	0.999638	1	P > 0.25

Table 5: X^2 test for "Within species" differences

Test	X^2-Value	Degrees of Freedom	P-Value
Within Species	5.3633E-17	2	P > 0.25

DISCUSSION

The results of the statistical analyses indicated that there was no significant difference in freezing survival rates both among and within bdelloid species. This suggests that the genotype of a particular bdelloid species does not influence freezing survival. However, given the relatively small number of different species used for the "among species" test, these results should not be deemed conclusive. Ricci et al. (2005) demonstrated that there is a difference in fitness among species in a similar experiment involving dessication of bdelloid rotifers with a larger number of species.

The next step in this area of research should be to increase the number of species tested in these freezing experiments in order to obtain more conclusive results. Additionally, an in-depth microscopic and biochemical analysis would reveal the mechanisms involved in initiating cryobiosis. The results of this research will have implications for the ecology and evolutionary biology of bdelloid rotifers. One such example is that freezing tolerance in asexual compensates for the disadvantage associated with losing the ability to reproduce sexually.

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