The X Philes

Morel Dilemma Episode 14 Script. Written and copyright Elizabeth S Gall 2018.

[Music begins]

Izzie: Welcome to Morel Dilemma, where we explore why some fungi are so highly sought, some are so heavily cultivated, and some are so very dangerous. In the last episode, we talked about how well-suited fungi are for traveling cosmic distances. We concluded on the note that yeah, microbes could actually do quite well for themselves in space.

That's weird, right? If panspermia isn't how life originated on Earth, then Earth organisms have never been to space before. And even if panspermia *is* how life got here, that was billions of years ago! Why would microorganisms still be so well suited to life under extremely high and low gravitational pressures, UV radiation, crazy temperatures, and all the rest?

[Music ends]

Izzie: Well, as I hinted last episode, there are some extremely tough areas to live on our own little blue marble. Modern gene sequencing techniques are letting biologists find entire ecosystems where once we only saw glacial ice, or in the poorly named Dead Sea. Before we dive into some of the weird and wonderful places where fungi make their homes, let's talk about some of the factors that help organisms *anywhere* live and adapt.

[Music begins]

Izzie: The environment exerts a lot of pressures on living beings. These pressures can be things like intense UV radiation, near-boiling temperatures, or quick and pointy predators. They can also be things we humans might not see as particularly stressful, like a temperature of 68 degrees Fahrenheit, which would be deadly cold to reptiles. The environmental pressures are equal to all of the organisms but affect them to different extents - when we are both at 68 degrees I might not even need a jacket, while an iguana would be very distressed. The organisms that can handle the stresses of an environment will have a better chance to survive and reproduce there than the organisms that can't. The better opportunity to thrive is commonly known as "survival of the fittest", because only the organisms that are fit to resist the environmental pressures survive. More formally, this idea is called "selective advantage", because the stresses are, in a way, "selecting" for organisms with particular traits.

The theory of evolution by natural selection hinges on this idea. As a species is exposed to the pressures of the environment over generations, mutations will arise in the population that offer a greater advantage over the pressures, and by way of natural selection, organisms with these traits will become more common in the population. This is why antibiotic resistance can arise so quickly: microorganisms grow and reproduce really fast, so any favorable mutation in one bacterial cell can rapidly lead to a bacterial population with increased fitness under the stress of a drug.

[Music ends]

Izzie: However, selective advantages have a limit: they're only advantages for as long as the selection stress is applied. If the environment stops exerting a particular stressor, then organisms resistant to the stress are no longer at an advantage. If we remove the antibiotic from our bacterial population, then a mutant bacterium *without* antibiotic resistance won't be at a disadvantage, so it could reproduce at the same rate as the resistant strain. Meanwhile, spending energy to maintain the antibiotic resistance could be a disadvantage when the drug isn't around. Eventually, the species could entirely lose the resistance it once had.

[Musical tone]

Izzie: So what does this mean for our spacefaring microbes from last episode? If our terrestrial micro-buddies are able to survive the intense UV radiation, dryness, and temperature and pressure changes they'd experience traveling through space, it's likely because they are living in places that exert those pressures on a regular basis. After millions of generations, favorable mutations have accumulated in the population that allow our micro-buddies to resist these stresses, with defenses that are either always present or activated when the stress appears. Organisms that thrive in extreme environments are called *extremophiles,* because they apparently 'love' to bask in hardship, and *philo* is Greek for "love".

Enough generalities! Now it's time to look at some specific fungi, and thee amazing abilities and adaptations that let them explore extreme environments without even leaving our atmosphere.

[Musical tone]

Izzie: There are so many different kinds of environmental stresses that we could talk about, so I asked my Twitter followers to vote. Based on their responses, I've crafted this episode around water stress, extreme temperatures, and high salt concentrations. These stresses are often related - for example, high salt concentrations lead to water stress. They can also combine with other stresses in the environment, like when desert organisms are exposed not only to extreme heat and dryness, but to UV radiation. But if there's one single most important factor in life's ability to survive anywhere, it's probably access to water.

[Music begins]

Izzie: Water is necessary for cell metabolism - for getting food in the right places, making proteins, having the proteins work correctly, getting rid of waste… et cetera. The problem is, water likes to go where it isn't. It "diffuses down the gradient", which means that it moves from areas of high water concentration to areas of lower relative water concentration. Think about how far spilled water spreads on a dry tablecloth. The water molecules want everything to be evenly wet, not caring about the distinction between a glass and a tablecloth - or between the environment and a cell. If a cell is growing in a desert, it needs to keep water inside, but the water wants to leave the cell and bring moisture to the dry air. If a cell is growing in a freshwater lake, the water wants to rush *into* the cell to dilute all the cell particles like DNA and proteins - but if too much water flows into the cell, it will pop like an overfilled water balloon. The movement of water through a membrane is called osmosis, and the different water situations a cell can be in are called different osmotic conditions.

There are two kinds of water in any environment. "Bound" water is strongly bound to the substrate, so it's not available to cells. Imagine trying to drink drops of water from some wood that's been soaking overnight. By contrast, *free* water is only weakly bound to the substrate, so it's totally available for organisms to use - imagine drinking through a wooden straw. The *water activity* of a substrate tells us how much free water is available. Pure distilled water is entirely unbound, so it has the highest possible water activity of 1. But water with something dissolved in it is partially bound to the dissolved particles, so its water activity will be less than 1. An organism that can live some part of its life cycle at a water activity of 0.85 - the same water activity you'd find in a salami [10] - is considered *xero*philic [9], or extremely tolerant to water stress. The single most xerophilic fungus we know about is *Xeromyces bisporus*, which can handle environments with water activity of 0.61, the lowest water activity known to support life [11]!

[Music ends]

Izzie: The optimal water activity for a species may be different depending on the *solutes*, or particles dissolved in the surrounding water. For example, some decomposing fungi can grow just fine at high concentrations of the sugar-alcohol glycerol, but not at the same concentration of potassium chloride salt [9]. In fact, many cells living under water stress *produce* glycerol to reduce their internal water activity, keeping water inside the cell. Molecules that cells produce or absorb in high quantities to lower their own water activity are called *compatible solutes* [9]. Some fungi, such as brewer's yeast, will keep some compatible solutes inside themselves and secrete the same molecules into their environment to keep the osmotic gradient approximately equal [9]. Others actively pull glycerol into their cells from the environment [9], expending valuable energy to maintain that water activity. Most of the compatible solutes used by fungi are alcohols, with glycerol followed in popularity by arabitol, erythritol, and mannitol [9].

Survival in lots of extreme environments comes down to water availability. Freezing, long dry spells, and high salt concentrations all lead to dehydration in addition to other problems [1]. So a lot of extremophiles use the same coping mechanisms whether they're in the Atacama Desert or Antarctica.

[Musical tone]

Izzie: It's summertime here in the northern hemisphere, and all these record-breaking heat waves have me thinking about… well, a lot of things, but partially I've been thinking how nice it would be if I were a little more heat-resistant. What if 104 Fahrenheit was where I just started sweating?

[Music begins]

Izzie: That's life for *thermophilic* fungi, which grow best between about 100 and 120 degrees Fahrenheit and cannot live in temperatures below about 70 [9]. Even these heat-loving fungi can't make it past 150 Fahrenheit, the temperature at which non-bacterial cell membranes completely break apart [9]. So where in nature will these fungi find cozy, 100-degree homes? Remember from Episode 2 that industrial compost piles slowly climb up to 180 degrees Fahrenheit, purely through microbial activity. Smaller piles of compost that occur in nature don't get quite as hot, but most of the thermophilic and thermotolerant fungi we know were first isolated from compost.

[Music ends]

Izzie: There are plenty of other places that reach those temperatures, and are a little more permanent than compost heaps. I'm excited to finally talk *in depth* about hydrothermal vents! I feel like the last three episodes have just been bringing us to this point. Hydrothermal vents are places where water that has been heated deep within the earth emerges in the deep ocean. Down there, the water is very close to freezing - about 36 to 40 degrees Fahrenheit [7]. By contrast, the water shooting up from the vents can reach 750 [7]! As you might imagine, some of the super duper hot stuff mixes into the really cold water, creating zones of livable temperatures where microbes and larger organisms can hang out. Large food webs surround these vents, including tube worms, crabs, and bacteria - and fungi, of course [7].

[Music begins]

Izzie: In terrestrial habitats, fungi are the major recyclers of organic material, and these vents in the ocean are host to a lot of organic material. You would expect to find some recycling organisms, right? But though the first hydrothermal vent was discovered in the 1970s, it wasn't until 2009 that a team of researchers looked into the fungal inhabitants of the biome [7]. Dr. le Calvez and his team from Université de Rennes used both culture-based and culture-independent techniques to sample the diversity in deep-sea hydrothermal vents from oceans around the world [7]. All of the isolates that grew in the study were ascomycetes, including black yeasts and fungi that are known to be marine parasites in tidal zones [7]. Perhaps they are also parasites of the organisms that live near hydrothermal vents.

The culture-independent approach involved searching the water near hydrothermal vents for fungal rRNA [7]. rRNA is a molecule related to protein creation in cells, and it is used in a range of genomic studies to identify organisms all the way down to the species level. In this case, Le Calvez' team turned up 7,425 fungal sequences [7], including members of every fungal genus present in the GenBank database at the time [7]! Definitely a lot of fungal action going on around these vents.

[Music ends]

Izzie: Unfortunately, while le Calvez' study - and others like it - can survey the incredible diversity of fungi thriving near hydrothermal vents, they haven't yet given us insight into how the fungi are able to live in this extreme environment, with all the layered pressures it poses: heat, water pressure, salt, et cetera. However, we do know some ways that thermophilic fungi, from the seafloor to sea level and beyond, handle high temperatures.

[Music begins]

Izzie: Some species tolerate sizzling heat by incorporating more saturated fatty acids into their cell membranes [9]. You may know that fats come in two varieties: saturated and unsaturated. Unsaturated fats, like those in olive oil, are great for cell membranes at the temperatures where humans hang out. At room temperature, they are nice and fluid, which makes it easy for membranes to bring in nutrients and get rid of waste. *Saturated* fatty acids, like those in butter or bacon, are solid at room temperature. At room temperature, too many saturated fats can make membranes rigid and reduce their ability to perform normally. But at high temperatures, when unsaturated fats just fall apart, *saturated* fats are just becoming fluid. By incorporating more saturated fatty acids into their membranes, thermophilic organisms can function at well above 100 degrees Fahrenheit [9]!

[Music ends]

Izzie: Thermophilic fungi can also make use of specialized "heat shock" proteins, which have specific sequences that protect the proteins from 'denaturing', or melting, in high heat [3]. Some of the heat shock proteins are called "chaperones". As the name suggests, their job is to follow around more heat-susceptible proteins and keep them from unraveling. If proteins have unraveled in the heat, chaperones can keep them from sticking together into problematic clumps until they can be fixed or broken down [3].

Whether on dry land, in a compost pile, or deep in the ocean, heat shock proteins and saturated fats help thermophilic fungi keep doin' their thing.

[Musical tone]

Izzie: Now, listeners in the southern hemisphere, don't go thinking I've forgotten about you. After spending early 2015 in Boston I will forever be haunted by the thought of ten-foot-tall snow piles and twelve-foot icicles. In weather like that, it sure would be nice to be psychrophilic. "Psychro" is a root word from the Greek *psychros*, which means "cold". While thermophiles love the heat, psychrophilic organisms can't survive anything hotter than 68 degrees Fahrenheit [4]. But their characteristic cold-loving nature is most obvious at 32 Fahrenheit - the temperature where water freezes, but these organisms thrive.

Psychrophilic fungi live in the places you'd expect, such as the Arctic and Antarctic circles, but they can also be found in temperate and tropical regions, as long as humans are around. Yep, if you've ever left yogurt in the refrigerator for too long, you've met a psychrophilic fungus. Refrigerators, which usually maintain temperatures between 39 and 50 degrees Fahrenheit, are common hosts of psychrophilic food spoilage fungi [9]. Some fungi, including species from the genera Cladosporium and Sporotrichum, can even contaminate meat that is stored below freezing temperature [9]. It's not uncommon for frozen foods to be contaminated with ascomycete yeasts [1] – even though those species are rarely found in frozen natural environments [1]. Modern packaging and quality control practices help us deal with the psychrophiles in human-controlled areas. So what's out there in the wild?

[Musical tone]

Izzie: Way up north in the Arctic, so-called "snow molds" are prevalent. The species *Sclerotinia borealis*, commonly found in high latitudes like British Columbia and Sweden, has been grown in lab studies between 19 and 60 degrees Fahrenheit. Their favorite temperature is, of course, the freezing point. In subarctic regions, soil fungi and fungi living on plant surfaces can comfortably overwinter, surviving at temperatures lower than negative 58 degrees Fahrenheit [9]!

Polar fungi are usually found in association with vegetation like berries, mosses, and some grasses [1], but aquatic species have also been found in polar *fresh*water [1]. Yes, there is such a thing. The sun can melt the very top layer of ice and snow, forming little rivulets and tiny films of liquid water. The water can support tiny ecosystems on glaciers and inside of permafrost, which is basically "perma"-nently frozen soil. But the ideal polar environment for microbes seems to be under glaciers.

[Music begins]

Izzie: When the freshwater runs through a glacier, salts and mineral-rich rock sediments can get mixed in. These vital materials get concentrated down at the bottom of the glacier [1], supporting fungal populations one hundred times denser than those on the glacier's surface [1]. In 2003, The University of Ljubljana's Dr. Gunde-Cimerman took a team to survey the fungi in subglacial ice of Kongsfjorden, Norway [1]. *[Sarcastic]* That was the easiest sentence I've ever read on this podcast. In the native ice, 85% of the fungal population were basidiomycetes [1]. However, culture tests revealed that the ratio of ascomycete and basidiomycete fungi in the frigid waters is highly dependent on water activity; when Kongsfjorden ice samples were plated on heavily salted media, the ascomycetes began to dominate [1]. The most common ascomycete from the study was the black yeast *Aureobasidium pullulans*, which has been isolated from other salty environments, including those in much warmer areas [1]. After those yeasts, the most common fungi were darkly colored, yeast-like fungi, including 24 Penicillium species [1]. The most common is *Penicillium crustosum*, which is also a dominant species in glacier melt water and is a common food spoilage fungus [1].

[Music ends]

Izzie: At the other end of the world from Norway, the average year-round temperature is negative four degrees Fahrenheit [1], there are fewer than four inches of precipitation each year [1], and there are at least four penguins at any time. Welcome to Antarctica. When I was younger, by which I mean before I researched this episode, I thought Antarctica was basically a big ice sheet. No, actually - it's a continent with mountains, valleys, rocks, and dirt. It isn't even fully covered in ice. The McMurdo Dry Valleys of Antarctica contain large areas where the permafrost is exposed, so even though it is really cold there, it's also very dry. In some of these valleys, any ice in the soil just comes from the water in the air, and hasn't contacted ice melt, rain, or any other liquid water [4].

The McMurdo Dry Valleys are very similar to the terrain expected to exist on Mars, so scientists in a range of disciplines are interested in what's living there [4], and the life there is pretty impressive. Some ascomycetes isolated from Antarctic rocks can survive at negative 112 degrees Fahrenheit [9]! Despite how hardy extremophiles can be, only 65% of Antarctic soils are known to harbor life [1].

[Music begins]

Izzie: In 2016, Dr Goordial of McGill University conducted a soil study in the University Valley of Antarctica, which is considered one of the harshest environments on Earth [4]. Dr. Goordial's team tried to culture microbes from six samples of the permafrost, which has an average temperature of negative 13 Fahrenheit and rarely thaws [4]. The soil samples were given culture media with everything a heterotrophic organism could ever ask for, but after two years of culturing, the 1000 sample plates only produced *six colonies* [4]. The fungal isolate from the order *Chaetothyriales* was the only one that grew in the first culturing attempt; the other five strains only appeared after being incubated at 41 degrees Fahrenheit for more than *three months*, so they may not have been actively growing when they were collected [4].

In 2006, a team led by Dr. Fell, of the Rosenstiel School of Marine and Atmospheric Science, investigated the microbial yeasts in the dry Taylor and Wright Valleys of Antarctica. The dominant ascomycetes from the samples were generalist saprobes [1], which means they can decompose a wide range of organic compounds. The most common basidiomycete was the yeast genus *Malassezia* [1], which has a wide range of substrates - you may recall that it's also common on human and animal skin.

[Music ends]

Izzie: Dr. Fell's study was most intriguing to me because the team didn't only look at the species present - they looked into the ecological relationships within the frozen soils.

As in Norway, the best spot for Antarctic microbes is near glacial melts and streams - places where fresh water accumulates [1]. Remember that in the Gunde-Cimerman study of Norwegian glaciers, the proportion of ascomycetes and basidiomycetes in glacier ecosystems depends on the salt concentration. Well, based on Dr. Fell's study, it seems Antarctic permafrost ecosystems also change with moisture content [1]. Wetter soil supports a greater variety of fungi and other microbes [1]. With 0.2 to 1.3 percent moisture, which is around the same water content as lard [2], only one genus of fungi is present [1]. But between about 3 and 5 percent moisture, about the same water content as a raw cashew [2], the soil can support entire food webs - from primary producers like lichens to single-celled predators, decomposers, and even some pathogenic fungi [1].

[Musical tone]

Izzie: So let's talk about exactly what makes super-low temperatures problematic for most organisms, and how psychrophiles avoid those problems. Just like getting out of your cozy bed in dead winter, cell metabolism goes more slowly in the cold. The creation of new proteins slows down and the existing proteins function more slowly, and cell membranes become more sluggish as the fats in them solidify. Low temperatures can also make cells shrink - up to 60% in some cases [9]. Plus, there's water in every cell, and when temperatures go below freezing, that water can turn into ice. Since frozen water is less dense than liquid water, freezing can cause cells to expand by about 10% [9]. Couple that with the shrunken membrane, and a cell can split like a frozen tomato.

[Musical tone]

Izzie: Organisms can produce specialized solutes that prevent freezing damage. That's right: fungi make their own antifreeze! Cells in frigid environments can protect themselves with some of the same solutes that work under other types of water stress, namely arabitol, erythritol, glycerol, and trehalose [9]. These molecules can reduce the amount of ice that forms inside the cell, mitigating the damage [9]. Trehalose, in particular, is used by a lot of species that live in cold habitats, like Arctic members of the genus Hebeloma and samples of *Mortierella elongate* known from Signy Island, Antarctica [9]. In lab studies, *Mortierella elongate* fungi grown at 41 Fahrenheit accumulate 75% more trehalose than those grown at 59 degrees [9], which is how we know that it's a compatible solute used for cold tolerance.

[Musical tone]

Izzie: To wrap up our discussion of stressed out cells, let's talk salt. Salts, like the sodium chloride you may sprinkle on your food, are composed of ions - atoms that are held together by their charge rather than by the firm bonds that hold together the atoms in water, DNA, or other molecules. One unit of 'table salt' is, very basically, a sodium ion and a chlorine ion magnetically held together. Water molecules have some internal magnetism of their own, so salts dissolve readily in water, splitting into sodium ions and chlorine ions. Remember that water wants to travel down its osmotic gradient, diluting everything equally. Well, from the water's perspective, each unit of 'table salt' is actually *two* things, so the water is extra eager to flow where the salt is. That water gets bound to the ions and is less available to cells [11]. If the water starts out in a living cell, we obviously have a problem. And yet, seventy percent of the earth is covered in salt water! Obviously, life has, uh… found a way. Even in extremely salty environments – like brine lakes on the ocean floor or hypersaline areas like the Dead Sea – some organisms thrive [8]. These microorganisms are "halophilic", from the Greek word *halos*, meaning "salt".

[Musical tone]

Izzie: Remember that xerophiles can grow and reproduce at a water activity of 0.85 or lower. On a sugary medium, that's about 50% glucose [5]. But because of the ions in salt, you don't need very much at all to reach the same water activity – only about 17% of the solution needs to be sodium chloride [5] to reach water activity of 0.85, and for some salts it takes even less. Most natural hypersaline environments – that is, extra salty places – are high in sodium chloride, so that's the salt that's used most often in lab tests of halophilic organisms [11]. Some other salts are common in nature, too, like magnesium chloride, calcium chloride, and magnesium sulfate.

[Music begins]

Izzie: Not all salts are created equal in terms of effects on living cells. *Xeromyces bisporus* is the xerophilic fungus that grows at the insane water activity of 0.61 if it's on sugar media, but in sodium chloride solution, it can only handle a water activity of 0.9 [original calculation based on source 11]. That's because, in addition to posing water stress, the ions that make the salts can be directly toxic to cells. Magnesium, chlorine, potassium, and calcium ions destabilize biological molecules, making them toxic to even halophilic organisms [11].

Only a few fungal species, spanning ten orders on the tree of life, are known to be tolerant to low water potential [1]. The most halophilic eukaryotic species we know about is the basidiomycete *Wallemia ichthyophaga*, which simply can't grow in an environment with less than 10% sodium chloride [1]. Because it cannot grow without salt, *Wallemia ichthyophaga* is known as an *obligate* halophile. Its favorite environment is completely salt-saturated water, with sodium chloride making up 30 to 35% of the solution[1]!

[Music ends]

Izzie: While many halophilic organisms were first identified from human environments, like contaminated foods [1], they are also fairly common in the wild, in areas where you might not think anything could live. For instance, there's an inland sea in the Middle East with a water activity of 0.68 [11] that's about 19% magnesium chloride and more than 5% calcium chloride salt [11]. This so-called Dead Sea actually hosts plenty of filamentous fungi, like members of the genera Cladosporium, Aspergillus and Penicillium [11]. There is also life in hypersaline lakes in Antarctica, like Lake Wanda [1]. Obviously, halophilic fungi living at the South Pole have some problems that those in the Mediterranean or the Middle East don't, but salt tolerant microbes do exist all over the world. Some species pop up over and over. For instance, *Debaryomyces hansenii* has been found in Antarctic soils, the Great Salt Lake in North America, and on the Skeleton Coast of Namibia [5]!

Halophilic microbes like *Wallemia ichthyophaga* [11] are well known in salterns, shallow pools where seawater collects and evaporates to create increasing concentrations of salt. Salterns can occur naturally, but most studies focus on the man-made variety. In Mediterranean salt production, water is moved along a series of salterns throughout the summer, with continual water evaporation leading to increasing concentrations of several salts. Water that starts in May with 3% sodium chloride may reach August with 35% salt [6] - the point where no more salt can go into the water, so it falls to the bottom of the pool. From the human perspective, that's sort of the point, because it's how we collect sea salt. But from a microbial perspective, that means starting in a water activity of 0.99 [5] and ending up somewhere around 0.78 [original calculation]. That's a lower water activity than *soy sauce* [10]. Not to mention that by September, salterns also have high levels of magnesium, potassium, and sulfate ions [6]. Who the heck would want to live *there*?

[Music begins]

Izzie: In 2000, the University of Ljubljana conducted a study led by Dr. Gunde-Cimerman again, to investigate the most halophilic organisms living in salterns throughout the year. Anything that grew on their 17% salt media, withstanding a water activity of 0.85, was given further study [6]. As with our psychrophilic friends, it seems the community composition of saltern fungi is driven by the amount of available water.

The ecosystem goes through seasonal fluctuations, with the most halophilic fungi dominating the pools at the end of the evaporation season [6]. *Hortaea werneckii*, one of the most halophilic fungi known, is very common at the end of the season [6] and was the only tested species that grew in 32% sodium chloride [6]. *Phaeotheca triangularis* and *Trimmatostroma salinum* were the dominant species in September, but they only survived up to 24% salt solution [6] – though that is still quite impressive, as that's a water activity of about 0.84 [original calculation based on source 5]! Interestingly, the study didn't turn up any known marine fungi, which means the fungi that live in salterns probably *didn't* evolve from ocean fungi [6].

[Music ends]

Izzie: To learn more about the halophilic fungi in the natural world, Dr. Zajc and a team from the University of Ljubljana conducted a study on salt tolerance in worldwide fungi. The study, published in 2014, surveyed 47 fungi collected from the Dead Sea, 13 from subglacial ice, 44 from salterns around the world, and a few strains from freshwater, animal skin, and human food to round the sample out [11]. The strains were first cultured on normal media without salt and were later cultivated on plates with increasing concentrations of various salts [11]. While many of the tested strains were able to withstand one or two salts, very few of them were able to grow in high concentrations or in multiple salt types.

[Music begins]

Izzie: Remember the obligate halophile *Wallemia ichthyophaga*. Its favorite water activity on sodium chloride is between 0.96 and 0.77 [11]. It turns out that that salt tolerance extends somewhat to magnesium chloride - the strains of *ichthyophaga* that Dr. Zajc tested could tolerate more than 17% of that salt [11]. However, *ichthyophaga* can't handle high levels of *calcium* chloride [11]. Cladosporium species isolated from the Dead Sea and from salt marshes could grow in media with up to 20% sodium chloride or 34% calcium chloride but couldn't handle high magnesium chloride [11]. Neither could species in the Aureobasidium genus, even though they could handle 30% potassium chloride and 36% magnesium *sulfate* [11]. Far and away the best halophilic generalist tested was *Hortaea werneckii*, which grew on the highest concentrations of all the salts tested: 19% calcium chloride, 20 percent magnesium chloride, 30 percent sodium chloride, 34% potassium chloride, 36% magnesium sulfate, and 41% sodium bromide [11]. Not bad! No wonder they're one of the most abundant fungi in salterns [11]!

[Music ends]

Izzie: Part of a halophile's remarkable tolerance comes from proteins in the cell membrane that specifically expel dangerous ions that have leaked in, usually once their concentration crosses a livable threshold [11]. Some halotolerant fungi, like *Hortaea werneckii* and *Debaryomyces hansenii*, have multiple versions of these exporters just for sodium [11]. It's not a requirement, though; *Xeromyces bisporus* doesn't have any pumps that expel sodium ions [11], and *Wallemia ichthyophaga* doesn't have many [11]. Halophiles with or without ion pumps can also do well if they stick together. This isn't just a metaphor for teamwork; *Hortaea werneckii* and *Wallemia ichthyophaga* each grow colonies in clumps [5] to reduce the amount of each cell's surface that is exposed to salt [11].

[Music begins]

Izzie: Remember how psychrophiles deal with low water availability by churning out *compatible solutes*? Fungi in salty environments do the same thing! When baker's yeast is grown in 10% salt media, the cells generate glycerol at 40 times the normal rate [9]. Halophilic fungi have specialized channels for the quick creation and uptake of glycerol [11], making sure water still wants to chill inside them without letting them take in toxic ions. The glycerol can also buffer cell enzymes from the damaging effects of the ions that do get in [8]. Various fungi can use different sugar alcohols, like arabitol, trehalose, and erythritol. *Wallemia ichthyophaga* mostly relies on glycerol, but also produces arabitol and mannitol [5]. *Hortaea werneckii* also chiefly uses glycerol [5], and in *Debaryomyces hansenii*, glycerol accumulation is directly triggered by low water activity [9] and by high concentration of sodium ions in the environment [5].

Halophilic fungi can make sure they keep a good stock of compatible solutes by changing the composition of their cell membranes. *Hortaea werneckii* and *Debaryomyces hansenii* accumulate less wax in their membranes than non-halophilic fungi do, which helps them retain the glycerol they make [5]. Halophilic fungi can also *de*-saturate their fatty acids to maintain a fluid membrane in high salt concentrations [5], and then increase the amount of melanin pigment to make membranes less porous so that good, good glycerol can't escape [11]. Levels of melanin directly relate to salt tolerance in some fungi, like the genus Aureobasidium [11], and the halophilic fungi isolated in Dr. Gunde-Cimerman's saltern study were almost exclusively melanized [6].

[Music ends]

Izzie: Well, my friends, that about wraps it up. There are plenty of places on Earth where fungi have to deal with temperature extremes and low water availability, which could be how some of them have trained for space travel. I love the complicated, yet overlapping, ways that fungi deal with extreme terrestrial environments - like how glycerol can help psychrophiles reduce ice formation *and* keep halophiles from overdosing on salt ions. And that's true for many stresses we didn't cover this episode, but are still relevant to both Earthlings and space ex-spore-ers!

In addition to buffering against a salty environment, melanin can help fungi resist damage from ultraviolet radiation, which they may deal with on a space station, in the desert, or on the leaves of plants. Maybe mechanisms of pressure resistance are similar for deep-sea fungi and the ones that can survive an asteroid impact. Maybe life uses the same pathways to tolerate low oxygen levels in space and in marshes, in hot springs and high altitudes. So next time you see a psychrophile in your fridge, give yourself a moment to appreciate the wonderful quirks of evolution that have enabled that organism to live in such a strange environment…

[Music begins]

Izzie: ...before getting rid of it and making a mental note to eat your yogurt faster.

Morel Dilemma is written and produced by me, Izzie Gall. Our theme music is "Fungi Among I", composed and performed by John Bradley, with additional music written and performed by Mihai Sorohan. You can find more of Mihai's music at mihaisorohan.bandcamp.com. If you like what I'm doing here, please tell a friend to lend an ear! You can also leave a rating on your podcast app of choice, and please consider donating to the Morel Dilemma Patreon, which takes donations whenever an episode is released. You can also help by calling the hotline at 347-416-6735 and leaving a mushroomy message, a question, or a topic you'd like me to talk about. You can find other ways to contribute, and other Morel Dilemma content, at moreldilemma.org.

Guys, I love making this podcast and teaching folks about how awesome fungi are. Thank you so much for listening and hopping on the mycology train with me. Mycelia later!

[Music ends]

Resources

1. Cantrell S.A., Dianese J.C., Fell J., Gunde-Cimerman, N., & Zalar, P. "Unusual fungal niches." *Mycologia*, vol. 103, no.6, 20 Jan 2011, pp. 1161-1174, DOI: 10.3852/11-108
2. "Food Data Chart – Water." *Asia Pacific Journal of Clinical Nutrition*, National Health Research Institutes, Accessed 4 Sep 2018. http://apjcn.nhri.org.tw/server/info/books-phds/books/foodfacts/html/data/data2b.html
3. Fox-Skelly, Jasmin. "What is the hottest temperature life can survive?" Earth. *BBC.com*, BBC, 10 Feb 2016. www.bbc.com/earth/story/20160209-this-is-how-to-survive-if-you-spend-your-life-in-boilin-water
4. Goordial, J., Davila, A., Lacelle, D., Pollard, W., Marinova, M.M., Greer, C.W., DiRuggiero, J., McKay, C.P., Whyte, L.G. "Nearing the cold-arid limits of microbial life in permafrost of an upper dry valley, Antarctica." *The ISME Journal*, Nature Publishing Group, vol. 10, no. 7, Jul 2016, pp.1613–1624. doi: 10.1038/ismej.2015.239
5. Gunde-Cimerman, N., Ramos, J, Plemenitas, A. "Halotolerant and halophilic fungi." *Mycological Research,* vol. 113, no. 11, Nov 2009, pp 1231-1241. https://doi.org/10.1016/j.mycres.2009.09.002
6. Gunde-Cimerman, N., Zalar, P., de Hoog, S., Pleminas, A. "Hypersaline waters in salterns – natural ecological niches for halophilic black yeasts." *FEMS Microbiology Ecology,* vol. 32, no., 3, 1 Jun 2000, pp. 235-240.
7. Le Calvez, T., Burgaud, G., Mahé, S., Barbier, G., Vandenkoornhuyse, P. "Fungal Diversity in Deep-Sea Hydrothermal Ecosystems." *Applied and Environmental Microbiology,* American Society for Microbiology, vol. 75, no. 20, Oct 2009, pp. 6415-6421. http://aem.asm.org/content/75/20/6415.full
8. Lever, Mark. "In Chaotropy Lies Opportunity." *Frontiers in Microbiology*, 5 Jan 2016. https://doi.org/10.3389/fmicb.2015.01505
9. Magan, N. "Fungi in Extreme Environments." *Environmental and Microbial Relationships,* Springer, 2007, pp. 85–103. The Mycota.
10. "Water Activity (a_W) in Foods". *FDA.gov,* U.S. Food and Drug Administration, 27 Jun 2015. Accessed 4 Sep 2018. https://www.fda.gov/iceci/inspections/inspectionguides/inspectiontechnicalguides/ucm072916.htm
11. Zajc, J., Dzeroski, S., Kocev, D., Oren, A., Sonjak, S., Tkavc, R., Gunde-Cimerman, N. "Chaophilic or chaotolerant fungi: a new category of extremophiles?" *Frontiers in Microbiology*, vol. 5, no. 708, 23 Dec. 2014 https://doi.org/10.3389/fmicb.2014.00708