Day 4 - Food

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Food!

Humans are used to having a diverse diet - we get food from all over the world in just a few meals a day, in space we need to work on meeting our dietary needs and set the diversity in diet on hold during the developmental stages.

Some notes - Astronauts often end up with high levels of iron in their blood because their blood condenses during space travel, loss of bone density is also common. Keeping this in mind when planning a diet will be important.
https://www.nasa.gov/mission_pages/station/research/experiments/912.html
https://www.nasa.gov/mission_pages/station/main/bone_study.html

Daily nutritional needs are different for everyone but we are using typical numbers for adult men and the recommended vitamins/minerals. Anything in bold can be found in Spirulina
3kg food per person daily

- **58 grams of protein**
- 44-78 grams of fat **(7.72 grams)**
- 225-325 grams of carbohydrate **(24 grams)**
- 2,000 calories (up to 3,000 because of increased activity) (**290)**
- **Vit A**
- Vit D - STUCK
- **Vit C**
- **Vit E**
- **Vit K**
- **Vit B1**
- **Vit B3**
- **Vit B5**
- **Vit B6**
- Vit B12
- **Folic acid - covered by folate aka B9**
- *Biotin - lentils*
- 9 Essential amino acids (**histidine**, **isoleucine**, **leucine**, **lysine**, **methionine**, **phenylalanine**, **threonine**, **tryptophan** & **valine**) - Found in meat - but how are we going to get it with what will certainly be a vegetarian and likely a vegan diet.
- **Calcium**
- **Magnesium**
- **Phosphorus**
- **Potassium**
- **Sodium**
- *Boron - lentils*
- Chromium - *Kelp*
- *Copper - lentils*
- Germanium - *Kelp*
- Iodine- Kelp
- **Iron**
- **Maganese**
- Molybdenum - *lentils*

- *Selenium - lentils*
- Silicon - *Cabbage*
- Sulfur - *Cabbage*
- Vanadium - may not be required
- **Zinc**

Cyanobacteria might be the solution to giving us more of those vitamins. http://iimsam.org/en/
https://ndb.nal.usda.gov/ndb/foods/show/11667?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=Spirulina&ds=&qt=&qp=&qa=&qn=&q=&ing=
Nutritional information for Spirulina
http://www.whfoods.com/genpage.php?tname=foodspice&dbid=52 **lentils**

https://www.bbcgoodfood.com/howto/guide/vital-vitamins - Vitamins
http://www.simple-approach-to-healthy-living.com/list-of-minerals.html - Minerals
https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/19920016718.pdf - NASA specific problems
http://marsforthemany.com/project/living-on-mars/farming-on-mars/ - nutritional estimate by food group

WHAT WE ARE WORKING WITH ON MARS

Screen Shot 2018-06-25 at 3.12.21 PM.png

Table 2 Environmental parameters on Mars and Earth surfaces

Parameter		Mars	Earth
Surface gravity		0.38*g*	1.00*g*
Mean surface temperature		−60 °C	+15 °C
Surface temperature range		−145 to +20 °C	−90 to +60 °C
Mean PAR photon flux		8.6×10^{19} photons m^{-2} s^{-1}	2.0×10^{20} photons m^{-2} s^{-1}
UV radiation spectral range		>190 nm	>300 nm
Atmospheric pressure		5–11 hPa	1013 hPa (mean at sea level)
Atmospheric composition (average)	N_2	0.189 hPa, 2.7 %	780 hPa, 78 %
	O_2	0.009 hPa, 0.13 %	210 hPa, 21 %
	CO_2	6.67 hPa, 95.3 %	0.38 hPa, 0.038 %
	Ar	0.112 hPa, 1.6 %	10.13 hPa, 1 %

Adapted from Kanervo et al. (2005) and Graham (2004), and reproduced from Verseux et al. (2015) with permission from the editor of the International Journal of Astrobiology

Questions for your students

- How will we create food with 30% of our normal light
- How will we create food with limited water
- How will we create food when the soil doesn't have the normal nutrients readily accessible
- How will we raise crops without natural pollinators'
- How do we make it taste good? Cyanobacteria aren't known for being delicious - at best they are flavorless.

The Martian film proposes using human waste to grow potatoes - that could work, human waste could be a part of our compost, provided there were other things mixed into it. But, we would need so much more than just potatoes to give us our nutritionally complete diet.

Spirulina would help us get the protein we needed into our diet, although not super high in fat or calories, but it is very nutrient rich, anything in bold above is naturally occurring in this Spirulina cyanobacteria. HOWEVER, we would need to engineer this cyanobacteria to reduce the iron content because space travelers are already high in iron, and spirulina has such a large concentration of iron. With a combination of Spirulina, lentils, kelp and cabbage we could get all of our vitamin/mineral and calorie needs met (we would likely need potatoes for carbs and bacteria discussed later will provide lipid requirements), we would need to engineer each of the foods to be able to live in martian regolith, human waste (both compost and fecal waste) and bioleaching bacteria.

We know that plants do not just magically create all of the minerals they provide, they have to come from somewhere. Fortunately, Martian soil and rocks include all the building blocks for life, (C,H, O, N, P, S) and smaller elements needed in smaller amounts (Mg, Fe, Ca, K, Mn, Cr, Ni, Mo, Cu, Zn....) and carbon in methane and nitrogen is present in the atmosphere. Solar radiation provides the energy needed for photosynthesis, human waste could provide the necessary organic material and fixed nitrogen for growth. Cyanobacteria like our Spirulina are autotrophs, they can photosynthesize using atmospheric CO2 and solar radiation but don't require organic material. Some cyanobacteria have been grown just in water and simulated martian rocks. This means our cyanobacteria can provide both a food a nutrient resource both from direct consumption and to act as a organic medium to grow our other plants in. Currently, we have used lysed cyanobacteria to grow ethanol-producing yeast, it would be more sustainable if we could get the cyanobacteria to secrete the nutrients without the need for lysing the cells. Brown-Stanford iGEM has engineered *Anabaena* PCC7120 to secrete sucrose so this thinking is possible. Fixed nitrogen compounds is also naturally released by some cyanobacteria. When grown on Martian rock substrate, cyanobacteria have released inorganic elements Ca, Fe, K, Mg, Mn into the water, meaning they are free to use by plants which cannot leach them from the rock. The fixed nitrogen and micronutrients could be used to grow our heterotroph plants, waste from these plants (either stool post consumption or from discarded organic matter) could be recycled back into the soil to continue the cycle. We would likely need to bring a highly concentrated "started soil" with ample quantities of minerals like Calcium, manganese, zinc etc. which could then be put into our closed system and recycled repeatedly. Bioleaching bacteria could be bioengineered to extract select minerals found in regolith that cyanobacteria and our plants cannot extract. This would be necessary if our organisms would be overwhelmed by the quantity genome editing required to give them the ability to directly utilize martian soil.

Our cyanobacteria would also need to be engineered to utilize lower levels of iron, to produce less iron. **There are blood letting practices which can help reduce iron levels but we want to avoid these. We would need to look into processing plants that can leach iron out of our food - specifically our spirulina.**

Another necessary soil treatment component will be the removal of toxins. Martian soil is covered in toxic perchlorate which is detrimental to the thyroid gland, metabolism and hormonal balance so we cannot consume it or allow it to enter our food. Fortunately, it can be used as an energy source for some microbes and some reduce perchlorate under anaerobic conditions. The process is well studied and is three steps. ClO^{-}_{4} reduction, chlorite dismutation and then oxygen reduction. Step one is done by perchlorate reductase, converting perchlorate to chlorite. Chlorite is then converted to chloride and oxygen by chlorite dismutation. Then oxygen is reduced to water by oxygen reductase. This all happens in the periplasmic space of a cell. The process can be used to detoxify the soil and also provide oxygen for the astronauts. **This cannot be done in the classroom because of the toxic quality of perchlorate.** We can utilize these bacteria which already do this process and engineer them to work faster - it works very slowly.

Cleaning up toxins
(PDF) Perchlorate on Mars: A chemical hazard.... Available from:
https://www.researchgate.net/publication/242525435_Perchlorate_on_Mars_A_chemical_hazard_and_a_resource_for_humans
[accessed Jul 25 2018].

https://www.sciencedirect.com/science/article/pii/S0944501310001114
Options for biological reduction (section 8)

https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-10-351
What our cleaning bacteria need to survive - See what other iGEM teams have done.

Cyanobacteria
(PDF) Synthetic Biology for Space Exploration:.... Available from:
https://www.researchgate.net/publication/281494184_Synthetic_Biology_for_Space_Exploration_Promises_and_Societal_Implications
[accessed Jul 25 2018].

Topics for activities

Bio leaching

Growing Spirulina

Growing things ON spirulina

Removing toxins

K-5

Growing your own spirulina

Supplies

- Bucket full of water for your spirulina
- Spirulina starter
- Spirulina culture medium
- Windowsill to grow your spirulina on

Talk about the use of spirulina as a food source for space travel. Fill your bucket with water, starter and culture medium. Allow to grow for a week on your windowsill.

Bacteria friends removing toxins

Supplies

- Growth media with a basic pH
- Yeast - representing our Friendly bacteria, but for labs that aren't BSL 1
- pH indicator (either strips or drops)
- Incubator (if possible - but this should work at room temperature as long as we aren't using any antibiotics - it will just take longer)

Show students our friendly bacteria and introduce bacteria as a tool for breaking down toxins which could hurt humans. Perchlorate is the toxin we are worried about for Mars. For demonstration purposes we are going to be using pH as our "toxin" and watching pH levels drop thanks to our helpful bacteria (currently using bakers yeast). Begin with pH testing your media, it should be basic. Activate and add your yeast. If you have a constant pH monitor add it to the broth and allow students to check in on the pH periodically. If the monitor is not available, test with pH strips occasionally or by doing a titration or by adding drops. For elementary settings checking throughout the day is great, for teacher who teach class periods, have each class complete the experiment (doing one or two before the first class period shows up - about an hour apart - can provide the first class with a "time lapse" experience, letting them see how the yeast [friendly bacteria] alter the pH [toxin] levels).

Alternatively, we can measure glucose using glucose strips.

6th-8th

Growing spirulina in natural light vs. 30% light (like what we would have on Mars)

Supplies

- Container for your spirulina (two - one for each growth condition)
- Spirulina starter
- Spirulina culture medium
- Outdoor "Farming" area
- Cloth filter for harvesting
- UV light source (two - one for each growth condition)

Talk about the use of spirulina as a source of calories, nutrients and macromolecules. Discuss the limited natural light available on Mars (roughly 30% of what we have on Earth). Set up two growth conditions mixing your culture medium, spirulina starter, and water. Place one bucket in a space of the room where you will shine a UV light on the container continuously. The second bucket should be placed near the first but where the light from the first set up will not impact the second set up. The second set up should have a UV light which is OFF 70% of the time. It is helpful if these set ups can be kept in a separate room or unlit space in the classroom. (in a cabinet could work). After 1 week of growth, pour the control spirulina mixture through a cloth filter and weigh the contents after 10 minutes of dry straining. Repeat the pour and weighing process with the experimental 30% light condition.

Bacteria friends removing toxins

Supplies

- Growth media with a basic pH
- Yeast - representing our Friendly bacteria, but for labs that aren't BSL 1
- pH indicator (either strips or drops)
- Incubator (if possible - but this should work at room temperature as long as we aren't using any antibiotics - it will just take longer)

Show students our friendly bacteria and introduce bacteria as a tool for breaking down toxins which could hurt humans. Perchlorate is the toxin we are worried about for Mars. For demonstration purposes we are going to be using pH as our "toxin" and watching pH levels drop thanks to our helpful bacteria. Begin with pH testing your media, it should be basic. Activate and add your yeast. If you have a constant pH monitor add it to the broth and allow students to check in on the pH periodically. If the monitor is not available, test with pH strips occasionally or by doing a titration or by adding drops. For elementary settings checking throughout the day is great, for teacher who teach class periods, have each class complete the experiment (doing one or two before the first class period shows up - about an hour apart - can provide the first class with a "time lapse" experience, letting them see how the yeast [friendly bacteria] alter the pH [toxin] levels).

9th-12th

Growing spirulina in Earth conditions and Mars conditions - CO2 concentration ***This is probably a challenge for most schools***

Supplies

- Container for your spirulina
- Spirulina starter
- Spirulina culture medium
- Sealable container
- Biosensor which measures CO2 and Oxygen levels (nitrogen is nice too)
- Dry ice

Talk about the use of spirulina as a source of calories, nutrients and macromolecules. Discuss the limited natural light available on Mars (roughly 30% of what we have on Earth). Also talk about the difference in oxygen and CO2 concentration. Set up two growth conditions mixing your culture medium, spirulina starter, and water. If resources allow, complete the 6th-8th conditions with 2 set ups, and the gas concentrations with 2 other set ups. In one sealed container plug in your biosensors and record current gas concentrations, over the class period gas levels should change. Have students record these changes at the end of the class period. In the second container, add the dry ice and some warm water, seal the container, CO2 level should rise dramatically as the dry ice evaporates, record the CO2 levels as the ice melts. Allow the spirulina to work over a class period. At the end of the period rerecord the gas concentrations (the recording of gas concentrations gives students a chance to think about cellular respiration). Depending on resources, continuing to add dry ice occasionally to increase CO2 levels during the growth phase. If you will be adding dry ice, when ever dry ice is added (likely at the start of the day) also open the control container to allow it to match atmospheric gas levels. After 1 week of growth, pour the control spirulina mixture through a cloth filter and weigh the contents after 10 minutes of dry straining. Repeat the pour and weighing process with the chosen experimental condition.

Growing **on** spirulina

Supplies

- Container for your spirulina
- Spirulina starter
- Spirulina culture medium
- Martian simulant (https://reprage.com/post/home-made-simulant-mars-dirt/https:/ **OR** www.themartiangarden.com/mars-simulant)
- Lentils or cabbage seeds (any seeds will work really)
- Potting soil

Grow spirulina for a week using the above lesson. Harvest the spirulina. Mix spirulina with martian regolith simulant and plant 10 seeds in the mixture. Plant another 10 seeds in pure martian simulant and another 10 in potting soil. Chart growth over a week. Keep all else equal - light, watering etc.

Bacteria friends removing toxins

Supplies

- Broth with a basic pH

- E. Coli
- pH indicator (either strips or drops)
- Incubator (if possible - but this should work at room temperature as long as we aren't using any antibiotics - it will just take longer)

Show students our friendly bacteria and introduce bacteria as a tool for breaking down toxins which could hurt humans. Perchlorate is the toxin we are worried about for Mars. For demonstration purposes we are going to be using pH as our "toxin" and watching pH levels drop thanks to our helpful bacteria. Begin with pH testing your media, it should be basic. Activate and add your bacteria. The inoculation can be of a colony from a plate or from a pre suspended culture. If you have a constant pH monitor add it to the broth and allow students to check in on the pH periodically. If the monitor is not available, test with pH strips occasionally or by doing a titration or by adding drops. For elementary settings checking throughout the day is great, for teacher who teach class periods, have each class complete the experiment (doing one or two before the first class period shows up - about an hour apart - can provide the first class with a "time lapse" experience, letting them see how the friendly bacteria alter the pH [toxin] levels). Track the pH change over the day and the next class period.

We could also talk about adding an additional component to our Mars bacteria, so that they glow in the presence of the toxin. I think we have a circuit which codes for GFP production in the presence of copper, we could use this as an example. I would love to give students the chance to create their own inducible circuit and test it out....