ELVIS Meltdown!
Microbiology Concepts of Culture, Growth, and Metabolism

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Part I—Return to Sender

Fresh out of college, with your degree in microbiology, you have landed your "first real job" as a scientist with DuPunt, a company that specializes in development and production of polyurethane derivatives (specialized plastics). You (and your boss) are not quite sure why DuPunt has a microbiologist on sta , but you are both about to find out why the company desperately needs one now. Your boss has called you into his office. "Read this article!" he says, pushing the front page of a major national newspaper across his desk to you.

ELVIS Naked, Skinless upon Return from Outerspace!

The recent return to Earth of the unmanned exploration probe ELV from the Nearby Previously Invisible Planet (NPIP) has provided scientists with important information about conditions on the surface of this recently discovered planet. Although physicists have made great advances in understanding how NPIP recently was "uncloaked," perhaps the most interesting discoveries are yet to come as biological scientists begin to analyze the soil samples collected by the probe's Extraterrestrial Landing Vehicle Integrated Sampler (ELVIS). Designed to gather samples and maintain them in their normal atmospheric and temperature conditions, this collection unit is a sophisticated robot and is the most expensive component of the ELV probe. Inspired by the acronym for the unit (ELVIS), workers constructed this robot to resemble music legend Elvis Presley, and even fashioned a white Spandex jumpsuit to clothe it.

These design features have proven to be a public relations coup, endearing the 2-foot-tall robot to the public and making it the unofficial mascot of the NASA space exploration program. This "human connection" has been instrumental in convincing Congress to provide the necessary funding for the ELV and many related space exploration programs.

However, the successful completion of the ELV mission has generated a mystery, one that has led to accusations of contractors providing substandard materials (in particular defective plastics) used in the ELV or of someone intentionally sabotaging the plastic components of the ELV in an attempt to embarrass the U.S. space exploration program.

Upon its return to Earth, the ELV capsule was opened to allow scientists to recover the soil samples. This was done as part of a special, televised "welcome home" ceremony, in which ELVIS was supposed to "dance" down the ELV exit ramp and speak his trademark words, "Thank you … thank you very much for supporting this critical space exploration mission."

NASA scientists and on-lookers at the ceremony were shocked to find that ELVIS's jumpsuit had been reduced to a slimy puddle. Even more distressing was the deterioration of ELVIS's "skin" (a version of Lycra specially developed to resemble human skin). This too was reduced to a slimy residue that dripped from the metal "skeleton" of the ELVIS unit. The deterioration of the plastic components of ELVIS ruined what organizers had planned to be a touching ceremony at the mission's completion. ELVIS's exit from the otherwise intact spacecraft was met by gasps and screams from the gathered audience. "It was a terrible sight!" said one member of the audience. "We expected to see the King, but we saw a horrible mess, a grotesque parody of Elvis. Without his plastics lips, I couldn't understand a word he said … and the smell was horrible!" said one NASA official who wished to remain anonymous.

Television viewers were spared much of the trauma of these sights as networks quickly switched to new episodes of Sponge Bob Squarepants in which a cartoon version of the ELVIS unit was featured. An investigation is underway.

Stifling your initial reaction ("Oh yeah, new Sponge Bob!") you manage to mumble, "What a tragedy!"

"Yes. Yes. And this could take an ugly turn for DuPunt!" your boss says. "I'm not sure what caused this mess, but I do know a couple of things that didn't make it into that news article: (1) the only plastics showing damage in the ELV were polyurethanes; and (2) our company provided those polyurethane products to NASA at a cost of $15, , . We're in big trouble if we can't prove that something from that planet is responsible for destroying ELVIS."

He continues, " e polyurethane products we provided were first-rate. We didn't cut any corners with this stu . Products from the same batches of polyurethane have been into outer space before, and they returned just fine. ere must be some explanation other than our incompetence. is is where you come in. I need you to find that explanation!"

"Why me?" you ask.

"Because of the stink!" your boss answers. "Some of the scientists present at the ELVIS disaster said the smell reminded them of an old fermenter or an autoclave. ose are microbiology terms, aren't they?" says your boss. " ose comments tell me that this whole stinking mess might be caused by microorganisms—you know, bacteria, fungi, viruses, germs … something like that. Get right to work on this! You and I will have to work closely on this, you know. I'll handle all the communications with the press, and you handle the science. Just make sure that you explain everything to me so that I can speak about it to the press without making a fool of myself and DuPunt!"

Okay. You are a trained scientist … you can do this! What do scientists do? ey answer questions by testing specific hypotheses. As a microbiologist at DuPunt you must determine what has happened to the polyurethane. Here is your hypothesis:

> *e degradation of polyurethane products was caused by a microorganism or microorganisms present in the soil samples collected by ELVIS.*

Questions

1. Using light microscopy, you examine the soil samples and the "goo" from the degraded polyurethane. Will this approach allow you to observe all microorganisms present in the samples? Why or why not? What are the limitations of this approach?
2. You use phase contrast microscopy to observe a wet mount of a soil sample (the first picture below) and a "goo" sample (the second image below) from the ELVIS. In what ways are the potential ET microbes similar to microbes previously characterized on Earth? In what ways are they di erent? How could you determine whether the microbes present in the soil or goo samples are phylogenetically similar or distant from known microorganisms on Earth?

Figure 1—Soil sample

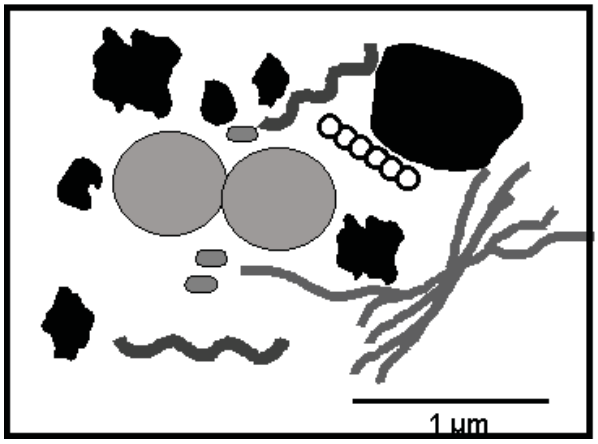
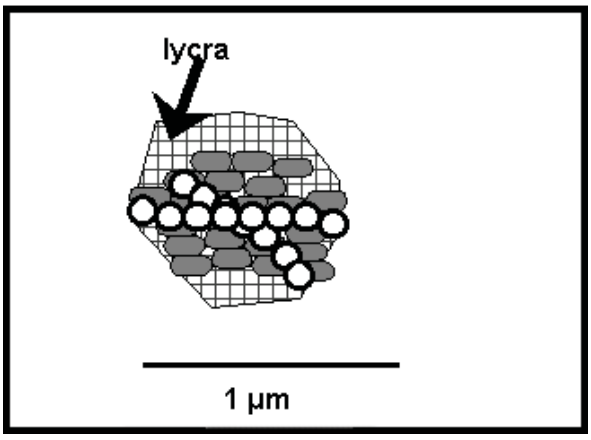
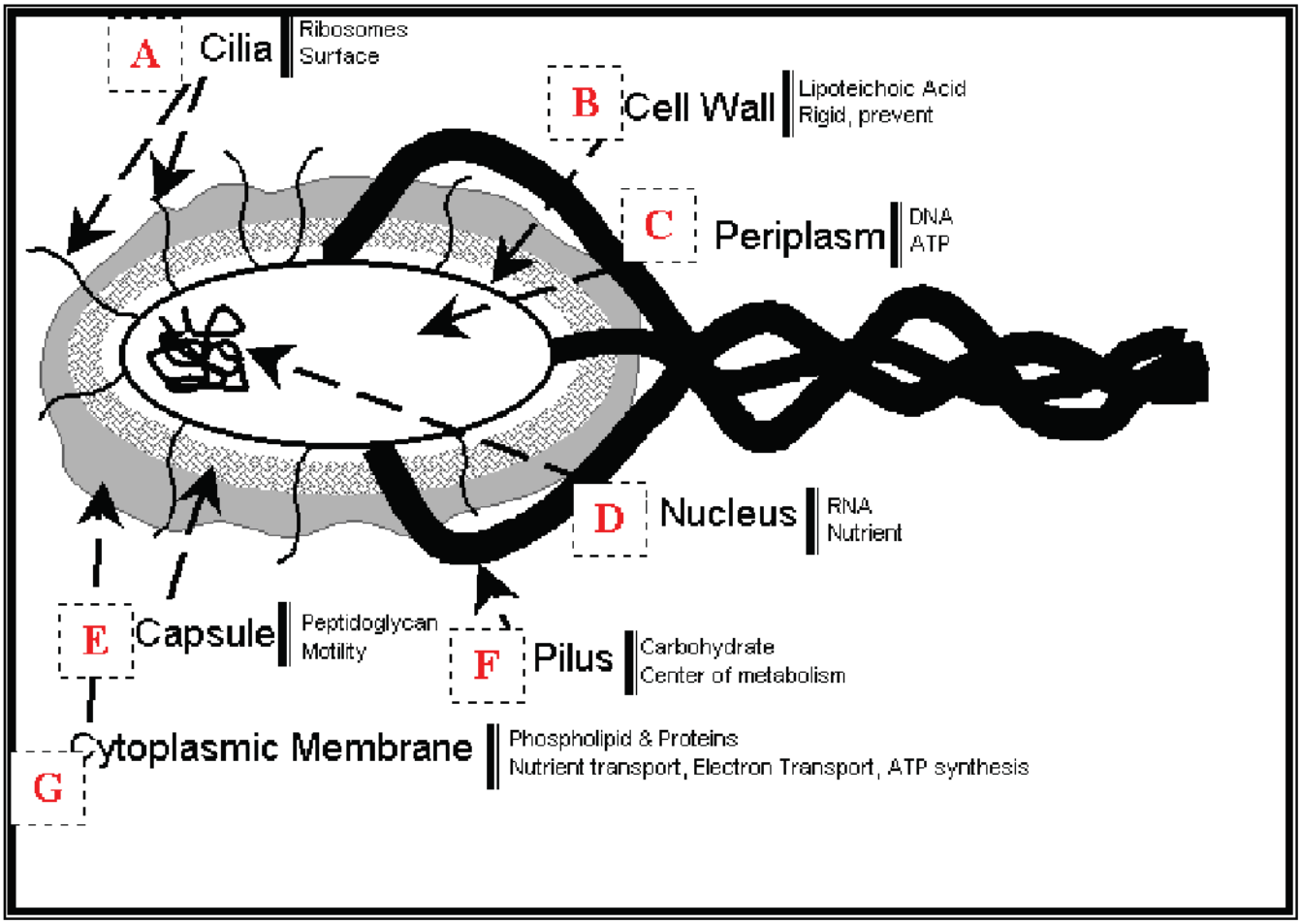


Figure 2—Goo sample



3. Your boss has done a little reading about microorganisms, but he finds it all pretty complicated. "It's like a foreign language!" he complains. "I have to face the press to explain our idea that microbes might be responsible for all the damage to ELVIS. I think that it will help my press conference presentation a lot if I can use some visual aids. What I want to do is explain to my audience what bacteria look like. You know… the functional architecture of bacteria and how they might be able to degrade polyurethane. I think that eukaryotes might be too complicated for this audience, so I just want to show them what Gram-negative bacteria look like in a schematic diagram. I've put together this diagram of a typical Gram-negative cell. Take a look at it and make any corrections you think are necessary. Notice that I not only labeled the features, I also indicated the major biochemical composition and function(s) of each main feature. Oh yeah … this figure will probably make it into lots of newspapers, magazines, and web sites, so it needs to be scientifically correct. We wouldn't want to make DuPunt look stupid, would we? I've already done most of the work. Just proofread it and make any necessary corrections." *(See Figure 3 next page.)*

Figure 3—Uncorrected Sketch



Part II—Suspicious Minds

Your direct microscopic observation of microorganisms in the soil samples has sparked your boss's interest. He is eager to determine what type of microorganism(s) is present: eukaryotic or prokaryotic, Gram-positive or Gram-negative, or maybe even something new, never before seen on Earth. He sends a sample of the soil o to a biochemistry laboratory for direct analysis.

You are equally interested in the nature of the microbes, but instead of directly analyzing the soil, you first isolate a pure culture of a microorganism that you demonstrate has the ability to degrade polyurethane. You send a sample of this pure culture to the same biochemistry laboratory for analysis.

Later, you receive the results of the analysis of your boss's sample and your pure sample.

Table 1

Test	Boss's sample	Your sample
S ribosomes	+	–
70 S ribosomes	+	+
Circular DNA	+	+
Linear DNA	+	–
RNA	+	+
Phospholipid membranes containing electron transport proteins	+	+
Peptidoglycan	+	+
LPS	+	+
Lipoteichoic acid	+	–
Flagellar basal body proteins	+	+
Pilus proteins	+	+
Nuclear pore proteins	+	–
Histone proteins	+	–

"I'm not sure what's wrong with your sample, but my results prove that we are dealing with a new kind of life form here … I'm calling it the "preuk-aryote" because it has components characteristic of both prokaryotes and eukaryotes. It's time for a press conference!" boasts your boss.

Later on, as you are getting ready to head home after a long day in the lab, you hear your boss bellow, "What the H-E-double hockey sticks is going on here!"

You ask him what happened.

" is morning I put a few thousand cells from your pure culture of Extraterrestrial PolyUrethane-Degrading Microbe (ETPUM) onto two slides in some water, but then I had to go to that press conference, and I didn't have enough time to look at the cells carefully except to notice that they were uniformly distributed under the coverslip. I didn't want the slides to dry out so I sealed the edges of the coverslips. On this slide I used

a rubber gasket to make the seal, and on this slide I used a Lycra gasket. Now look at the cell distribution! On the rubber-sealed slide, the cells are still uniformly distributed, but on the Lycra-sealed slide all the cells have congregated around the edge of the coverslip. Look… they are all over at the edges; none are left in the middle part of the slide. Could somebody have come in here and moved all those ETPUM cells over to the edges? But who? Maybe someone small with really tiny tweezers. Did you see anyone like that lurking around this scope? Nah… I need to get a grip on reality here. No tweezers could be that small."

Questions

1. How would you go about isolating your pure culture?
2. If your goal is to characterize the ETPUM, whose results are more informative: yours or your boss's? Why? What do your results indicate about the nature of this microbe? Does its biochemical composition most closely resemble that of a prokaryote or a eukaryote? Gram-positive or Gram-negative? Do you agree with your boss's conclusion that the ETPUM is a prokaryotic-eukaryotic hybrid? Why or why not?
3. Come up with at least two possible alternative explanations for the "amazing" redistribution of the ETPUM on the Lycra-sealed slide. Both of your explanations should consider how the microbes "sensed" the presence of polyurethane. One of your answers should not involve flagella.

Part III—All Shook Up

You have found media that support growth of pure cultures of ETPUM in your laboratory. e recipes for these media are shown below:

Table 2

Medium #1	Medium #2
g yeast extract	10. g K_2HPO_4
20 g tryptone extract	4. g KH_2PO_4
. g NaCl	1 g $MgSO_4$
3.6 g glucose	10 g polyurethane
1 l H_2O	1 l H_2O

Growth in these media:

Table 3

Growth	Medium #1	Medium #2
ETPUM growth—aerobic	+	+
ETPUM growth—anaerobic	+	–
E.coli growth—aerobic	+	–
E.coli growth—anaerobic	+	–

You are excited because, in Medium #2, ETPUM utilizes polyurethane as its energy source and its sole source of carbon and nitrogen, a finding that raises the possibility that ETPUM could be a useful tool for bioremediation of polyurethane-containing wastes (in landfills, etc.). You have also made some progress in characterizing the central metabolic pathways and related biochemical activities of ETPUM. In particular, you have discovered that:

- ETPUM secretes an enzyme (polyurethanase) that catalyzes degradation of polyurethane, generating citric acid (citrate) as a product.
- e cytoplasmic membrane of ETPUM contains an ABC transport system capable of transporting citrate across the membrane at the expense of 4 ATP molecules (hydrolyzed to form ADP and phosphate) per molecule of citrate transported.
- e cytoplasm of this organism contains all of the enzymes required for glycolysis, and for the TCA cycle.
- e cytoplasmic membrane of ETPUM contains proteins that form a functional electron-transport pathway (that uses O_2 as the terminal electron acceptor).

Questions

1. Which medium would you consider to be "complex" and which "defined"? Which is "rich" and which is "minimal"? Explain your answers.
2. Given that polyurethane is a huge polymer (MW >>100, Daltons), why is it important that the polyurethanase is a secreted enzyme? If we assume that the polyurethane is the source of energy for the

organism, how can material (carbon atoms) from it find its way into the central metabolic pathways of this microbe? What is the "entry point"? What happens after its entry into the metabolic pathway?

3. Why does growth of ETPUM in Medium #2 require oxygen? ink about this in terms of how ETPUM can generate a net gain in ATP by processing polyurethane. Remember that the degradation of polyurethane by polyurethanase does not expend ATP. In order to answer this question, address each of the following questions in your answer:

   a. Is there a net gain or loss of ATP during the transport of the citrate?

   b. Consider the ATPs that can be generated via substrate-level phosphorylation. Will glycolysis be useful for generating any ATPs during growth on polyurethane? How many ATPs can be generated via TCA? Is this enough to support growth (is there a net positive in the ATP tally)?

   c. Now consider how else ETPUM can generate ATPs (if not by substrate- level phosphorylation). Can this process generate a net positive in the ATP tally?

   d. Now explain the importance of oxygen as relates to the ATP tally.

Part IV—A Little Less Conversation

Questions

1. At a press conference announcing your company's successful isolation and characterization and cultivation of ETPUM, a reporter raises an important question: "How do you know that this microbe actually came from NPIP (the Nearby Previously Invisible Planet) and not from Earth? Could this microbe really be an Earth microbe that was present in/on ELVIS before it was launched into space?"

   " at's impossible," your boss answers. "Prior to launch, we wiped down the entire ELV and ELVIS with the disinfectants ethanol and triclosan. In addition we used plastics impregnated with disinfectant chlorhexidine. I had our chemists check! ese chemicals do all kinds of nasty things to cells: coagulate proteins and destroy membranes. No microbes could have survived that."

   What do you think? Were these treatments adequate to rule out the possibility raised by the reporter?

2. You are a consultant for a second ELVIS mission to NPIP. Describe what physical and/or chemical treatments you would require prior to lifto to minimize the opportunity for contamination of the ELV (the landing module of the spacecraft) and ELVIS (a new version of the robot) by Earth microbes.

   HINT: Keep in mind the following: ELVIS's skin cannot withstand temperatures above 90°C; the ELV itself is approximately the size of a minivan and includes many metal parts. Use scientific terminology as you discuss this answer: e.g., are you trying to achieve disinfection or sterilization? Are you recommending use of antiseptics or disinfectants? Specify how each of your treatments would achieve killing of microbes.

3. In your opinion, is EPTUM an earthly isolate or an extraterrestrial isolate? Some things to think about: Have bacteria been isolated from outer space to date? What is this field of study? What agencies fund this field? What is "Planetary Protection"? Are there earthly microbes that have not yet been isolated and grown in culture? If so, what is the predicted percentage? Are there methods to study microbes that cannot be cultured?