NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Evolution in Action: The Power of Mutation in *E. coli*

by
Merle K. Heidemann, College of Natural Science, Emeritus
Peter J. T. White, Lyman Briggs College
James J. Smith, Lyman Briggs College
Michigan State University, East Lansing, MI

Introduction

Dr. Richard Lenski, a researcher at Michigan State University, has shown, in real time, evolution in cultures of the widely known bacterium, *Escherichia coli*. This required immense patience over decades to see if different phenotypes would emerge from separate, but initially identical cultures of *E. coli.* After about 33,000 generations, one of the cultures gained an advantage: it could also use another molecule in addition to glucose, citrate, to fuel its activities under conditions where it previously could not. Dr. Lenski's prediction was supported by a change in *E. coli* metabolism and showed that evolution could take place in an unchanging environment.

An acute reader of this paragraph will likely have questions about this finding. These include:

- What happened to *E. coli* to produce a new phenotype?
- Is there a genetic basis of this new phenotype?
- How do bacterial genes function?
- What role did chance play in producing the new phenotype?
- How does metabolism "work" in *E. coli?*
- Was the *E. coli* with the new phenotype able to outcompete its fellow flask dwellers?

Of course, as you learn more about Dr. Lenski's research, more interesting questions will arise.

Instructions

1. *E. coli* is often in the news, associated with both good and bad events. Make a list of everything you know about the bacterium *E. coli.* This can include where it lives (besides in a flask), its basic structure and functions, its genetic makeup, and possible answers to the questions above. We will discuss this list as a class.
2. Next, based on your lists (above) and the handout Expert Facts provided by your instructor, generate an additional list of questions in your groups that should, when answered, complete your understanding of how *E. coli* evolved the ability to use citrate as a food under new conditions. These questions may include those posted above, but you should have thought of many more in your group.

Over the next several days we will gain a complete understanding of Dr. Lenski and his group's work, which we'll call *Evolution in Action*. It doesn't really matter where we begin since this understanding will encompass everything from ecology to molecular genetics.

Module: *E.coli* Basics and the Long Term Evolution Experiment or LTEE

Introduction

A key aspect of *Evolution in Action* is that *E. coli* has long been a model organism for studying biochemistry, genetics and more recently, evolution, which, of course is interwoven with all sub-disciplines of biology. Your task is to find out why and how *E. coli* is a perfect organism for Dr. Lenski's studies.

Instructions

Sort through your questions and findings from the case study introduction and identify information that applies to the basic biology of bacteria in general and *E. coli* specifically. This information will be the basis for your small group investigations. You may generate additional questions, which you can also investigate in this section of the case study.

Investigation Phase

1. Download the *E. coli* Citrate Metabolism Evolution slide set from:
   http://www.evo-ed.com/Pages/Ecoli/index.html
   These slides will be used in this module and throughout the case study. As you study these slides, pay attention to the specific information in the notes pane for each slide.

   Guide to Slides:
   Slide numbers are visible when viewing the presentation in "normal view."

   - Slides 5–15 provide background about bacterial structure, diversity and metabolism, as well as detailed information about *E. coli* as a model organism.
   - Slides 16–27 show how *E. coli* is being used to study *Evolution in Action* by Richard Lenski at Michigan State University.

2. After viewing these slides and revisiting your questions and hypotheses related to *E. coli* as a study organism, complete the following questions.

Questions

1. Dr. Lenski's study requires an understanding of how *E. coli* obtains metabolic energy from food. Organisms obtain this energy by aerobic respiration, anaerobic respiration or fermentation. Do a little outside research and compare and contrast each of these.
2. Is *E. coli* a "good" or "bad" bacterium? Explain your answer.
3. What attributes of *E. coli* make it a good organism for research purposes?
4. Describe the protocol for Dr. Lenski's Long Term Evolution Experiment (LTEE) studies.
5. What was observed in flask #9?
6. *Application question:* Provide a reasonable explanation, based on what you learned from Question 1, for the sudden increased growth of *E. coli* population in flask #9.
7. *Challenge question:* How does a change in growth rate lead to a change in fitness?

Module: Citrate Metabolism in *E.coli*

Introduction

One of the cultures in Dr. Lenski's study gained an advantage in that it could use citrate to fuel its activities under conditions where it previously could not. This "new" condition was the utilization of citrate when oxygen was present, where previously, *E. coli* could use citrate only when oxygen was absent. This ability added a new food to their regular diet of glucose. The question to pursue is: How do these *E. coli* utilize citrate?

Instructions

Sort through your findings from the case study introduction and identify information that applies to metabolism in *E. coli.* This information will be the basis for your small group investigations. You may generate additional questions about the utilization of both glucose and citrate by *E. coli*, which you will investigate in this section of the case study.

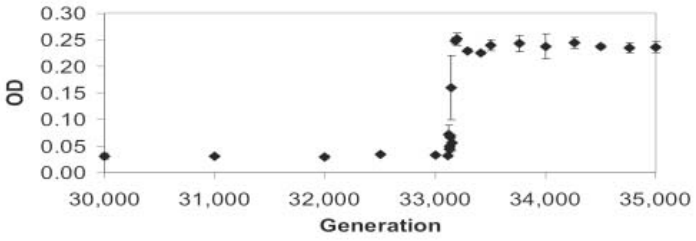
Investigation Phase

1. Download the *E. coli* Citrate Metabolism Evolution slide set from:
   http://www.evo-ed.com/Pages/Ecoli/index.html
   These slides will be used in this module and throughout the case study. As you study these slides, pay attention to the specific information in the notes pane for each slide.

   Guide to Slides:
   Slide numbers are visible when viewing the presentation in "normal view."
   - Slides 28–35 compare glucose and citrate as food sources in the medium in which *E. coli* are growing.
   - Slides 36–46 introduce the CitT transport protein and the consequences for *E. coli* growth when it can be produced in conditions with oxygen present as well as conditions without oxygen. The slides also introduce the citric acid cycle and changes that occur when the CitT transport protein is produced and active in the cell.
2. After viewing these slides and revisiting your questions and hypotheses related to the cell biology of *E. coli*, particularly in terms of metabolism, complete the following questions.

Questions

1. The figure to the right shows what happened in flask #9 in Dr. Lenski's studies. Based on this figure:
   a. What does OD mean and what does it represent?
   b. What do higher OD values at generation ~33,000 tell us about the population of *E coli* growing in flask #9?
   c. What changes in cell function allowed this happen?



2. Acetyl Co-A is the normal input to citric acid cycle reactions. What is the original molecule that *E. coli* breaks down to produce Acetyl Co-A? How does it get into the cell?
3. What are the inputs and outputs of the citric acid cycle? Be sure to keep track of "energy management" molecules, those that carry energy. What happens to the outputs of the citric acid cycle?
4. What happens to citrate in *E. coli* when oxygen is not present?
5. In Dr. Lenski's studies, *E. coli* evolved a way for citrate to enter a cell from the medium in conditions with oxygen. How does citrate get into the cell? Fully describe how the protein involved works.
6. Follow both the matter and energy as citrate enters the cell and then is incorporated into citric acid cycle reactions under aerobic conditions.
7. What is the advantage to *E. coli* of being able to import citrate under conditions both with and without oxygen?
8. *Application question:* Why is succinate important when explaining that *E. coli* evolved to take up citrate under conditions with oxygen?

Module: The Molecular Genetics of Citrate Metabolism in *E. coli*

Introduction

Researchers in Dr. Lenski's lab have determined that the robustness of *E. coli* from flask #9, central to *Evolution in Action*, can be explained by changes in the operon controlling citrate transport. It's a mutant! However, understanding the magnitude of this discovery requires that we be able to explain the control of gene expression in bacteria generally and of the citrate transport operon particularly.

Instructions

Sort through your findings from the case study introduction and identify information and questions that apply to the genetics of *E. coli* metabolism. This information will be the basis for your small group investigations. You may generate additional hypotheses about the related molecular genetics, which you will investigate in this section of the case study.

Investigation Phase

1. Download the *E. coli* Citrate Metabolism Evolution slide set from:
   http://www.evo-ed.com/Pages/Ecoli/index.html
   These slides will be used in this module and throughout the case study. As you study these slides, pay attention to the specific information in the notes pane for each slide.

   Guide to Slides:
   Slide numbers are visible when viewing the presentation in "normal view."
   - Slides 47–54 introduce the *E. coli* citrate operon and its mechanism of control.
   - Slides 55–63 discuss the genetic changes that allow the *E. coli* citrate *(cit)* operon to be functional in aerobic conditions.

2. After viewing these slides and revisiting your questions and hypotheses related to molecular genetics underlying citrate metabolism of *E. coli,*, complete the following questions.

Questions

The citrate (cit) *operon*

1. Operons are the basic unit of gene expression in prokaryotes. Explain the role of each of these operon components in prokaryotic gene expression: *structural genes, promoter, repressor,* and *operator.*
2. Fully describe the *E. coli cit* operon as it functions in conditions without oxygen. Include *promoter*, *repressor* and *operator* in your description.
3. The *cit* operon is under negative control. What does that mean and how is this operon negatively controlled?
4. What is the result of the *cit* operon being negatively controlled under conditions were oxygen is present?
5. What is the relationship between the *cit* operon and synthesis of the CitT transporter protein?

Genetic changes in the cit *operon: Mutation*

6. A section of the citrate operon, including the *citT* gene, was duplicated in an individual in flask #9. What precisely was duplicated? What happened in terms of operon structure after this duplication?
7. Draw a sketch of both the pre- and post mutation citrate operon.
8. How did this new arrangement facilitate the transcription of the *citT* primary transcript under conditions *without* oxygen?

   How did this new arrangement facilitate the transcription of the *citT* primary transcript under conditions *with* oxygen?
9. *Application question:* Was the rearrangement within the citrate operon the result of a single or multiple genetic event(s)? Explain.
10. *Synthesis question:* Explain the advantages and disadvantages of the citrate operon mutation in *E. coli* living in a flask with *E. coli* that do not have this mutation.

Module: The Ecology and Phylogenetics of Citrate Metabolism in *E. coli*

Introduction

We know that organisms living together form an ecosystem, even when the organisms are from different populations of *E. coli* and the ecosystem is as simple as a flask with nutrients. To further our investigation of *Evolution in Action*, we need to know if *E. coli* populations with different food utilization abilities (those in flask #9) co-exist. Will one outcompete the other?

Instructions

Sort through your findings from the case study introduction and identify information that applies to the ecology of *E. coli.* This information will be the basis for your small group investigations. You may generate additional hypotheses about the ecology and phylogenetics of *E. coli*, which you will investigate in this section of the case study.

Investigation Phase

1. Download the *E. coli* Citrate Metabolism Evolution slide set from:
   http://www.evo-ed.com/Pages/Ecoli/index.html
   These slides will be used in this module and throughout the case study. As you study these slides, pay attention to the specific information in the notes pane for each slide.

   Guide to Slides:
   Slide numbers are visible when viewing the presentation in "normal view."

   - Slides 64–70 show the niches of *E. coli* bacteria in the Flask #9 ecosystem.
   - Slides 71–73 show the phylogenetic relationships of the evolving populations in the LTEE study.

2. After viewing these slides and revisiting your questions and hypotheses related to the LTEE ecosystem and Flask #9 descendants, complete the following questions.

Questions

1. What molecule(s) did the original LTEE population use for food?
2. What molecule(s) did the *newly* evolved Cit+ population use for food?
3. What molecule(s) did the established populations derived from Flask #9 use for food?
4. Can the Cit- and Cit+ populations co-exist in the same flask? Explain your answer.
5. Was the original LTEE population that occupied the 12 flasks homogeneous or heterogeneous? First, define these two terms and then explain your answer. Were the populations that occupied flask #9 likely homogeneous or heterogeneous after 33,000 generations? Explain your answer.
6. *Synthesis question:* What if the Cit transporter was not an antiporter and succinate was not exchanged for citrate. How would this affect the relationship of Cit- and Cit+ living together in the same flask?