Bacterial Fermentation of Carbohydrates Activity

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Overview

The gut microbiome plays an important role in carbohydrate digestion. Different types of bacteria produce different digestive enzymes, which enable them to break down different carbohydrates during a process called fermentation. Fermentation releases energy from the carbohydrate molecules (which the bacteria use) and produces waste products including short chain fatty acids (SCFAs), which the host can use either directly as an energy source, or to support other physiological processes.

Learning Objectives

After completing this activity, students should be able to:

- Compare the role of different bacteria in the digestion of different carbohydrates
- Explain the process by which bacteria ferment carbohydrates to produce short chain fatty acids
- Predict the consequences of different diets and bacteria in the digestive system for overall health

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Part 1: Carbohydrate metabolism

Materials Needed
- Carbohydrate K'nex kit
- Bacteria card deck (found within kit)
- Kit instructions – posted to Sakai

Carbohydrate Structures

1. Review pages 1-3 of the Instructions and identify the different pieces in your kit.

2. Assign one of the carbohydrates to each member of your team to build using your K'nex pieces in the kit. Refer to the Kit Instructions document for details on how to build the carbohydrate.
    a. Amylose (Section 1, pages 3-12)
    b. Amylopectin (Section 2, pages 13-24)
    c. Cellulose (Section 3, pages 25-40)

3. All carbohydrates are made up of glucose subunits. Referring to your carbohydrate models, answer the following questions:
    a. How many glucose units do you count on the amylopectin model?
    b. How many glucose units do you count on the amylose model?
    c. How many glucose units do you count on the cellulose model?

4. Glucose subunits are connected by different types of chemical bonds, which can be digested with different enzymes. Refer to your carbohydrate models and answer the following questions:
    a. Which bond(s) do you see on the amylopectin model?
    b. Which bond(s) do you see on the amylose model?
    c. Which bond(s) do you see on the cellulose model?

5. Spend some time reviewing the complexity of each carbohydrate molecule (i.e., how many parts you used to build it) and structural integrity (i.e., how hard it is to break the connections). Which carbohydrate do you think is easiest and hardest to digest? List the carbohydrates in order of digestive difficulty (or complexity) below, starting with the easiest to digest.

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Bacterial digestion

6. Look through your card deck. Which bacteria are able to digest each type of bond? Record your answers below.

Bacterial taxon	**α-1,4**	**α-1,6**	**β-1,4**	**Carbohydrates**

7. Different foods are considered more <u>accessible</u> or <u>selective</u> based on the number of species that can digest them. According to your card deck, are simple or complex carbohydrates more selective?

8. Which phylum does each of the bacteria belong to? Do you see greater evolutionary diversity in the bacteria that digest simple or complex carbohydrates?

9. Based on this information, would you consider fiber digesters to be <u>generalists</u> or <u>specialists</u>?

Apply your understanding

12. Based on their carbohydrate composition, which food is the easiest to digest *without the help of any gut microbes*? Explain why.

13. Based on their carbohydrate composition, which food(s) do we need the most microbial help to digest?

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Fermentation products

After bacteria digest the bonds that connect the glucose units that make up carbohydrate molecules, they split each glucose unit into two pyruvate molecules. The bacteria then convert the pyruvate into one or more short chain fatty acids (SCFA): acetate, propionate, and/or butyrate. Just as different bacteria produce different digestive enzymes, they also produce different fermentation products. And each SCFA has a different effect on the host!

Deal out the deck of cards among your group, so that each student receives a randomly generated microbial community. Read the descriptions on the front of each card (including favorite food, what each taxa digests and which short chain fatty acids it produces), then flip the cards over to convert the pyruvates from glucose to short chain fatty acids (SCFA).

12. Do you notice any relationships between which carbohydrates bacteria can digest and which SCFA they produce from those carbohydrates?

13. Add up the total number of each SCFA that your "hand" will produce. Compare your results with those of your group.

SCFA	**Student 1**	**Student 2**	**Student 3**
Acetate			
Propionate			
Butyrate			

Apply your understanding

14. Consider your own diet (either today, or your typical / average overall intake).
    a. What proportion of each carbohydrate do you think you consume?

    b. Which bacteria does your typical diet favor?

    c. Which fermentation products do your gut microbes likely produce from your diet?

    d. How might the gut microbial community and fermentation products differ for a classmate who consumes a different diet?

STOP: Do not proceed to part 2 until instructed.

Part 2: Effects on the host

Compare the destination (i.e., which host tissues use each SCFA) and impacts (i.e., mental and/or physiological effects on the host) of each fermentation product in the figure[1] below.

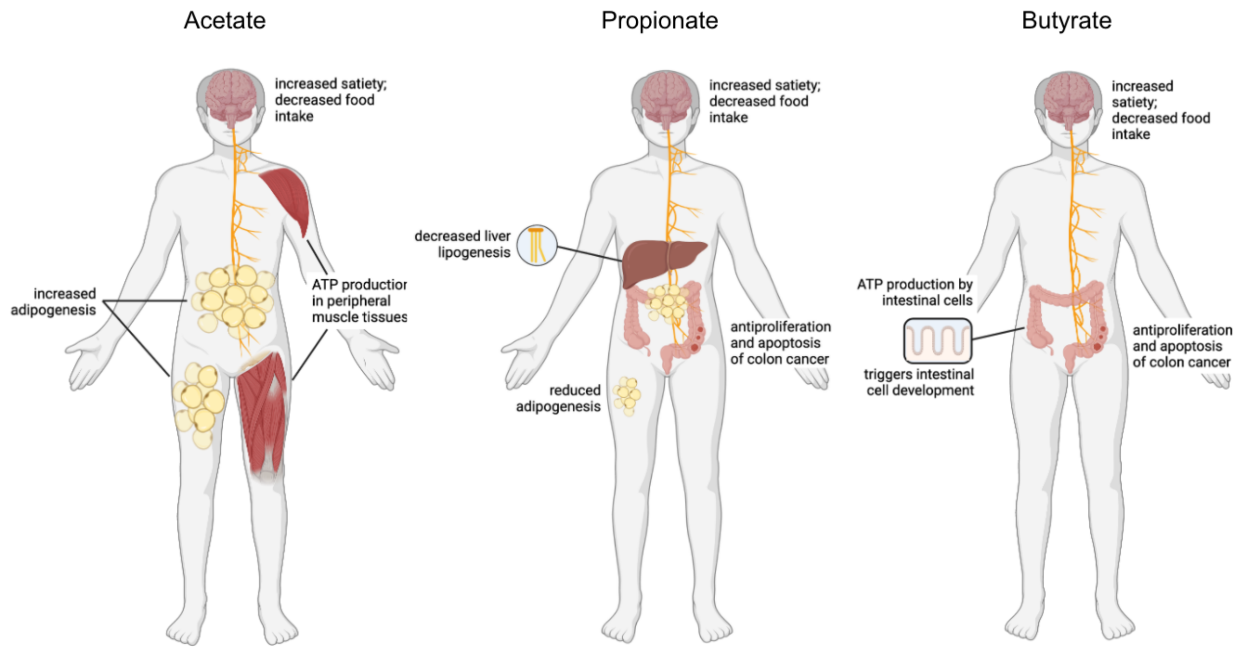

Figure 1. Comparison of the bodily effects of three short chain fatty acids: acetate, propionate, and butyrate.

15. What are the benefits of each SCFA?

    Acetate

    Propionate

    Butyrate

16. Are there any potential drawbacks of over- or under-production of these SCFAs?

17. Refer to the fermentation products you tallied from your “hand” in **Q13** above. What physiological effects would you predict will result from the SCFAs produced by that example microbial community?

[1] Figure generated by Erin McKenney via BioRender

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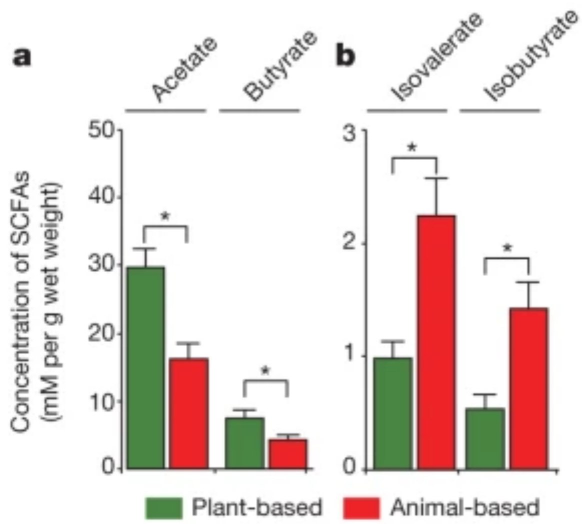
Case Study

18. Spend some time reviewing the figure below from a 2014 study[2] that explored the impact of different diets on the production of short chain fatty acids (SCFA). Summarize this figure in a single sentence. (Note, isobutyrate is a precursor for butyrate biosynthesis and isovalerate is a precursor for valerate, a five-carbon SCFA).



19. What could this figure mean for an animal who is an herbivore vs. an animal who is a carnivore?

20. Based on what you have learned in the sections above, what do you think may be different about the microbiome of the individuals who had a plant-based vs. animal-based diet?

[2] David, *et al.* (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559

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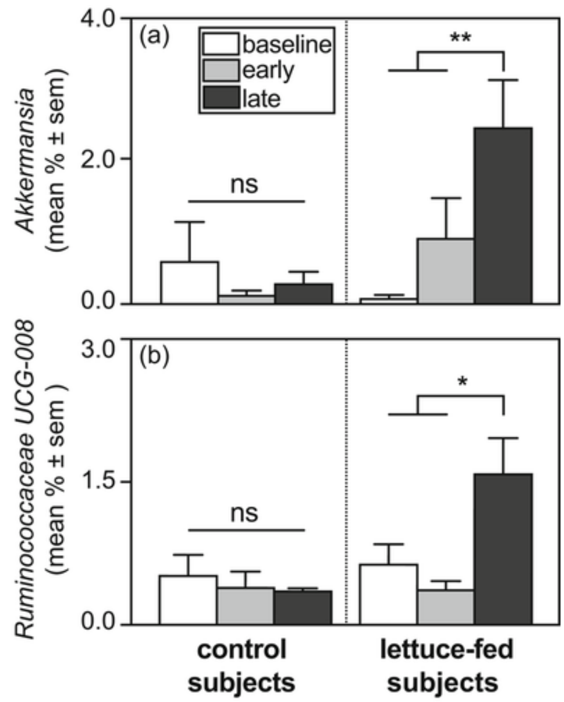
21. Spend some time reviewing the figure below from a 2020[3] study that compared the gut microbiomes of frugivorous ruffed lemurs (*Varecia rubra* and *V. variegata*) consuming standard fruit-based diets (control subjects; limited in fiber) with individuals receiving supplemental lettuce. Summarize this figure in a single sentence. (Note: *Akkermansia* are bacteria known to produce anti-inflammatory compounds.



22. Based on the carbohydrate content of lettuce (i.e., leafy greens), which SCFA(s) would you predict that these gut bacteria are producing?

[3] Greene, *et al*. (2020). Daily lettuce supplements promote behavior and modify the gut microbiota in captive frugivores. *Zoo Biology* 39, 334