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**October/November 2015 Teacher's Guide for**

***Probiotics: Good Bacteria, Good Health***

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# About the Guide

Teacher’s Guide editors William Bleam, Regis Goode, Donald McKinney, Barbara Sitzman and Ronald Tempest created the Teacher’s Guide article material. E-mail: [bbleam@verizon.net](mailto:bbleam@verizon.net)

Susan Cooper prepared the anticipation and reading guides.

Patrice Pages, *ChemMatters* editor, coordinated production and prepared the Teacher’s Guide. E-mail: [chemmatters@acs.org](mailto:chemmatters@acs.org)

Articles from past issues of *ChemMatters* can be accessed from a DVD that is available from the American Chemical Society for $42. The DVD contains the entire 30-year publication of *ChemMatters* issues, from February 1983 to April 2013.

The *ChemMatters* DVD also includes Article, Title and Keyword Indexes that covers all issues from February 1983 to April 2013.

The *ChemMatters* DVD can be purchased by calling 1-800-227-5558.

Purchase information can be found online at [www.acs.org/chemmatters](http://chemistry.org/chemmatters/cd3.html).

# Student Questions

* 1. What do the terms probiotic and microbiome mean?
  2. What are some of the benefits that some think may be provided to our bodies by some of our gut bacteria?
  3. Who first researched the role of probiotics in maintaining health?
  4. Name some foods that contain probiotics.
  5. According to the UN World Health Organization, how are probiotics defined?
  6. Explain what is meant by probiotics acting as miniature chemical factories.
  7. In addition to energy, what are the chemical products of bacterial fermentation?
  8. What are two different mechanisms by which certain probiotics may control or reduce obesity in mice?
  9. We usually associate the term pasteurization with milk, but for which drink did Louis Pasteur actually begin his research to kill bacteria with heat?
  10. How might certain bacteria known as *Lactobacillus rhamnosus* reduce anxiety in mice?
  11. What mechanism is thought to reduce anxiety when mouse gut bacteria, *Bifidobacteria*, are increased in numbers?

# Answers to Student Questions

* + 1. **What do the terms probiotic and microbiome mean?**

*The term probiotic refers to “…live strains of bacteria similar to the ones that live naturally in the human gut.” The microbiome is the name for all the bacteria living inside our body.*

* + 1. **What are some of the benefits that some think may be provided to our bodies by some of our gut bacteria?**

*Different bacteria in the gut are essential to digestion, provide energy, back up the immune system and synthesize chemicals that our body can use.*

* + 1. **Who first researched the role of probiotics in maintaining health?** *Russian physiologist and Nobel Prize winner Elie Metchnikoff first studied the effects of probiotics on people’s health in Bulgaria.*
    2. **Name some foods that contain probiotics.**

*Some foods containing probiotics include yogurt, sauerkraut, pickles, Kefir (a fermented milk drink), kimchee, kombucha, cottage cheese (with added probiotics), and buttermilk.*

* + 1. **According to the UN World Health Organization, how are probiotics defined?**

*Probiotics are defined as “… live microorganisms administered in adequate amounts which confer beneficial health effects on the host.”*

* + 1. **Explain what is meant by probiotics acting as miniature chemical factories.**

*Probiotics provide chemical reactions that convert carbohydrate molecules into alcohols or acids as they release energy through the chemical reaction, fermentation.*

* + 1. **In addition to energy, what are the other chemical products of bacterial fermentation?**

*Carbon dioxide and either lactic acid or ethanol are produced in bacterial fermentation, in addition to the energy.*

* + 1. **What are two different mechanisms by which certain probiotics may control or reduce obesity in mice?**

*It is thought that “… certain genetically modified probiotics can produce a hunger-suppressing compound which signals the brain to want less food. Other probiotics stimulate the release of a hormone called GLP-1 which also causes a reduction in food intake.”*

* + 1. **We usually associate the term pasteurization with milk, but for which drink did Louis Pasteur actually begin his research to kill bacteria with heat?**

*Louis Pasteur began his research on killing bacteria using wine, which neighboring winemakers had found was turning sour. They asked him to help them find out why.*

* + 1. **How might certain bacteria known as *Lactobacillus rhamnosus* reduce anxiety in mice?**

*When mice are fed the bacterium, Lactobacillus rhamnosus, it was found that the mice had half the normal amount of a stress hormone called corticosterone in their brains. This is due to the fact that the bacteria secrete a neurotransmitter, GABA (gamma aminobutryric acid), which influences the brain as an anti-stress compound, travelling from the intestine to the brain via the vagus nerve.*

* + 1. **What mechanism is thought to influence a number of different brain perceptions when mouse gut bacteria, *Bifidobacteria,* are increased in numbers?**

*With an increase in this particular bacterium in the gut of mice, there was a matching increase in a protein called brain-derived neurotrophic factor. This factor in turn increases the production of another chemical called serotonin which is involved in mood regulation, pain perception, and perception of hunger and satiety (feeling full).*

# Anticipation Guide

Anticipation guides help engage students by activating prior knowledge and stimulating student interest before reading. If class time permits, discuss students’ responses to each statement before reading each article. As they read, students should look for evidence supporting or refuting their initial responses.

**Directions:**  *Before reading*, in the first column, write “A” or “D,” indicating your agreement or disagreement with each statement. As you read, compare your opinions with information from the article. In the space under each statement, cite information from the article that supports or refutes your original ideas.

|  |  |  |
| --- | --- | --- |
| **Me** | **Text** | **Statement** |
|  |  | 1. Probiotics are similar to bacteria that live naturally in your gut. |
|  |  | 1. There are fewer than 100 species of bacteria living inside you. |
|  |  | 1. The idea of adding probiotics to your diet is only about 30 years old. |
|  |  | 1. All probiotics are made from dairy products |
|  |  | 1. Very specific rules have been established by authorities in the United States for commercial probiotics. |
|  |  | 1. The chemical process of fermentation releases the energy that was stored in plants through photosynthesis. |
|  |  | 1. As much as 10% of the energy you need daily may come from fermentation inside your body. |
|  |  | 1. In a healthy person, there are about twice as many beneficial bacteria as harmful bacteria. |
|  |  | 1. Because of pasteurization, store-bought milk can’t be used to produce probiotics like sour milk. |
|  |  | 1. There is a nerve that goes from your abdomen to your brain. |

# Reading Strategies

These graphic organizers are provided to help students locate and analyze information from the articles. Students’ understanding will be enhanced when they explore and evaluate the information themselves, with input from the teacher if students are struggling. Encourage students to use their own words and avoid copying entire sentences from the articles. The use of bullets helps them do this. If you use these reading strategies to evaluate student performance, you may want to develop a grading rubric such as the one below.

|  |  |  |
| --- | --- | --- |
| **Score** | **Description** | **Evidence** |
| 4 | Excellent | Complete; details provided; demonstrates deep understanding. |
| 3 | Good | Complete; few details provided; demonstrates some understanding. |
| 2 | Fair | Incomplete; few details provided; some misconceptions evident. |
| 1 | Poor | Very incomplete; no details provided; many misconceptions evident. |
| 0 | Not acceptable | So incomplete that no judgment can be made about student understanding |

***Teaching Strategies:***

1. Links to **Common Core Standards for Reading**:
   1. ELA-Literacy.RST.9-10.5: Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
   2. ELA-Literacy.RST.11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11-12 texts and topics.
2. Links to **Common Core Standards for Writing**:
   1. ELA-Literacy.WHST.9-10.2F: Provide a concluding statement or section that follows from and supports the information or explanation presented (e.g., articulating implications or the significance of the topic).
   2. ELA-Literacy.WHST.11-12.1E: Provide a concluding statement or section that follows from or supports the argument presented.
3. **Vocabulary** and **concepts** that are reinforced in this issue:
   1. Solution chemistry
   2. Chemical equilibrium
   3. Acids and bases
   4. pH
   5. Buffers
   6. Molecular structures
4. The infographic about autumn leaves on page 19 will engage students with more information about some of the natural dyes found in “Eating With Your Eyes.”
5. To help students engage with the text, ask students which article **engaged** them most and why, or what **questions** they still have about the articles. The Background Information in the *ChemMatters* Teachers Guide has suggestions for further research and activities.

**Directions:** As you read the article, complete the graphic organizer below to describe probiotics.

How are they made?

What are they?

Probiotics

How do they work?

What are some of their advantages?

**Summary:** On the back of this sheet, describe probiotics you might try after reading the article, and why you might eat them.

# Background Information

**(teacher information)**

**More on** **the science of probiotics**

The term probiotics is a general one which requires the specific names of bacteria and the chemicals they emit that may produce a variety of effects mentioned in the *ChemMatters* article. In general parlance, the role of probiotics often strays from documented evidence of what these substances can do. This is particularly true when non-scientific publications report on the properties of the probiotics. The present state of affairs hints at what these organisms might be capable of doing. What is badly needed is scientific documentation (i.e., isolation) of the particular chemicals that are produced by various probiotics and specifically what biological targets respond to these chemicals. As mentioned in a government report by NIH (National Institutes of Health), “…it is important to stress that the biological effects of probiotics are strain specific and the success or failure of one strain cannot be extrapolated to another strain. Thus proper identification using novel molecular and based technologies is imperative.” (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4053917/>).

Then again, some of the functions of the microbiome in the human digestive tract are well documented.

Research suggests that the relationship between gut flora and humans is not merely commensal (a non-harmful coexistence), but rather a mutualistic relationship. Though people can survive without gut flora, the microorganisms perform a host of useful functions, such as fermenting unused energy substrates, training the immune system, preventing growth of harmful, pathogenic bacteria, regulating the development of the gut, producing vitamins for the host, such as biotin and vitamin K, and producing hormones to direct the host to store fats. In return, these microorganisms procure within the host a protected, nutrient-rich environment in which they can thrive….Not all the species in the gut have been identified because most cannot be cultured, and identification is difficult.

*(*[*https://en.wikipedia.org/wiki/Gut\_flora*](https://en.wikipedia.org/wiki/Gut_flora)*)*

The standard bacteria used for making yogurt, an oft-cited source of probiotics, have been well studied for years. They are considered to be probiotics because they help lactose-intolerant people digest yogurt by breaking down the sugar lactose, which these people cannot do because they lack the enzyme lactase. However, these bacteria—*Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*—don't make it through the intestines alive. That's why many yogurt producers add other probiotics that can survive in the gut. Lactic acid bacteria (LAB) are the most common microbial genera used in probiotics.

(<http://www.livescience.com/46298-the-lowdown-on-probiotics.html>)

Research shows that feeding mice with yogurt containing probiotics does not produce colonization of the cultures in the gut. But ingesting the bacteria directly does produce beneficial effects related to obesity and possibly sugar metabolism (i.e., reducing insulin resistance in diabetes). VSL#3, a commercial product name, has been shown to increase probiotic bacteria, which in turn reduce certain circulating inflammatory cytokines that are related to inflammation associated with diabetes.

Research scientists suggest certain criteria for probiotic bacteria.

The bacterial strains that are selected for probiotic milk products by the culture supplier should fulfill the following requirements:

1. Survive the gastric acidic conditions (pH 1–4).
2. Be resistant to the action of bile salts.
3. Resist degradation by digestive enzymes present in the intestines (e.g. lysozymes).
4. Survive action of toxic metabolites, primarily phenols, produced during the digestion process, antibiotics and phage, anaerobic growth conditions and storage conditions of the food carrier.

(<http://www.nature.com/icb/journal/v78/n1/full/icb200012a.html>)

What other things can probiotics do that have some scientific validity? Scientists have already demonstrated that the gut microbiome is importantly involved in the development of the human immune system, and that abnormalities in microbial diversity are correlated with several inflammatory diseases. Some probiotics have been shown to be effective or possibly effective in preventing pediatric antibiotic-associated diarrhea, necrotizing enterocolitis in preterm infants, and upper respiratory tract infections. They have been shown to be effective or possibly effective in treating acute infectious diarrhea and persistent diarrhea in children. To date, probiotics, such as *L. acidophilus* (found in yogurt) have not been shown to be effective against bacterial vaginosis, ulcerative colitis, Crohn’s disease, or preterm labor. These conclusions are based on reports from the National Center for Biotechnology Information. (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3859987/>)

Additionally, many studies have shown that different probiotics can impact immune function in different ways. But, because the effects of probiotic strains can vary broadly, and immune-system effects are incredibly diverse, an international panel of researchers have decided that an immune-system boost should not be considered a core benefit of probiotics,

And from an article by one of the medical authorities at Tufts University, Joel Mason, who is a physician and senior scientist at the Jean Mayer USDA Human Nutrition Research Center on Aging and a faculty member at Tufts School of Medicine and the Friedman School:

Many advertisements and Internet postings say probiotics are effective for the treatment of asthma, dermatitis, irritable bowel syndrome and other conditions. At best, there is marginal evidence that probiotics help these conditions.

Where they have been convincingly shown to be beneficial is in the treatment or prevention of certain kinds of diarrhea, as well as in ulcerative colitis and with certain problems that can accompany fat malabsorption….Many people eat yogurt because it contains probiotics, but studies looking at the potential usefulness of probiotics in a rigorous scientific manner have generally used pure preparations, not yogurt. So whether the helpful bacteria you get by eating yogurt are really as effective as pure probiotics is up in the air right now.

(<http://now.tufts.edu/articles/can-probiotics-keep-gastrointestinal-health#sthash.S9ekHblO.dpuf>)

The *ChemMatters* article mentioned a number of probiotic food products that contain live bacteria. Researchers feel that these foods should not be labeled as containing probiotics unless the bacterial cultures have been shown to provide health benefits as probiotics. Rather they should be labeled as sources of particular bacteria and not sources of probiotics. Three of the products (Asian), Kefir, kimchee, and kombucha have not been shown to have any beneficial effects other than being of nutritional, rather than probiotic, value. And one of them, kombucha, presents some health risk, according to the Mayo Clinic. On the other hand, combining yogurt with kefir, both fermented milk products, create a symbiotic situation, in that the two contain live bacteria and the fuel they need to thrive.

A basic problem with identifying these beneficial bacteria is that not all the species in the gut have been identified because most cannot be cultured. So this fact limits identification. Bacteria make up most of the flora in the colon and up to 60% of the dry mass of feces. Somewhere between 300 and 1000 different species live in the gut, with most estimates at about 500. However, it is probable that 99% of the bacteria come from about 30 or 40 species. Fungi, protozoa, and archaea also make up a part of the gut flora, but little is known about their activities. (<https://en.wikipedia.org/wiki/Gut_flora>)

A healthy gut is mostly composed of bacterial species that fall within two different groups of bacteria: the phyla Bacteroidete*s* and Firmicutes.

A large, government-funded project known as the Human Microbiome Project is in operation to determine the genetic markers on the many bacteria in the body. The purpose of the Human Microbiome Project is to collect all the microorganisms living in association with the human body. These communities consist of a variety of microorganisms including eukaryotes, archaea, bacteria and viruses. An understanding of what microbes exist in the body as well as their genetics and biochemical activity could lead to the development of pharmaceuticals that can either control their behavior (think antibiotics) or enhance their beneficial functions. To date, “… the Human Microbiome Project has published an analysis of 178 genomes from microbes that live in or on the human body. The researchers discovered novel genes and proteins that serve functions in human health and disease, adding a new level of understanding to what is known about the complexity and diversity of these organisms.” (<http://www.sciencedaily.com/releases/2010/05/100520141214.htm>)

**More on** **the role of certain gut bacteria in affecting obesity and insulin-resistant diabetes.**

Another idea about a gut bacterium producing some product related to reducing obesity comes from research done at Vanderbilt University. The design of the research is described in a press release published by the American Chemical Society. ([www.acs.org/content/acs/en/pressroom/newsreleases/2015/march/special-microbes-make-anti-obesity-molecule-in-the-gut.html](http://www.acs.org/content/acs/en/pressroom/newsreleases/2015/march/special-microbes-make-anti-obesity-molecule-in-the-gut.html))

Related to the idea of weight or obesity reduction is the discovery of a lipid produced by certain gut bacteria that reduces feeling of hunger. A chemical called N-acyl-phosphatidylethanolamines (NAPEs) is produced in the small intestine after a meal and is quickly converted into N-acyl-ethanolamines (NAEs), potent appetite-suppressing lipids. Researchers have been able to genetically alter certain probiotic bacteria to produce NAPE. Then they have special mice, bred to gain weight on a regular diet, ingest these altered bacteria. Compared with controls, these mice do not gain excess weight (in fact, a weight *reduction* of 15%) while on their normal diet. In addition, these mice do not show the usual development of fatty livers and diabetes.

One study shows that the use of a commercial product (one of many) called VLS#3 which contains viable probiotics both reduces or controls obesity gains as well as reducing the development of insulin-resistance in mice. The manner in which this product works is that it causes an increase in a short chain fatty acid, butanoic acid, which in turn stimulates the production of an appetite-suppressing hormone called GLP-1 that reduces food intake and improves glucose tolerance.

The VLS-#3 product contains eight different bacterial strains which are able to survive in the gut. The mix of bacteria Include:

4 strains of *Lactobacilli:*

*L. acidophilus,*

*L. paracasei,*

*L. delbrueckii subsp. bulgaricus,*

*L. plantarum*) and

3 strains of *Bifidobacteria:* (*B. breve, B. infantis, and B. longum*) and

1 strain of *Streptococcus thermophiles*

Four formulations are available including capsules with 112.5 billion live bacteria, packets with 450 billion live bacteria, double strength packets and junior packets, each with 225 billion bacteria. A commercial description of VLS#3 can be found at <http://www.vsl3.com/discover/about-probiotics/>. And drugs.com provides a fact sheet on VLS#3 here: <http://www.drugs.com/mtm/vsl-3.html>.

**More on preventing intestinal infections with probiotics**

There is increasing evidence that probiotics can benefit the human host by acting as a first line of defense against disease-causing pathogens by improving the intestinal microflora. Biologically effective probiotic bacteria exert their influence by inhibiting the growth of enteric pathogens through the production of lactic acid and antimicrobial peptides, known as bacteriocins.

Lactobacillus acidophilus and bifidobacteria exert antagonistic effects on the growth of pathogens such as *Staphylococcus aureus, Salmonella typhimurium, Yersinia enterocolitica* and *Clostridium perfringens*. Probiotic bacteria enhance resistance against intestinal pathogens via antimicrobial mechanisms. These include competitive colonization and production of organic acids, such as lactic and acetic acids, bacteriocins and other primary metabolites, such as hydrogen peroxide, carbon dioxide and diacetyl. By competitive colonization, probiotic bacteria inhibit the adhesion of gastrointestinal pathogens to the intestinal mucosa. Production of organic acids, such as lactic and acetic acids, by probiotic bacteria lowers intestinal pH and thereby inhibits the growth of pathogens.

(<http://www.nature.com/icb/journal/v78/n1/full/icb200012a.html>)

And probiotics can provide Immune enhancement at the gut level.

The epithelial lining of the gastrointestinal tract offers a vast surface area for the absorption of molecules and presents a barrier to the countless number of extraneous antigens that may pass through the gut. The exclusion or elimination of potential foreign antigens is mediated by the gut immune system, known as the gut-associated lymphoid tissue (GALT). It is well known that a variety of dietary antigens and commercial and pathogenic micro-organisms can cross the gut mucosal barrier and cause disease or stimulate an immune response. … (A researcher) has reported that there is sufficient evidence to suggest that lactic acid bacteria exert their immunity enhancing effects by augmenting both non-specific (e.g. phagocyte function, NK cell activity) and specific (e.g. antibody production, cytokinase production, lymphocyte proliferation, delayed-type hypersensitivity) host immune responses.

(<http://www.nature.com/icb/journal/v78/n1/full/icb200012a.html>)

Another function of probiotics, in terms of the gut mucosal barrier, is to restore the integrity of the intestinal mucosa if it loses its ability to block passage of pathogenic bacteria because of intestinal inflammation. Ingesting probiotic bacteria that then establish themselves (colonize) in the intestine have been shown to effectively treat patients with intestinal disorders including rotavirus diarrhea in children, persons with food allergies, and patients undergoing pelvic radiotherapy. All these health conditions have their etiology in a disturbed intestinal mucosa and altered gut permeability.

**More on fecal transplants**

One infectious condition that can be cured using gut bacteria is *clostridium difficile* infection, CDI. Fecal Microbiota Transplant (FMT) is a procedure in which fecal matter, or stool, is collected from a tested donor, mixed with a saline or other solution, strained, and placed in a patient, by colonoscopy, endoscopy, sigmoidoscopy, or enema. *Clostridium difficile* is a very serious infection, and its incidence is on the rise throughout the world. The CDC reports that approximately 347,000 people in the U.S. alone were diagnosed with this infection in 2012. Of those, at least 14,000 died. Fecal transplant is considered to be an inexpensive and low risk treatment for a clostridium infection. (<http://thefecaltransplantfoundation.org/what-is-fecal-transplant/>)

CDI infections are considered a very serious health situation for the elderly (those over 65). This population segment develops complications with the disease and attended relapses, compared with the under-65 group of patients. These patients often are missing certain gut flora, most likely due to antibiotic usage. It is thought that some kind of signaling takes place between healthy bacteria and the mucosa of the gut, and without that signaling, *C. difficile* can take over. Restoring the missing flora seems to be the key. And that is what happens when fecal transplants are done. It has been shown that patients having undergone a fecal transplant are cured within 24 hours to several days. This research is from respected health organizations such as the Mayo clinic; it is not anecdotal information. (<http://www.mayoclinic.org/medical-professionals/clinical-updates/digestive-diseases/quick-inexpensive-90-percent-cure-rate>)

On the other hand, there is interest in using the fecal transplant procedure for treating a number of other disease conditions such as irritable bowel syndrome (IBS), celiac disease and ulcerative colitis as well as rheumatoid arthritis, obesity, and diabetes. These are some of the same medical conditions that are being investigated for links to specific probiotic control. But the situation is still in the anecdotal stage—sound data is difficult to come by at this point.

**More on using gut bacteria for weight loss**

The previous section described using a transplant of fecal bacteria to change the gut biota (bacterial) to cure a serious infection due to *Clostridium difficile.* The same idea or technique is now being considered to reduce obesity rather than using the surgical procedure called gastric bypass.

A bypass operation separates off a small part of the stomach and connects that directly to the intestines. Recipients tend to feel less hungry, fill up more quickly and burn more calories at rest, and they often lose up to 75% of their excess fat. Counter-intuitively, this is thought to be caused by a change in metabolism, rather than by the reduced size of the stomach. Gut microbes are thought to be part of this picture. People who have had bypasses are known to experience changes in the selection of microbes in their guts. Fat people have been shown to host a different selection of gut bacteria from people who are obese, and transferring the gut bacteria of fat mice into thin ones can cause the thin mice to pack on extra weight. But no one knew whether the microbes in bypass patients changed because they got thin, or if the patients got thin because the microbes changed.

(<http://www.scientificamerican.com/article/gut-microbe-swap-helps-weight-loss/> )

In experiments done at Massachusetts General Hospital, two sets of mice were set up, one having had bypass surgery. The other group was specially bred mice that lacked any gut flora. Fecal bacteria from the bypass mice were introduced into the mice that lacked gut flora. As a result, this recipient group of mice lost 5 % of their body weight. That these mice did not lose as much weight as the surgical group suggests that there are other factors involved in weight loss. The amount of weight loss however is considered significant. Should this technique be refined, it will require methods to keep the special gut flora intact and not be replaced by other, perhaps harmful, gut bacteria. The next step in the research is to isolate the four different types of bacteria that were instrumental in affecting weight change and introduce them into obese mice or people.

**More on** **the relationship between certain gut bacteria and autism**

While the occurrence of autism remains a big mystery as to its etiology, some studies suggest a link between certain bacterial types and their possible influence on the nervous system.

A ... 2013 study from Mazmanian’s lab found that a mouse model with some features of autism had much lower levels of a common gut bacterium called *Bacteroides fragilis* than did normal mice. The animals were also stressed, antisocial and had gastrointestinal symptoms often seen in autism. Feeding *B. fragilis* to the mice reversed the symptoms. The group also found that the mice with these symptoms had higher levels of a bacterial metabolite called 4-ethylphenylsulphate (4EPS) in their blood, and that injecting that chemical into normal mice caused the same behavioral problems.

(<http://www.nature.com/news/gut-brain-link-grabs-neuroscientists-1.16316>)

These same bacteria influence the body’s use of vitamin B-6 which is associated with the normal development of nerve and muscle cells. In autism there are reports that there is an alteration of these bacteria resulting in gastrointestinal problems which, in turn, influence behavior. One test for autism is based on certain end products from bacterial metabolism. (The B vitamins including B-6, folate, and riboflavin are synthesized by lactic acid bacteria and bifidobacteria in the gut.)

# Connections to Chemistry Concepts

**(for correlation to course curriculum)**

1. **Bond breaking and reforming**—Fermentation and respiration reactions both involve the rearrangement of bonds within molecules.
2. **Chemical Thermodynamics**—The breaking and reforming of chemical bonds in the cellular respiration process could be used to illustrate, with chemical equations (see article for these equations), exothermic reactions in which the energies for bond breaking are less than the energy resulting from bond formation. Students can be asked to calculate the energy changes in these reactions.
3. **Alcohols**—The specific alcohol, ethanol, a product of probiotic activity in the gut and part of fermentation is a great example for alcohols in organic chemistry.
4. **Organic acids**—In the gut, probiotics produce organic acids such as lactic acid due to their metabolic activity to generate energy. Lactic acid is a byproduct, as are butyric, propionic, and acetic acids. These are relatively simple organic acids to illustrate the carboxyl group in organic chemistry.
5. **Carbohydrates**—These organic compounds are formed in plants through photosynthesis and are used by other organisms to produce energy. Carbohydrates are good examples to use in class discussions involving bond breaking and reforming and the accompanying energy changes, and in developing structural formulas in organic chemistry,
6. **Fermentation**—This is an important energy-generating process (exothermic), used by bacteria and fungi. This process could be used in the chemistry curriculum to illustrate energy changes (exothermic, endothermic) in chemical reactions.

# Possible Student Misconceptions

**(to aid teacher in addressing misconceptions)**

1. **“All probiotics are basically the same.”** *Some probiotics have a single strain of organisms, while others contain multiple strains. Different strains of the same species may even be different, and could have different effects on health.*
2. **“Probiotics can be used in place of medications.”** *Although some people may prefer natural treatments, probiotics have typically been studied in conjunction with medications—not as a substitute for them.*
3. **“Most yogurts are generally a good source of probiotics.”** *Just because it is yogurt does not mean it contains probiotics. If probiotics are present in yogurt, the container label will say “live and active cultures”. All yogurts with live bacteria contain Lactobacillus bulgaricus and Streptococcus thermophilus, but some manufacturers add other probiotic bacteria after pasteurization, such as L. acidophilus, L. bulgaricus, L. rhamnosus and L. casei.*
4. **“Taking probiotics might help prevent colds.”** *There is no solid evidence that probiotics either prevent or mitigate the severity or length of time for the illness.*
5. **“Single strain probiotics are as effective as multiple strain probiotics.”** *Because different locations in the gut provide different microenvironments for different probiotic organisms, a single strain approach will not obviously cover all the different loci in the gut for the cultivation and activity of any one probiotic.*
6. **“Diet does not play a part in the effectiveness of a probiotic.”** *In fact, depending on the components of a particular diet, the effectiveness of a probiotic is definitely enhanced by the contents of a meal or snack. For instance, consuming certain amino acids such as lysine and methionine (found in fish and whey protein [e.g., tofu]) provide nutrition for certain probiotics. The same is true for the sugar galactose that supports probiotic metabolism.*
7. **“You need to take probiotics every day like a multivitamin.”** *Various studies suggest that you take the probiotic daily for about two weeks to establish cultures in the gut. After that, taking the probiotics just twice a week seems sufficient to maintain good colonies of probiotic bacteria.*

# Anticipating Student Questions

**(answers to questions students might ask in class)**

1. **“What is the difference between a prebiotic and a probiotic?”** *A prebiotic is non-biological, most often a carbohydrate of the fructo-oligosaccharide category. These are plant sugars as found in cereals, fruit, and vegetables. They are consumed as a fiber which means they can reach the intestine without being degraded (digested) before aiding the probiotics’ growth and activity. A combination of the two is known as synbiotics.*

([*http://sitn.hms.harvard.edu/flash/2015/the-human-microbiome-and-media-confusion/*](http://sitn.hms.harvard.edu/flash/2015/the-human-microbiome-and-media-confusion/))

1. **“Since probiotics are actually bacteria, are they really safe to add to our body?”** *Probiotics are generally safe. But they may cause problems for young children, seniors, and those with a serious illness or a weak immune system. Always check with your doctor.*
2. **“For how long does one have to take probiotics?”** *Depending on what reason you are taking a probiotic source, the time span for the probiotic will vary. The effects of supplements last for just a few weeks after you stop taking them.*
3. **“What other available foods contain probiotics besides those mentioned in the article?”** *Certain juices and cereals have probiotics added to them. Other sources include the Japanese Miso soup, tempeh (Indonesian meat substitute), soy-based foods, pickled cabbage (other than sauerkraut) such as kimchi, curtido, and choucroute, brine pickles and olives.*
4. **“What is the difference between live cultures and probiotics?”** *Live cultures are microbes associated with foods, often as food fermentation agents. Many of these have not been directly tested for health benefits. Probiotics are live microbes that have been shown to have a health effect.*
5. **“Can dead bacteria function as a probiotic?”** *No, by definition probiotics must be alive when ingested.*

## In-Class Activities

**(lesson ideas, including labs & demonstrations)**

1. The fermentation process is an energy-generating process which some bacteria and certain fungi use. Students can study fermentation as a chemical process, evaluating the various components that determine both rate of the process including the specific substrate, its concentration, pH, and the temperature of the reacting medium.
   1. There are several different lab procedures for doing this activity, including electronic monitoring of products using software and hardware from companies like Pasco or Vernier, if you have such. The Web site for the lab book for Vernier’s activities can be found here: <http://www.vernier.com/products/books/bio-i/>, while the Pasco lab book is described here: <http://www.pasco.com/prodCatalog/PS/PS-2870_biology-through-inquiry-teacher-guide/index.cfm>.

The Pasco site provides brief descriptions of the activities, while the Vernier site allows you to see evaluation copies of each of the activities.

The Vernier fermentation experiments ask students to research the various parameters of the fermentation reaction, then decide on a particular lab procedure to measure carbon dioxide production or ethanol production over time, plotting the data using Vernier software.

The Pasco activity has students study the process under aerobic and anaerobic conditions and at different temperatures.

Both of these lab books and their lab activities require company-specific software and hardware.

* 1. A less technical approach to measuring the rate of the fermentation process by measuring volumes of gas production over time is found at <http://www.math.unl.edu/~jump/Center1/Labs/FermentationExercisesforTeachers.pdf>.
  2. Instead of using balloons to determine volume of generated gas, a respirometer using a pipette in the fermenting solution can be used. Refer to <https://www.google.com/search?q=Fermentation+lab+exercise&ie=utf-8&oe=utf-8>.
  3. A college-level reference on fermentation as a lab activity is found at <http://www.instruction.greenriver.edu/kmarr/biology%20211/Labs%20and%20ALEs/B211%20Labs/B211%20Labs/5%20_Lab%205_Alc%20Ferm%20in%20Yeast_F2009.pdf> . Included in this reference are ideas about yeast metabolism, the experiment design (the variables to be considered), and the chemistry involved, including equations. At the very least, teachers will find this a useful background resource if not used directly by students.
  4. Another lab on fermentation which includes using distillation to isolate the alcohol product is found at <http://www.nuffieldfoundation.org/practical-chemistry/fermentation-glucose-using-yeast>.

1. A very informative and practical series of lab activities on fermentation, involving a large variety of foods, is found at the National Centre for Biotechnical Education (UK), at <http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/fermentation.html>. There is a student lab manual here as well as a teacher guide that can be downloaded.
2. Another interesting exercise for students is to make yogurt. Again the lab exercise can be used to illustrate the control of variables in the design of the experiment—temperature, types of bacteria cultures if listed on the inoculation source (other yogurts). Does adding sugar make any difference in the yogurt-forming process? Guidelines for making yogurt with suggestions for making the exercise a bit of an investigation are found at <http://www.math.unl.edu/~jump/Center1/Labs/FermentationExercisesforTeachers.pdf> (same reference noted above for fermentation of sugar).

There’s another lab for making yogurt at this college site: <http://biology.clc.uc.edu/fankhauser/Cheese/yogurt_making/YOGURT2000.htm>.

Students might be able to examine the yogurt culture for bacteria before and after the yogurt-making process (see <http://www2.mrclmb.cam.ac.uk/microscopes4schools/yoghurt.php>).

1. Students could also read the labels of different yogurt products for any information about added probiotics, whether pasteurized or not (dead bacteria are not of any value as a probiotic source!), and how effective a given yogurt product is in terms of being the inoculating source for starting a yogurt culture. Students can compare their yogurt-making process to that of commercially prepared yogurt. The details associated with the commercial yogurt-making process are found at <http://milkfacts.info/Milk%20Processing/Yogurt%20Production.htm> and <http://science.howstuffworks.com/innovation/edible-innovations/yogurt1.htm>.

# Out-of-Class Activities and Projects

**(student research, class projects)**

1. For students who would like to combine their interest in biology and chemistry, an involved series of experiments using lactic acid-producing bacteria (LABs) requires the experimenter(s) to collect this category of bacteria from a variety of common sources, then examine the conditions for cultivation of these bacteria. Students will monitor three parameters: growth (as determined by culture turbidity), culture diversity (as determined by microscopy), and pH. For a complete guide to this activity, consult <http://serc.carleton.edu/microbelife/k12/LIMW/lactic_acid.html>. Because of the need for specialized equipment and teacher oversight, this activity is best done in school.
2. Students could research the literature that supports the ideas for the influence of gut microbe metabolites on the nervous system, particularly the brain, and a person’s mood or behavior. A starting read for students is found in an article from the *Atlantic* magazine, titled “When Gut Bacteria Change the Brain”. Refer to <http://www.theatlantic.com/health/archive/2015/06/gut-bacteria-on-the-brain/395918/>.

Here’s a research article that details an experiment done to test the idea that certain gut microbes influence behavior (in mice): <http://www.nature.com/news/gut-brain-link-grabs-neuroscientists-1.16316>. This research report illustrates for students how some of the ideas about microbes come about through scientific investigating.

# References

**(non-Web-based information sources)**



**30 Years of *ChemMatters***

Available Now!

**The references below can be found on the *ChemMatters* 30-year DVD (which includes all articles published during the years 1983 through April 2013 and all available Teacher’s Guides, beginning February 1990). The DVD is available from the American Chemical Society for $42 (or $135 for a site/school license) at this site:** [http://ww.acs.org/chemmatters](http://www.acs.org/chemmatters)**. Click on the “Archive” tab in the middle of the screen just under the *ChemMatters* logo. On this new page click on the “Get 30 Years of ChemMatters on DVD!” tab at the right for more information and to purchase the DVD.**

**Selected articles and the complete set of Teacher’s Guides for all issues from the past three years are available free online at the same Web site, above. Simply access the link and click on the aforementioned “Archive” tab.**

An article from a 1989 issue of *ChemMatters* describes the history of yogurt making (including a modern recipe for making yogurt), as well detailing the important bacteria present in yogurt. This includes the important role of these yogurt bacteria in changing 25–50% of the disaccharide lactose (milk sugar), found in milk products, into glucose and galactose so that lactose-intolerant people will not have as serious a digestive problem eating yogurt compared with other milk products. (Evan, G.D. Yogurt. *ChemMatters,* 1989, *7* (3), pp 9–12.

Another article from this 2013 issue is all about lactose intolerance and its relationship to digestion of milk products in the gut. Tests for lactose intolerance and ways around the problem using foods without lactose or taking a pill containing the missing enzyme, lactase, are described. Refer to Rohrig, B. Not Milk? Living With Lactose Intolerance. *ChemMatters,* 2013, *31* (2), pp 18–19.

# Web Sites for Additional Information

**(Web-based information sources)**

**More sites on** **probiotics**

A useful summary article about probiotics in terms of what probiotics can and cannot do is found at <http://www.livescience.com/46298-the-lowdown-on-probiotics.html>.

A second general article on the balanced view about the potential of probiotics is found at <http://www.theguardian.com/lifeandstyle/2014/nov/30/probiotics-myth-or-miracle-prebiotics>.

A scientific article that elaborates on separating myth from medical evidence about the role of probiotics in human health is found at <https://www.mja.com.au/journal/2008/188/5/probiotics-sorting-evidence-myths>.

A handout for patients to answer their possible questions about probiotics and their use is provided by the University of Wisconsin School of Medicine at <http://www.fammed.wisc.edu/sites/default/files/webfm-uploads/documents/outreach/im/handout_probiotics_patient.pdf>. Among other things is a listing of probiotic-containing products and the specific bacteria that are present. Naturally one needs to know what bacteria are needed, which may not be known to the patient unless suggested by a knowledgeable clinician.

**More sites on** **gut bacteria effects**

A useful site that describes the various types of bacteria and their functions in the human body, other than those of the probiotic type (“5 Ways That Gut Bacteria Affect your Health”), is found at <http://www.livescience.com/39444-gut-bacteria-health.html>.

A generalized article from *The Atlantic* magazine that describes the various studies on the possible effects of gut bacteria on the brain and certain behaviors is found at <http://www.theatlantic.com/health/archive/2015/06/gut-bacteria-on-the-brain/395918/>.

Recent research has suggested that certain vaccines are more effective if specific bacteria are present in the gut. Specifically, a link has been established between the effectiveness of a vaccine used to treat infant diarrhea caused by rotavirus and certain flagellated bacteria of the Proteobacteria group. A description of the problem and potential solution is found at the *Scientific American* Web address, <http://www.scientificamerican.com/article/gut-microbes-may-help-determine-our-immune-response-to-vaccines/>.

A very scientific but informative article on all aspects of probiotics, in particular, their therapeutic potential and survivability, is found at <http://www.nature.com/icb/journal/v78/n1/full/icb200012a.html>.

Another different but interesting approach involving the gut and body bacteria is to use their metabolic end products to diagnose both the healthy as well as the disease state in humans. The body and its intestinal flora produce chemicals with hidden health information according to one investigator, Jeremy Nicholson. Someday, treating disease may mean treating those bacteria associated with different disease states, including neurological conditions. Knowing the chemical byproducts of bacteria would contribute to understanding their metabolism, from which pharmaceuticals could be designed. The story behind Nicholson’s research is found at <http://www.scientificamerican.com/article/jeremy-nicholsons-gut-instincts/?page=2>.

**More sites on informed and critical reporting of scientific research**

In part, the *ChemMatters* article illustrates how scientific research, particularly in the realm of medical issues, can be misrepresented to the public. One day coffee is dangerous (excess causes chromosomal damage), the next day, so to speak, it is found to be a good thing (a source of antioxidants). A cautionary note to explain the issue is provided by a Harvard researcher at <http://sitn.hms.harvard.edu/flash/2015/the-human-microbiome-and-media-confusion/>. One of the concerns is that some of the research (and results reported) is funded by a variety of commercial entities with an investment in positive outcomes for their product’s effectiveness.

A related article expressing caution about what probiotics can and cannot do is found at <http://now.tufts.edu/articles/can-probiotics-keep-gastrointestinal-health>.

A useful Web site that reports on the science behind news reports is found at <http://sitn.hms.harvard.edu/>. This Web site is run by graduate students in the sciences and reports on current science topics that garner both general interest and criticism. Among other topics is the much maligned and misunderstood topic of Genetically Modified Organisms, GMO’s. (<http://sitn.hms.harvard.edu/signal-to-noise-special-edition-gmos-and-our-food/>)

**More sites on probiotics and control of obesity**

For a complete description of the experimental procedure to determine the relationship between probiotics and their influence on controlling the development of obesity and diabetes, refer to <http://www.nutraingredients-usa.com/Research/Probiotics-may-counter-obesity-and-diabetes-NIH-study>. It could be used with students to illustrate both the design of an experiment and how data is generated and what the limits are to “cause and effect” conclusions.

A more recent experimental approach for controlling obesity without involving probiotics focuses on cellular metabolism through the lipid-controlling hormone leptin (the “thin” hormone) and a chemical called celastrol, isolated from a plant called the Thunder God vine. Normally, leptin suppresses food intake. Second, it increases energy expenditure by altering metabolic rate; both effects reduce calorie build-up or storage. The research is clearly described at the Harvard Web site <http://sitn.hms.harvard.edu/flash/2015/the-skinny-on-celastrol-a-potential-future-anti-obesity-drug/>. The content requires careful reading to understand. But it does illustrate what is involved in trying to discover connections in the complicated biological but chemically-dependent systems at the cellular level.

**More sites on the Human Microbiome Project**

The purpose of the Human Microbiome Project is to collect all the microorganisms living in association with the human body. For a complete description of this government project, refer to this Web site at Tufts University: <http://now.tufts.edu/articles/microbiome>.

**More sites on the life and work of Elie Metchnikoff**

Ilya Ilyich (Elie) Metchnikoff, who can be considered the “father” of the probiotics idea back in the beginning of the 20th century, was a pioneer in the field of human physiology, immunology, and medicine, winning the Nobel Prize in Immunology or Medicine in 1908. Teachers, as well as some students, might find Metchnikoff an interesting person to research, learning that there were creative scientists long before the age of computers and sophisticated lab technology! A starter reference is found at <https://embryo.asu.edu/pages/ilya-ilyich-mechnikov-elie-metchnikoff-1845-1916#sthash.EZ3K0di0.dpuf>.

A second biography about Metchnikoff comes from the Nobel Prize Web site. Refer to <http://www.nobelprize.org/nobel_prizes/medicine/laureates/1908/mechnikov-bio.html>.

# General Web References

**(Web information not solely related to article topic)**

As mentioned above, there is a Web site that elaborates on a wide range of science topics that are reported in the press, providing critical information that gives both perspective and validation (or not!) of ideas that are being promulgated for public consumption. This is a good reference for teachers to consult when students bring up a variety of questions about such topics as global warming, the “dangers” of GMO’s (genetically modified organisms), cell phones and cancer, and the like. Refer to <http://sitn.hms.harvard.edu/>.