



### SPLASH! milk science update September 2015 Issue

This month's issue features how probiotics may work better with milk, helping pre-term babies fight fungal infection with milk sugars, kefir consumption, and breastfeeding rates among African American women.

#### Probiotics May Work Better with Milk

- **The bacteria *Lactobacillus casei* are commonly used as probiotics and are thought to have several beneficial effects, including improved immune function and reduced intestinal inflammation.**
- ***L. casei* survived better in mouse guts when they were pre-incubated in refrigerated milk.**
- ***L. casei* also prevented intestinal inflammation in mice when ingested in milk, but not when delivered in a nutrient-free buffer.**
- **Delivery through milk thus appears to improve *L. casei*'s efficacy as a probiotic, which could have implications for how probiotics are consumed in humans.**

We respond differently to different environments; we might put on a thick coat when it's cold, or open an umbrella when it's raining. It turns out that probiotic bacteria also react differently depending on their environment, and this could have important implications for how we consume probiotics. Two new studies led by Maria Marco from the University of California, Davis, found that probiotic bacteria showed improved survival and efficacy when delivered through milk rather than in another medium [1,2].

The researchers conducted their experiments using a bacterium called *Lactobacillus casei*. "*Lactobacillus casei* are often incorporated into fermented dairy products as probiotics, and certain strains are among the most widely used probiotic strains," says Marco, an associate professor of food science and technology. *L. casei* is thought to have several beneficial effects, including improved immune function and reduced intestinal inflammation. The researchers used a specific strain of *L. casei* called BL23, which is closely related to other strains of *L. casei* commercially used as probiotics [3,4].



Marco and her team found that *L. casei* BL23 survived better in mouse guts when it was incubated in milk at 4 degrees Celsius before being fed to the mice [1]. The researchers also found that *L. casei* was better able to prevent intestinal inflammation in a mouse model of colitis when it was ingested in milk [2].

"I think it's very well understood that bacteria respond quickly to new habitats," says Marco. "So it made sense to think that bacteria would be responding to their environment in milk and making different proteins that we believe are really important for their performance as probiotics in the intestine," she says.

"I think it's an intriguing result, because it really begs the question, 'what happens then if people consume probiotics?'" says Marco. The current studies were done in mice, and Marco points out that it will be important for researchers to perform follow-up studies in humans. "Taking this back to humans again would be an important next step, particularly for those people who suffer from chronic inflammation of the intestine," she says. Future studies could help determine whether "*Lactobacillus casei* might actually perform better in people when it's in a dairy product, as opposed to when we have it in a nutritional supplement or candy bar or something like that," says Marco.

Marco has been studying probiotics for more than a decade, and started working on the current project a few years ago after conducting a literature review with a collaborator [5]. "What impressed me in reviewing the literature was how little we actually know about the context in which we consume these bacteria, and how that matters for their efficacy and their performance in the gut," says Marco. "We also know that probiotics are being added into other products besides dairy right now: there are supplements, juices, candy bars, cereals, all kinds of different foods," she says.

Marco decided to test whether probiotic bacteria would survive better in mouse guts after incubation in milk at 4 degrees Celsius. "We chose the 4 degree Celsius because that's the temperature of refrigeration, and we store yoghurt and milk in

the refrigerator,” says Marco. “We knew that *Lactobacillus casei* was able to survive in the gut, so we asked a simple question: does incubation in milk in the refrigerator improve this?,” she says.

“We fed *Lactobacillus casei* BL23 to mice, after incubation in milk at 4 degrees, or after incubation in its regular culture medium that we use to propagate it in the lab,” says Marco. The researchers also tested what happened when the probiotic bacteria were grown in a standard culture medium and put in milk right before being fed to the mice.

“We compared these different ways of consuming *Lactobacillus*, and we found that it survives better in the mice when it was consumed after incubation in milk,” says Marco. “That was intriguing to us, so then we started to look at what products the *Lactobacillus* was making as it was being incubated in milk.”

To do this, Marco and her colleagues decided to take a global look at the proteins being made by *L. casei* in different environments. “We found proteins that were made by *Lactobacillus* that were really interesting, because they were really more abundant or uniquely made when the cells were incubated in milk,” says Marco. The researchers also identified proteins made during incubation under cold conditions.

The researchers chose four proteins that were more abundant in milk or low temperatures and created mutant bacteria that didn’t make those proteins. “Interestingly, for three out of those four proteins the mutants were impaired or deficient in their ability to survive in the gut compared to the original wild type cells,” says Marco. The fourth mutant actually survived better, she says. The researchers plan to further characterize these mutants to find out the mechanisms that influence their survival in the gut. In addition, they are also continuing to study the other proteins that they found.

“We now have a collection of proteins that we’ve identified, and I think these are important pieces to the puzzle,” says Marco. “Some of these proteins are going to be important for *Lactobacillus*’s ability to survive and tolerate the conditions it finds in the digestive tract, and others we believe are going to be important for the communication between the *Lactobacillus* and the gut,” she says. “But there certainly are more targets and more proteins and more work to be done,” says Marco.

Marco also wanted to look beyond the probiotic bacteria’s survival in the gut to ask whether being delivered in milk actually affected their efficacy as probiotics. Probiotic bacteria appear to be a promising therapeutic approach in reducing the risk of ulcerative colitis, a form of inflammatory bowel disease, and so the researchers decided to test *L. casei* in a mouse model of ulcerative colitis [6,7,8].

The researchers fed the mice *L. casei* after incubating the bacteria in either refrigerated milk or a nutrient-free buffer that mimicked how they would be consumed in a nutritional supplement. “Lo and behold, we found that *Lactobacillus casei* was very good at preventing colitis or intestinal inflammation in this model, but only when it was delivered in the presence of milk,” says Marco.

Marco is interested in figuring out the mechanisms behind *L. casei*’s beneficial effects. “One of the proposed general mechanisms is that probiotics work because they can change the types and activities of the other bacteria in the intestine,” she says. “We couldn’t find any kind of real shift in the microbiota that would indicate that *Lactobacillus casei* is affecting these other bacteria that then can prevent inflammation,” Marco says.

Marco suggests that *L. casei* may be directly affecting the body’s inflammatory response, and the researchers plan to look for specific products made by *L. casei* that interact with the intestinal epithelium and immune cells to reduce inflammation. “We know that we need to be looking at *Lactobacillus casei* products being made in milk, so that’s where we’re working, and we’re looking at not only the bacterial side but also the host side, trying to put the pieces of the puzzle together,” says Marco.

“You can imagine that with all of the different kinds of benefits that people have been ascribing to these bacteria, there’s going to be different kinds of cell products and different kinds of host response pathways that are important,” Marco says [9]. “So it’s not going to be just one metabolite or one protein that’s being made, but a whole collection,” she says. “It’s going to be a really exciting time ahead, as we start to identify what these molecules are that are so important.”

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*Contributed by*

*Dr. Sandeep Ravindran*

*Freelance Science Writer*

*Sandeep.com*

## Milk Sugars May Help Pre-term Babies Fight Fungal Infection

- **Pre-term babies are susceptible to life-threatening infections, including fungal infections.**
- **Human milk contains a complex mix of sugars, also known as oligosaccharides.**
- **Oligosaccharides can help prevent fungal infection of intestinal cells.**
- **Human milk oligosaccharides, either as purified preparations, or in colostrum or milk, can potentially help pre-term babies avoid infection.**

The human body is a host for billions of microbes, including the very common fungus, *Candida albicans*. Harmless most of the time, these yeast cells may suddenly transform into many cells, chained together as long and branching filaments that invade body tissues. This has been reported in many vulnerable individuals, including pre-term babies. A recent study by Gonia et al. [1] found that, in the laboratory, human milk oligosaccharides protect cells, similar to intestinal cells from pre-term babies, against infection with *Candida albicans*.

Human milk contains about 7% carbohydrates, mostly in the form of the simple milk sugar, lactose. Other components of milk carbohydrates are the more complex sugars, the oligosaccharides. Oligosaccharides constitute about 1% of human milk, and more than 2% of colostrum [2,3]. Even though these sound like small amounts, the total quantity of sugars in human milk is actually similar to the quantity of proteins, both being abundant components.

One of the most important roles for breast milk is to support the immune system of a newborn, and provide bioactive



**Candida albicans**

molecules that allow the gastrointestinal tract of the baby to mature. A normal part of this maturation is the development of a healthy balance of microflora. *Candida albicans*, a fungus found in the human gastrointestinal tract, is one of the mix of microbes that are considered to be commensal. Normally held in balance, like the many other commensal microorganisms found in the gastrointestinal tract, if allowed to grow unchecked it becomes life threatening. One such critical circumstance is in the immature gut of pre-term babies.

Pre-term babies have both an immature immune system and an underdeveloped gastrointestinal tract, which makes them highly susceptible to infection. Their journey to become happy healthy infants depends on nutritional support and preventing the growth of microbes that may lead to sepsis.

*Candida albicans* is a common problem for pre-term babies. It has the capacity to over grow in the gut and invade the bloodstream through the intestinal wall. Gonia et al. [1], from the University of Minnesota, along with colleagues from UC San Diego, designed experiments to test the capacity of human milk oligosaccharides to protect the immature intestinal wall against invasion by this pathogen. To do this, they added purified milk oligosaccharides to cell cultures in the lab. They used a cell line derived from the intestinal lining corresponding to the gut of a pre-term baby. In this way they could control the conditions under which the cells were exposed to the fungi, and infer how the cells of the intestine would behave when *Candida albicans* was present.

The scientists first developed a combined purified stock of oligosaccharides from pooled breast milk samples. This provided an opportunity to examine the effect of different doses of the treatment. Cells were grown with escalating doses of oligosaccharides pre-mixed with the *Candida albicans*. The cells were allowed to incubate for set periods of time, and then the scientists examined the cultures for evidence of invasiveness. Invasion is the term given to a specific stage of fungal infection when it actually penetrates the cell. The scientists also looked for binding of yeast cells to the intestinal cells, and measured the size and presence of hyphae—the branching projections that fungi use to enter the intestinal wall, and ultimately into the bloodstream.

When they examined the cells, they found that the addition of the oligosaccharides markedly reduced the association of yeast and intestinal cells by 40%. They also noticed that the lengths of the hyphae were reduced by 30%. This also corresponded to the inhibition of yeast gene activity. However, for the human milk oligosaccharides to have an effect, they had to be present at the time the yeast cells came into contact with the intestinal cells. In other words, the effect was due to the direct interaction of the oligosaccharides and yeast, and did not prevent the yeast cells from growing. The scientists also noted that the treatment delayed the formation of hyphae, rather than completely preventing it.

Although there is further work to be done to understand how to get the most from milk oligosaccharides, these results are very encouraging for clinical application. Additional studies will determine if purified preparations have practical benefits, but until then we can be reassured that the concentrations of oligosaccharides in colostrum and human milk are going to help pre-term babies to survive during those crucial early weeks of life.

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*Contributed by*  
*Professor Peter Williamson*  
*Associate Professor, Physiology and Genomics*  
*University of Sydney, Australia*

## Kefir Consumption-a Growing Culture

- **Kefir is a fermented dairy beverage with the consistency of thin yogurt, a mild acidic flavor, natural carbonation, and—traditionally—a small amount of alcohol.**
- **A natural probiotic drink with a range of purported health benefits, kefir is increasingly gaining acceptance and popularity worldwide—as a home-brewed as well as a commercially produced drink.**
- **Kefir originated thousands of years ago in the Caucasus Mountains at the border of Europe and Asia.**
- **The traditional fermentation of milk to produce kefir is based on a starter culture of “kefir grains”, made up of bacteria and yeast cells embedded in milk proteins and complex sugars.**

Kefir, an ancient cultured dairy drink touted as a health-promoting probiotic, is coming back into fashion in Europe and gaining popularity in the US. With its fizzy freshness and mildly acidic flavor, kefir (pronounced “keh-FEAR”) likely owes its name to a similar Turkish word meaning “good feeling.” Fermented by yeast and bacteria in a unique way, kefir has been shown to promote gut health and boost the immune system, among other beneficial effects (1, 2). Kefir has even been credited with beginning “a new dawn of food” (1), while commercial producers and home brewers are experimenting with modern recipes and flavored variants.

Perhaps not since the dawn of time, but at least for some 12,000 years, humans have exploited fermentation of certain foods, including milk, to keep it from spoiling. Apart from making the milk safe to consume and store for extended periods, fermentation also gave rise to appealing flavors and textures, and made the milk more digestible. Yogurt, for instance, is a fermented milk product that even lactose intolerant people can easily digest (3). Kefir has been called “the yogurt of the 21st century” (4) in view of its renaissance in Western countries; however, its roots stretch back thousands of years to the Caucasus Mountains, at the border of Europe and Asia.

In Russia and other parts of Eastern Europe, kefir has long traditions and, owing to its purported health benefits, is commonly given to patients in hospitals and recommended for babies and the ill (2). While scientific studies have demonstrated a number of beneficial effects of kefir—including immune-boosting, antimicrobial and anti-cancer effects, as well as improved gut health and lactose digestion (1)—more studies are needed to provide conclusive evidence. The drink, which has the consistency of thin yogurt, found its way into Western Europe decades ago, but its popularity has waxed and waned. In today's Scandinavia, for instance, many remember it as an old-fashioned drink favored by their parents or grandparents back in Abba's heyday of the 1970s. But as Abba did, kefir is regaining popularity—although it's not yet produced commercially in all of Europe. In some places, it's only sold in occasional corner stores, often run by immigrants. Perhaps this somewhat variable availability has contributed to the growing “subculture” of health-conscious aficionados who brew their own kefir, share recipes online, and even send clumps of starter culture to fellow kefir lovers.

### The “immortal kefir grain”

So, what exactly is this kefir culturing all about, and what's special about it? The traditional way of making it is based on an “immortal” substance called kefir grains—not to be confused with food grains. Resembling tiny, slimy cauliflower florets or clumps of sticky rice, kefir grains are a kind of compact curd made up of lactic acid bacteria, acetic acid bacteria, and yeast cells embedded in milk proteins and kefirans—a type of complex sugars produced by bacteria. The first kefir grains arose spontaneously inside goatskin bags used for fermentation of milk; the grains have since been passed along and propagated during the long tradition of kefir making.



Added to a batch of fresh milk, the grains kick-start the fermentation of lactose and other milk ingredients, resulting in kefir after a day or so. The grains are then filtered out and used to inoculate the next batch. Kefir grains can be used with a wide

range of temperatures and types of milk, including cow, goat, or sheep milk. Furthermore, they practically never die—making kefir ideal for home brewing.

This is different from the traditional fermentation of yogurts and other milk products, which commonly starts with the practice of “back-slopping,” or inoculation of fresh milk with a portion of the previous fermented batch. But yogurt cultures quickly lose their potency, and new starter culture must be obtained regularly.

Adding to kefir's uniqueness is the presence of yeast in the kefir grains, producing a naturally carbonated drink containing a tiny amount of alcohol. Owing to its carbonation and alcohol, kefir has aptly been called “the champagne of milk” (5). Commercially available brands of kefir, however, do not always contain carbonation and alcohol.

### An artisanal beverage going global

Traditional kefir culturing using kefir grains does not lend itself easily to large-scale commercial production. Commercial producers have therefore developed a method using freeze-dried starter cultures of microbes isolated from kefir grains. This eliminates the need to recover grains and ensures greater consistency of the product. Commercial methods also give the product longer shelf life.

But commercial starter cultures lack the “immortality” of kefir grains, as well as some of the mystery and individuality of the walnut-sized “villages” that the grains are—housing some 30-odd microbial species. This tends to remove some of the drink's “kefirness”—especially when commercial starter cultures without yeast are used, resulting in a flat and non-alcoholic product very similar to yogurt. The particular microbial populations of individual kefir grains may also contribute to the sensory, as well as the potentially therapeutic characteristics of the final product (6). Perhaps that's part of the reason kefir is commonly produced as an artisanal beverage. So far, artisanal production seems to be the most common form in the US, where the beverage is beginning to gain a foothold (2).

With kefir's popularity on the rise globally—coinciding with an all-time high focus in the food industry on probiotics, or beneficial bacteria—we can no doubt expect more research on kefir in years to come. This will help scientists better understand and document its health-promoting potential, and could also lead to improved technological processes that might enable commercial production of “true” kefir.

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*Contributed by*  
*Lillian Sando, PhD*  
*Freelance science writer & editor*  
*Online editor, [www.technologist.eu](http://www.technologist.eu)*

## Success Stories

- **New qualitative research has cast light on the low breastfeeding rates of African Americans in the United States.**
- **This research employed Black Feminist Theory to analyze the contents of many long interviews, in which participants were given space to explain and expand on their views.**
- **Unlike the vast majority of studies on the topic, the research looked at the experiences of African American women who continued breastfeeding for an average of 10 months.**
- **The study suggests that engaging faith communities and social media, and addressing conflicting understandings of the breast as both nurturing and sexual, may help to increase breastfeeding rates in the African American community.**

As the benefits of breast milk have become better known and the world has become more connected, sharing or selling it has concomitantly become much more common. Milk banks are the most institutionalized method of milk sharing, and probably the safest, but different countries run them very differently. As the third item in SPLASH!'s series on the subject—the other pieces can be found [here](#) and [here](#)—this article explores those differences, and picks out lessons offered to the rest of the world.

Anyone involved in healthcare in the United States has probably heard that breastfeeding rates among African American mothers are much lower than those of any other racial grouping. As it happens, breastfeeding initiation rose by 8% in the African American community between 2000 and 2008, but the aforementioned gap still didn't narrow. Research into the topic tends to focus on why these women aren't breastfeeding, with studies often emphasizing statistical results, sometimes based on narrowly worded questionnaires. Recently, however, a nurse named Becky Spencer turned this perspective on its head: she has sought to understand African American breastfeeding successes.



Spencer's paper [1], which she co-authored with two other researchers at Texas Woman's University's College of Nursing—Karen Wambach and Elaine Williams Domain—also doesn't read like a typical 'medical' study. She chose methods more common to anthropology. The idea was to approach the topic free of assumptions, to ask open-ended questions, and to let interviews and focus groups run on for an hour or two.

"I was not interested in just continuing the narrative of asking what's wrong with this population, and what I can do to fix it," she says. "I really like the positive deviance approach. In this case, finding the outliers that match the rest of the population—the African American mothers who do have successful breastfeeding experiences." The

mean length of breastfeeding for the women who provided individual accounts for the study was 10 months.

Spencer was inspired to work on the topic by a shocking fact: In the United States, an African American infant is twice as likely to die before the age of one compared to a white infant—even when the mothers' education level is held constant using statistics. Breastfeeding rates certainly aren't the only contributor to this, but they are likely part of the answer. Receiving breast milk, as opposed to formula, reduces the odds of developing and dying from at least two prominent killers of young infants, necrotizing enterocolitis (a condition in which parts of the intestines start to die, mostly found in preemies), and SIDS (Sudden Infant Death Syndrome)—which is the second biggest killer of under one year olds in the country.

“One of the interesting things about our results,” says Spencer, “is that the women's experiences had very little to do with health considerations; they only mentioned health in the sense that they would talk about growth, or report developments like ‘my baby rolls over.’” Instead, breastfeeding was mainly about bonding with the baby, about the empowerment of accomplishing what sometimes wasn't well supported in the community, and about experiencing femininity in a way that wasn't sexual. In the words of one interviewee, “Breastfeeding solidified and helped me to appreciate my womanhood and the way my body is different that allows me to do these awesome things. And not having to reject it or saying that my body's only... this sexual object.”

This dual cultural identity of the breast—as a nurturing organ and as a sexual one—was front and center of many of the study participants' experiences of breastfeeding. It presented itself as a tricky tension to navigate socially, tempting many to give up. “Just google the word ‘mother’ and then the word ‘woman’,” says Spencer. “African American women are very sexualized in U.S. culture—and they have been since slavery. Showing one's self in public is so taboo. The resistance to breastfeed in public is bound up with resistance to being sexualized.” And the absence of visual examples of mothers breastfeeding in the community has a kind of feedback effect, discouraging other women from breastfeeding in public, or at all.

Spencer is also conducting focus groups of African American men who have fathered successfully breastfed infants, in order to understand their experiences of the period while their partners were breastfeeding. “Every single one brought up having to share their partner's body with the baby. You'd even hear the women say ‘They belong to the baby now but they will belong to him later’—referring to their own breasts,” notes Spencer.

The health benefits of breastfeeding were brought up spontaneously much more often in the male focus groups; these dads also often reported feeling a sense of pride in the fact that their child was breastfed. Perhaps one under-appreciated point, Spencer suggests, is the sense of struggle that fathers themselves experience while they watch their partner having difficulty or feeling pain breastfeeding. “It just kills them to watch women struggle,” she says. Why this might be especially prominent among African American men was suggested to her by a male teacher who runs a daddy boot camp in the same community. “So many of these men grew up in single-parent households, and often grew up watching their mothers struggle without being able to do anything about it. He thinks that some of that sense of powerlessness might be transferring to watching their partner breastfeeding,” Spencer explains.

Whether or not that's the case, solid support—from the infant's dad, from the grandma, and from friends and medical professionals—was extremely important to the women who started and managed to stick to breastfeeding. This conclusion rings true to researchers working in states other than Texas, where Spencer works.

Janelle Richardson has, for the past five years, been studying a sample of African American WIC mothers in Ohio. Unlike Spencer, Richardson is African American herself—a distinction that some of the academic literature suggests helps to acquire more in-depth answers in this kind of discursive research setting. She thinks Spencer's paper hit the nail on the head, though. “Her research is pretty well aligned with my findings... It captures the embarrassment that many African Americans feel over breast feeding.” In an attempt to reduce so-called ‘interviewer effects’, Spencer explicitly set out to approach the work using the framework of Black Feminist Theory, which, like mainstream feminist theory, questions the traditional insertion of women in society, but explicitly expands those discussions to consider intersections of race and gender (for a more complete treatment of Black Feminist Theory, see Collins (2002) [2]).

On the matter of social support, Richardson emphasizes that the whole of the health system has to be on board, and employers still need to make it easier for women to pump, despite legal changes in many states requiring them to provide appropriate time and space within the working day. “What happens after a mom goes home [from the hospital] matters,” says Richardson. “Studies show that nurse visits and peer telephone calls help moms stay focused on breastfeeding.” In the paper, Spencer's discussants put forward the idea of leveraging social media to offer information and road-tested tips to breastfeeding moms experiencing difficulties. Unlike friends and lactation support counselors, Facebook is there if you need it at 2am, after all.

Aloka Patel, who is also working to understand breastfeeding rates among African American mothers, concurs that support is really crucial. As an associate professor of pediatrics, her professional home is the NICU of Chicago's Rush University Medical Center. When mothers have a premature offspring being treated there, 98% of them provide the infant with milk, regardless of race, she explains. "So how they start doesn't vary. But we see that by the time of discharge, differences start to show up." Patel has conducted various types of analysis with her breast milk provision data, one of which is a spatial analysis of where mothers live in relation to the hospital. Before she crunched the numbers, distance seemed like an obvious explainer for why some women kept making the trip to the NICU, while others' visits trailed off. Yet distance didn't prove statistically significant. For reasons not yet fully understood, having access to a car was what really mattered for African American women with an infant in the NICU; it didn't matter for white or Hispanic women. Patel is hoping to understand this result through qualitative research. "The Spencer paper was really quite striking," she says. "It highlights how much we don't know. I think we need others like it, at other centers."

Richardson has an even grander vision. She believes the U.S. should plough more resources into measuring and seeking to understand initiation and duration of breastfeeding in the African American community. When asked what the most surprising item in Spencer et al.'s paper, Patel says that she did not expect to see that some churches fail to encourage breastfeeding. Some in the paper do, though: the supportive ones provide a room for privacy, and a few even have a TV in that room for mothers to keep up with the action in the main hall. The support of faith communities, the role of social media, and the need to tackle the often-reinforced idea that breastfeeding cannot be extricated from being sexually provocative, are all action points in the work. But the broader lesson from this paper is simpler. Spencer sums it up, "I don't think this is something that science can fix."

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*Anna Petherick*

*Professional science writer & editor*

*www.annapetherick.com*

#### **Editorial Staff of "SPLASH! milk science update"**

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