Dear Peter,

It was such a pleasure to finally meet you at Camp Bread—and what an event that was! Since we last spoke, I have been busy pulling together the research you requested. This is a long story, going back to early spring of 2002. As you know, I was a member of the King Arthur Baking Circle, and there was a lot of interest in sourdough there at the time. In March, Pat88 began posting of the difficulties she was having in getting a starter going. She had tried a few different recipes with no success, and now she was following the formula in The Bread Baker's Apprentice. But, still no luck. Newly armed with all of the advice that she was getting from others on the message board, she started over. And again, she got results that were nothing like the book describes. But, there was a pattern. On the second day, her seed cultures would fill with bubbles and expand to over three times the starting volume when minimal growth was expected, then do nothing on the third and fourth days when they were supposed to be expanding more and more. They came on strong, and then died at the same point each time. To me, patterns mean something, and so I offered to do the procedure myself to see if I could reproduce what she was seeing. I followed the directions to the letter, and lo and behold, my results duplicated Pat's perfectly. I may be the only person on the planet who would be excited about this, but it gave me something that I could study and troubleshoot. There was something unexpected going on at the microbial level. Living things are funny that way; microorganisms don't always follow directions.

One by one, other people on the message board began to speak up and post that they had seen the same thing. In fact, it seemed that many more experienced that scenario than the one described in the book. This phenomenon had nothing to do with local strains of lactobacilli and yeast as some had surmised, because Pat was making starter in Massachusetts and I was in Missouri. Others chiming in represented various regions of the country from coast to coast. This pattern is apparently quite common. We ran the gamut of theories on why the yeast were coming on like gangbusters, only to quit and become non-responsive. We tested each theory by trying different flours at various points, increasing the feeding frequency, changing the hydration and water source, cooling it down, and anything else that anyone thought might help. But in the end, nothing fixed the problem, and the results weren't making much sense to me.

At that point, I had to do what microbiologists are trained to do when things don't add up—go back to the microscope and take a look. That meant packing up my starters, taking them to work, and having to answer all the curious questions about what I was doing and why. But, that day proved to be the turning point. No wonder things didn't make any sense! We were operating on the assumption that we were growing yeast, and what I found was that there were no yeast or lactobacilli to be seen anywhere amidst all the activity on day two. Not a single one. But it was like a three-ring circus in there—different kinds of bacteria, some round, some rod-shaped, some motile, some not. Some were spinning, some were twirling, some flipping or zig-zagging, and some were just darting back and forth across the field. What were these bacteria, and which one was responsible for all the gas?

I knew, from having made so many starters by that point, that this pattern does turn into sourdough if given more time. So, I looked at the cultures each day in the process, comparing them to my established starter which was yeasty and stable. Everything quiets down in there and yeast emerges a few to several days later. They don't appear to be coming from the air as many people believe, because it happens even in a covered container. But, if they are already in the flour as the more reliable sources say, then why couldn't I find any? There was obviously more to it than just a symbiotic relationship between lactobacilli and yeast, gradually increasing in number, or the good guys out-competing the bad—the popular theories. No, there seemed to be something else going on and it was evident that there are many more bacterial and fungal species present in flour than sourdough lactobacilli and yeast. But where were the good guys? Why weren't they growing? It was time to close the cookbooks and open the textbooks.

I turned to a newly updated, graduate level food microbiology textbook. It's a huge volume, and yet I could find only two brief paragraphs on sourdough, and not much more on yeasted breads. It was a challenge to find information, mostly borrowed from chapters on wine, beer, dairy, and other food fermentations that share something in common with sourdough and its microorganisms. Rather like going on a treasure hunt for pieces of a puzzle, but I was able to narrow down the gas producer to a *Leuconostoc* species. I found the first clue in the chapter on spoilage of fruits, vegetables and grains. It turns out that almost 90% of spoiled doughs are caused by *Leuconostoc dextranicum* or *Leuconostoc mesenteroides*. I started to search for more information on these two organisms, and found that *Leuconostoc mesenteroides* is the primary organism involved in the fermentation of an Indian steamed bread called idli. (Spoilage is a subjective thing from culture to culture.) After soaking grains for a day and then grinding them with water into a paste, there is a 15-24 hour fermentation during which the idli batter increases in volume by about one and one half to three times—the same as our wild day two growth.

Leuconostocs are found to be occasional spoilage bacteria in wine making, *"but they undergo little or no growth during the alcoholic fermentation and tend to die off because of competition from yeasts. Nevertheless, these bacteria are capable of abundant growth in the juice and, if yeast growth is delayed, they could grow and spoil the juice or cause stuck alcoholic fermentation."*[1] Many microorganisms produce characteristic aroma compounds, and so smell is an important clue to a microbiologist. I had described an unamended, all-white seed culture as smelling like sour milk with a hint of rotten cheese. And then I found that some leuconostocs are used in dairy fermentations (cultured buttermilk, and cheeses like Gouda, Edam, blue cheese and havarti) for their carbon dioxide and aroma compounds. Together, these things all describe what we were seeing, and according to the chapter on fermented vegetables, leuconostocs are quite common in nature and found routinely on all kinds of produce and plant material. So, we can expect them to be present on grains and in flour.

Knowing how bland a flour-water mixture starts out, and seeing how the microscopic picture becomes more subdued as the sourness increases, it was clear that the shift in populations and activity are tied to changes in acidity. pH is a fundamental factor in microbial growth. Some like it neutral while others need more acidity or alkalinity, but each species has its own pH range. The reason that the starters had become quiet on day three was because the pH had fallen and the gas-producing bacteria were no longer growing. Even though I still wasn't sure what these bacteria were, it was clear that whenever the gas-producing one or ones grew, the starter would subsequently become still and take longer to finish—sometimes by several days. I reasoned that the best solution might simply be to keep them from growing. And, since they stop growing as the pH drops, why not add an acidic ingredient to the mixture to lower the pH and inhibit them from the outset?

It was May now and Evan (shacke) had entered the picture. Unaware that this was already a hot topic, he began posting to the message board, seeking help after having tried The Bread Baker's Apprentice formula and gotten the same result that we had. Evan was interested in knowing the science behind it, and he and Pat were both eager to get to the bottom of the problem, so they volunteered to do some testing. Soon after we joined forces, Gary (gwray) contacted me and we invited him to join our little task team. With so many different recipes to choose from, it was clear that there are several approaches to making sourdough starter, but we needed to pick a direction to focus our problem-solving efforts. Because so many people on the message board were loyal fans of The Bread Baker's Apprentice, the group decided the goal would be to take that formula, and alter it as little as possible to make it proceed as described in the book. The fix should be simple with ingredients readily available at home or in the average grocery store. Our choices for the acids were ascorbic (vitamin C), citric (sour salt), tartaric (cream of tartar), acetic (vinegar), lactic (yogurt), and mixed acids (fruit juices).

For our first trial we chose ascorbic acid, because it is readily available in the vitamin supplement section, known to be beneficial, and widely accepted in bread-making. Pat and I used vitamin C tablets that we had on hand. We crushed them and mixed the powder with the flour and water on day one. And, much to our amazement... it worked! No gassy bacteria, and we were both growing yeast on or before day four, where it had been taking about seven days. But I discovered a little problem with supplement pills, and that is that some are buffered without being labeled as such. I was not getting the pH to drop in mine even though I kept adding more and more vitamin C. When I took a closer look at the bottle, I found two ingredients listed, that together, form the buffer system that was keeping me from reaching the pH I was aiming for. Pat's vitamin C was not buffered and her starter took off in only three days.

Buffer problems aside, neither one of us enjoyed the task of crushing pills. And whirring them in a blender with the water only worked so-so. We also had no idea what the best dose would be. Gary and I both had some ascorbic acid powder. So we did another experiment testing different doses ranging from 1/8 to over 1 teaspoon mixed with the 4.25 ounces of flour on day one. It was a fun experiment to do. With the jars lined up next to one another, they looked like perfect stair steps as the starters began to rise. It was easy to see which doses were most effective by how fast and how high the cultures rose. For me, the most active jars were the ones with 1/4 and 1/2 teaspoon of ascorbic acid powder. For Gary, the best results came from 1/2 and 3/4 teaspoon, so we settled on 1/2 teaspoon as the recommended dose. While the ascorbic acid worked quite well, and may be the ingredient of choice for purists or professionals, the average person must go a little out of their way to find or mail-order it. So, we decided to press on.

All of the acids that we tried, inhibited the gassy bacteria effectively, but sour salt (sometimes found with canning supplies) was so strong that it was hard to measure the tiny amounts accurately. Cream of tartar (found in the spice section) was too weak, and required an impractically large amount to effectively lower the pH. We dismissed lactic acid because we didn't want to deal with dairy or go to the trouble of draining yogurt for the whey. And vinegar was so highly inhibitory to yeast in the doses required to lower the pH, that it was no solution at all. That left the fruit juices. I tested the pH of various juices and made a list for the group to try—apple cider, orange, lemon, grapefruit and pineapple juices seemed the most promising. Whenever trying out a new juice or acid, I had the group run what is called a *negative control* alongside the test, using plain water in place of the juice or other acid. This was to show that any changes in the result were due to the acid and not to chance or variation in experimental conditions. Time after time though, the control jars followed the familiar pattern while the test jars proceeded by the book.

I was hoping orange juice would perform well, since it is a good source of Vitamin C and a staple in many homes. But, it turned out not to be acidic enough to meet the group's objective, which was to use it only on the first day. However, Orange juice and apple cider do work well if they are used in place of the water for two to three days. Pat was the first to try pineapple juice, which has a lower pH than most other juices, and just happens to come in handy 6-oz cans (exactly the right measure for day one). She liked it so well that she stopped testing anything else and started recommending it to others. Most everyone who tried it was successful with it, and so pineapple juice became the solution that stuck. The group's mission was accomplished.

I wanted to know more, so while the trials were under way I went back to basics, monitoring the changes in acidity and examining seed cultures under the microscope every day. I recorded pH readings, growth measurements and observations at the beginning and end of each 24-hour feeding cycle. After a number of runs, I gathered my notes to compare and look for patterns. (My pH paper was only sensitive to the nearest 0.5 increment, so readings are approximate.) I found that when I acidified the day one mix to 4.5, it stayed at 4.5 until I fed it again on day two. If I didn't add more acid at that time, the freshly fed starter would read 5 and the gassy bacteria grew on day two and followed the oh-so-familiar pattern. If I acidified the day one mix to 4, it stayed at 4 until I fed it on day two, after which it read 4.5. The gassy bacteria did not grow and the culture started producing its own acid as other lactic acid bacteria were increasing in activity. During the second 24 hours, the pH dropped to 3.5 and the starter tasted sour. Yeast appeared one or two days later. When I acidified the day one mix to 3.5, I actually got some yeast growth on day two. I'm not sure that this is the best way to go, though. I've only done it once with citric acid and the yeast were not as vigorous the next day as I had hoped to see them. More testing could be done. The key points here are that the gassy bacteria grew at or above pH 5, but not at or below 4.5, and the cultures I was growing all failed to produce acid of their own in the first 24 hours. That is important because a day one flour-water paste measures about 6—quite inviting to leuconostocs. And even more importantly, in all my trials I have never seen yeast before a starter gets sour, but it usually appears very soon after.

By this time I wanted some real answers, so I had one of my flour-water cultures analyzed on day two during the big expansion phase. I took samples to two laboratories—one that could identify leuconostocs, and another that could identify lactobacilli and other bacteria of interest. Both labs found that there were three organisms growing. The *Leuconostoc* was identified as *L. citreum*. There were no lactobacilli or yeasts found, which supports what I observed time after time on microscopic examination. I didn't find any information specific to *Leuconostoc citreum* at the time, but it appears to share many characteristics with other *Leuconostoc* species found in foods, most of which will not grow at or below pH 4.8 (their optimum pH is 6.5-6.6).

In addition to the *Leuconostoc*, there was a large quantity of *Aerococcus viridans*, and a very small amount of *Enterobacter cloacae*. The first lab found the *Leuconostoc* to be in the highest number, so I think that earlier in the day it was. The *Aerococcus* was multiplying so fast by that time that it soon passed the *Leuconostoc* in number. That is important, because even though *Aerococcus* doesn't produce gas, and so was not responsible for any of the expansion, this organism may have been a major contributor to the delayed progress. It does not produce acid as the product of fermentation[2], so while it was using up a large portion of the available sugars, it was not helping the pH to fall. I can't find its lower pH limit in my reference books, but since pineapple juice seems to keep it at bay, I suspect that it must be around 4.5-5.0 as well. *Aerococcus* is an occasional spoilage organism in unpasteurized milk, which was the extent of the information that I could find on its involvement in foods.

Motility is the ability of bacteria to actively move about. I mentioned previously that some of the bacteria were flipping, twirling and zipping around under the microscope. Those were *Enterobacter cloacae*. *Enterobacter* produces gas, but since it was present in only a scant amount compared to the others, I think it is safe to say that the *Leuconostoc* was generating most of the gas. The *Enterobacter* was probably contributing to the odor, as well as the *Aerococcus* and *Leuconostoc*. Since some people report a very stinky smell and others not so much, I'd guess that not all starters growing *Leuconostoc*, necessarily have the same combination of bacteria that I had. There are others that can grow as well. Because the number and species of microorganisms in flour are influenced by conditions relating to weather and grain crop production, results may vary from flour to flour and year to year. I wish I could have all the organisms identified at every stage, but there aren't any laboratories here that are equipped to identify wild yeasts or sourdough bacteria. And, if they could, the cost would be prohibitive. I was fortunate to be in a position to have two of the organisms identified as a professional courtesy.

With all this new information and having watched the drama unfold under the microscope, I now view the process as a natural succession of microorganisms that pave the way for "the good guys" in the way that they transform the environment. There are microbes in flour that prefer the more neutral pH of freshly mixed flour and water (like *Leuconostoc* and company). They are the first to wake up and grow, some producing acids as by-products. This serves to lower the pH to the point that other bacteria will begin to grow. And they produce their acids, lowering the pH even more. Which then makes it impossible for the first bunch to keep going, and so they drop out. The more acid-loving bacteria, like lactobacilli can eventually take over. Like a relay, the baton is passed to the next group in line as conditions become suitable for them. The acidity increases a bit more with each pass, and the appearance of yeast seems to correlate in some way to low pH. Maybe directly, maybe indirectly. But the pattern is not random, which indicates that it's not about "catching" yeast or their gradually increasing in number.

In the late fall/early winter of 2004, I was coaching a group of people on Taunton's Fine Cooking forum. Seeing the starters developing a little more slowly this time around is what inspired me to describe the different stages a new culture goes through rather than to pin it to a time frame. Room temperature is different from one kitchen to the next, as well as season to season. Sometimes rye flour works faster, and sometimes whole wheat is faster. Sometimes a culture doesn't start producing its own acid for the first two days instead of one. And, I made a new observation; my starters often liquify the day before yeast starts to grow. The gluten disappears, which shows the work of a protease enzyme. At first, I thought maybe it signaled the appearance of lactobacilli and their proteases. But more likely, it was an indicator that the pH had dropped low enough to activate a cereal protease.[3] Since I use whole grain flour to seed a new culture for at least three days instead of only one, there would have been more cereal enzyme present than in a starter fed with white flour (because most of it is stripped away with the bran in the milling process). But either way, I think it is a sign of *Lactobacillus* activity, whether it is by production of proteases or by the organism's effect on pH. Because this process involves variable live cultures under variable conditions, it doesn't always work in a prescribed number of days, but it always seems to follow the same basic pattern. While this has been a discovery process for me, it is not a new discovery.

> "*There has been nice work done in Rudi Vogel's lab on the microflora of a freshly started sourdough: first, there are enterobacteria (Escherichia coli, Salmonella, Enterobacter), highly undesirable organisms that stink terribly. Then there are homofermentative lactobacilli (good lactic acid producers, but they don't produce gas or acetic acid), then acid-tolerant, heterofermentative lactobacilli that make lactic and acetic acid, as well as* CO_2*. I think this took about forty-eight hours at 30ºC in Vogel's study. The stink at the beginning does not matter as the organisms will be diluted out or die eventually. No L. sanfranciscensis appears by forty-eight hours, though: these will occur only after repeated refreshments. Peter Stolz told me that it takes about two weeks of repeated inoculations to get a good 'sanfranciscensis' sourdough.*"[4]

That paragraph didn't have much significance for me until I had gotten to this point. But now I could see how all the microorganisms fit into the four phases that I had described. Here is the updated version, marrying the two. You don't need a microscope for this, because there are outward signs that serve as useful indicators of progress.

The First Phase:

For the first day or so, nothing really happens that is detectable to the human senses. It doesn't taste any tangier or develop bubbles. It stays looking much the same as when it was mixed, except a little lighter in color if an acid was used. While nothing visible is happening, the first wave of organisms (determined by pH and the microflora in the flour) are waking up, sensing their new environment and preparing to grow.

This phase usually lasts one day, sometimes two.

The Second Phase:

The starter will begin to produce its own acid and taste tangy. Lactic acid bacteria are actively growing at this point, and I believe this is when homofermentative lactobacilli start growing. When using only water, this phase may represent two waves of microbes—first would be *Leuconostoc* and associates, followed by other lactic acid bacteria and homofermentative lactobacilli. If using an acid, you just by-pass the leuconostocs and other "*highly undesirable organisms that stink terribly,*" and go straight to the second wave. It will expand only if the pH is not low enough to prevent growth of the gassy bacteria, otherwise there won't be much else to see. There probably won't be much gluten degradation, and it may smell a little different on the surface, but shouldn't smell particularly foul unless using only water. This phase could last one to three days or more. If it is going to get hung up anywhere, this is the place it will happen, especially if it is put on a white flour diet too soon. If after three days, it still doesn't become more sour and show signs of progress, use whole grain flour instead of white at one or more feedings for its higher microbial count.

The Third Phase:

The starter will become very tart—an indication of more acid production by a more acid-tolerant bacteria. The gluten may disappear and tiny bubbles become more noticeable. These are signs that heterofermentative lactobacilli have picked up the baton. Once the starter becomes really sour, it usually transitions right into phase four. Note that lactic acid doesn't really have an aroma, and so smell is not a very reliable way to judge sourness.

The Fourth Phase:

Yeast start to grow and populate the starter quickly, causing it to expand with gas bubbles all over, and it will take on the yeasty smell of bread or beer.

Maybe wild yeasts are activated by low pH, or maybe the activator is something produced by lactobacilli, but it happens predictably at this point for me, as long as the whole grain flour has not been diluted out—white flour is not as quick in getting things going, because it has a much lower microbial count. There may be some variation among species of wild yeasts as to the exact pH or activating substance. That is something I have been unable to find in scientific literature, and my contact at Lallemand did not know (I could only find studies done with cultivated strains of *Saccharomyces cerevisiae*). The only useful information I have been able to find on the subject is this, on microbial spores in general:

> *"Although spores are metabolically dormant and can remain in this state for many years, if given the proper stimulus they can return to active metabolism within minutes through the process of spore germination. A spore population will often initiate germination more rapidly and completely if activated prior to addition of a germinant. However, the requirement for activation varies widely among spores of different species. A number of agents cause spore activation, including low pH and many chemicals... The initiation of spore germination in different species can be triggered by a wide variety of compounds, including nucleosides, amino acids, sugars, salts, DPA, and long-chain alkylamines, although within a species the requirements are more specific. The precise mechanism whereby these compounds trigger spore germination is not clear."*[1]

What this means is that before dormant cells can return to active growth, they have to break dormancy. That is initiated by different things for different microbes, and it sometimes requires more than just a food source. In the case of these wild sourdough yeasts, if all they needed were food, which is there from the get-go, then they would start growing immediately. The fact that they don't, is probably why people think they are caught from the air or that large quantities of flour must be used to round up enough of them. There are usually enough dormant yeast cells present in even a small quantity of whole grain flour, but it's kind of like the game Simon Says. You can try coaxing them to grow, with food and all the things that you fancy to be good for actively growing yeast. But, they are not actively growing yeast, and they won't grow until they get the right message from their environment. If you think about it, this could be a survival mechanism, since it greatly increases the likelihood that they will wake up in the company of lactobacilli, with which they share a mutually beneficial relationship. But, it is also important to note that active sourdough yeasts thrive in a much wider pH range than what seems to be required for activation of dormant cells.

None of this is unique to the formula in the Bread Baker's Apprentice. You'll see this progression, in whole or in part with all starter formulas, because the needs of the organisms must be met before they can grow. It doesn't matter how much flour you start with. In fact this can be done with small quantities of flour. It proceeds at the same speed with a tablespoon as it does with a pound. Procedures that have you feeding two or three times per day before yeast are active, actually slow the process, because this dilutes the acid, delaying the drop in pH, which keeps it from moving through the succession of microorganisms in a timely manner. Three to five days is about all it usually takes to sprout yeast at average room temperature. Somewhat longer if *Leuconostoc* and associates grow. The strategy is different from reviving a neglected starter, which is likely to have an overabundance of acid, with a large population of yeast and lactic acid bacteria, however sluggish they may be.

There are things you can do to facilitate the process, like provide conditions for the first two to three days, that are favorable to lactic acid bacteria—a warm spot, but not much higher than 80ºF, and a reasonably high hydration (at least 100%). Use an acid to control the pH and bypass at least the first round of bugs. Feed with whole grain flour for its higher microbial count, until yeast is actively growing. But, don't feed too much or too frequently, so as to allow the acids to accumulate and the pH to fall more rapidly. The ideal feeding quantity and frequency would depend on the temperature, hydration, and how fast the pH is falling (although, once a day at room temperature works fine and keeps mold at bay). The first days are not about developing flavor or even fostering the most desirable species. The object is simply to move through the succession and get the starter up and running. The fine-tuning comes after. Once yeast are growing, change to any temperature and hydration you like, and the populations will shift in response to the flour and conditions that you set up for maintenance. Another thing I have found is that new (white) starters seem to improve and get more fragrant right at about 2 weeks with daily feeding at room temperature. Maybe that coincides with the appearance of *Lactobacillus sanfranciscensis* as previously mentioned. A Fifth Phase? According to scientific literature, various strains are found commonly in sourdoughs all over the world, not just in San Francisco.

Well, I hope that I have addressed all of your questions. Please let me know if I can be of further help.

With best regards,
Debra Wink

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