ESSENTIAL KNOWLEDGE IN THE BATTLE AGAINST BIOFILMS
Teleclass Transcript

Hosted by Paul Webber
Sponsored by Johnson Wax Professional
Presented by Dr. Bill Costerton
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What follows is a transcript of a teleclass entitled "Essential Knowledge in the Battle Against Biofilms. The teleclass took place on April 4, 2002 and featured Dr. Bill Costerton of the Centre for Biofilm Engineering at Montana State University. Dr. Costerton is a global authority on biofilms, and was recently cited as one of the top 94 most quoted microbiologists in the world. If you want more information on this topic, or on Dr. Costerton's Centre for Biofilm Engineering, or on teleclasses in general, I would invite you to contact me by e-mail paul@maunco.com

Paul

Dr. Costerton:
I'm hoping that people have access to the images off the web site. If you want to bring the first picture up, (Slide #2) then that tells us what we are talking about with biofilms - in industrial systems, engineering systems, and environmental systems, the general consensus now is that bacteria live predominantly - we're talking about 99.9% - on surfaces. The left hand panel shows bacteria adherent to a surface without polysaccharides. And when they're adherent to a surface, they show their happiness with their situation by reproducing as we all do. And so in the right hand panel we see a 4 and 8 and 16. So basically we can take that there's a consensus now that bacteria prefer to live on surfaces.

Today I'm going to take this into the medical area, and some sterilization areas, and I'd like to start off with a neat story. Tom Marrie, down in Halifax, had heard my biofilm ideas, and he found the perfect patient for me. The patient, strangely enough, was a litigation lawyer who had had happier times. He had an alcohol problem, he lived in a cardboard box on the docks, and basically he was the poster-boy for a medical problem. The problem was that, if you have an infection associated with a device of some kind, you normally have symptoms, then you get treated for the infection, it resolves for a short length of time, and then it keeps coming back, and back. After a while, the medical wisdom is that you take the device out. This patient, whose name also is Tom, had a bacteremia of *Staphylococcus aureus*. We treated him with 14 grams a day of Cloxacillin. And Tom Marrie had noticed that he had in the upper quadrant of his chest, a little cigarette package sized battery pack. He must have a pacemaker in his system. So he had bacteria in his blood, and he had a pacemaker in his system, and we figured he probably would have a biofilm on that pacemaker. So for 3 weeks we gave him 14 grams a day of Cloxacillin, and when we took him off the Clox., back came the infection. Then another 3 weeks of Cloxacillin plus Rifampicin. We stopped the antibiotics, back came the infection again immediately.

So if we change the slide we'll see the pacemaker that we took out of his chest, and from 11:00 until 4:00 on the pacemaker, you'll see a large accretion. It was actually a macroscopic... the central target there is metal, the outside cowling is plastic. This was actually ten to the tenths living *Staph* bacteria when we grew it in the lab.

If we take the next slide, we see spherical bacteria, and we see bacteria embedded in large amounts of polysaccharide. This was the moment of truth for biofilms in the medical area. We had seen the enemy, as Paul Kelly would say, those spherical cells embedded in the plastic. And 6 weeks of antibiotic chemotherapy at huge levels had failed to actually resolve this infection. We were beginning to understand the resistance of these populations of bacteria to antibiotic therapy - we're talking 1978 here.

Looking at the next slide, we're looking at a Hickman catheter. These are used for cancer chemotherapy. There's the lumen and the Hickman, and you can see the sort of general curvature of the whole picture. The barnacle-like masses are huge masses - the bacterial biofilm. Here our star patient was in a situation where we could put fluids down the catheter by a Hickman catheter on the subclavian right into the heart. But we could never bring blood back up. So it was sort of a valve at the bottom of the catheter. What we did was to take this out after he had used the catheter for 8 ½ months. He didn't need it anymore, he hadn't had any very serious infections, and in this picture - sort of oval picture here, with a valve coming in from about 10:00 - you can see at the end of his catheter. As we look at that higher magnification, you'll see cocci. They turned out to be *Staph epidermidis*. We'll see some snaky looking masses up at about 11:00, which are *Candida.* In fact this large mass on his Hickman catheter was made out of *Staphylococcus* and *Candida*. It's very interesting that this patient never actually had a bacteremia. We didn't see any symptoms at any time. But if any device is placed in the body, we're going to see biofilms develop.

Here's an absolutely classic one, the bacteria held together with slime, the fungi going through them, and a huge accreted mass. We were interested in looking at other infections, and this is another champion infection of ours. If anyone wants to make sure they're on the right page here, we should have black cocci with dark slime material, should say JW Costerton in the bottom right hand corner. This is a post mortem picture from an osteomyelitis that went for 60 years. This person was kicked with a skate when he was 6 years old, but he died 4 years ago at the age of 66 with post mortem material from them. Here are the bacteria, here's the huge amount of slime, and this is a biofilm infection of osteomyelitis in the bone. It started way back, so he was treated with sulfa drugs and he was treated with beta-lactams and he finally died on fluoquinolone, not of osteomyelitis but something else. But he actually carried this chronic infection for a full 60 years. Three operations for debridation, that's scraping the slimy mass out of his bones, but basically this terrific bacterial population, you can see literally hundreds of little black cells here the slime that surrounds them that actually kept the bacteria alive through a very large amount of antibiotic therapy.

Now the next slide shows the valve and endocarditis. These are various end group *Streps* and you can see occasional slime around the bacteria. Now if you do unwise things with little white powders, you're likely to get endocarditis on your valves, this is a pure biofilm. And this is the only biofilm that we know of that gets successfully treated. If you have native valve endocarditis

and they give you huge doses of antibiotics for 8 to 10 weeks, sometimes these bacteria can be cleared. I hope I'm making the point that in our observation, basically speaking, if you have a device or a dead bone or a dead tissue of any kind, then it's really difficult to clear these bacterial infections. In a few cases, like in this one, we can clear it. We can now say that we've looked at virtually hundreds of chronic infections, particularly ones that were associated with medical devices, and found exactly the same pattern. Hold this picture in your mind, if you would: bacteria occupying about 30% of the volume, huge amounts of slime, and not much luck with antibiotics.

You can only go on telling stories and looking at pictures for a while. Now we want to look at the first experiment in a really scientific way. It says artificial urine, but we didn't use artificial urine, we used professorial urine and graduate student urine, mixed and carefully collected. And we added bacteria to this urine stream. These were not lab bacteria; these were islets from a chemical lab. They went around the peristaltic pump - you'll see a "P" there - went past the Robbins device, and the next slide shows at 2 hours bacteria creating the surface. The next slide shows at 8 hours a beautiful biofilm all over the surface, about 8 microns thick. These are the kinds of things that we studied in the lab.

Now here we see in the numerical slide that we're going after planktonic and sessile cells, which are *Pseudomonas aeruginosa* - sessile means biofilm - with tobramycin. But this could be any bacteria or any fungus with any antibiotic agent. Now if you look at the planktonic cells, and we look at 50 μg/ml, contact time of 8 hours, we get a complete kill. This is typical of floating cells. But if we use 1000 μg/ml, and contact time of 12 hours, on the biofilm bacteria – sessile cells - we really don't have any significant kill at all.

In the next slide we'll see that there is a patient and he has a device that has a biofilm on it. He's got a fever obviously, from the thermometer in his mouth. What's happening here is, we can only usually get the bacteria from the planktonic phase - the floating phase. That's the MBC that we're used to, and that's good value, very good value. If we then run the bacteria through a Robbin's device and make a biofilm and attack it with an antibiotic, we get the biofilm killing dose. So clinically, let's think about what these two values mean. The other patient has a device, he has a biofilm, and he's got problems because he had planktonic bacteria coming away from it. They're giving him the symptoms. So if we treat him with a planktonic killing dose, we're going to get the symptoms reverse because we'll go back to just a plain biofilm and he'll feel better for a little while. But then the planktonic bacteria will come away again from the biofilm and we'll have another planktonic episode, and another, and another. This is the pattern in these device-related infections. If we can ever get the biofilm killing dose, and sometimes we're doing it now in orthopedic patients by direct infusion of huge amounts of antibiotic, then we can actually cure the basic problem, which is the presence of the biofilm. So let me just sort of lean on that a little bit. The patient's got a device, biofilm growing on the device, not too many symptoms, then planktonic bacteria start to come away from the device, we get symptoms. We relieve them back with the planktonic killing dose, that's fine, but the infections can recur and recur, and we won't actually cure the basic problem, which is the biofilm, until we use the biofilm killing dose.

Change over to the next slide, and I'll answer a little question that probably has occurred to you, and that is, why doesn't the host defense system resolve these biofilm infections? Here we have a large number of bacteria in a biofilm in the lung in cystic fibrosis. If we look at this bunch of bacteria, there are about 50 of them in here surrounded by large amounts of slime. If you look up at about 12:00 and around at 3:00, you'll see a sort of crust of dark material around the outside of the biofilm mass. These are antibodies. These are IgG. They've accreted around the bacterial biofilm mass. The critical thing in cystic fibrosis is that there are huge amounts of antibody there, but they do not kill the bacteria, and they do not resolve the infection.

The next slide is a great pride to me, because you'll see in the bottom corner, K. Costerton. I believe in nepotism. I've always hired my kids to work in the lab. I hired my son and he promptly married my post-doc. This girl that was my post-doc is now my daughter-in-law, Kathy, and a dear, dear friend. She invented this lifesaver device. She was answering a pretty specific question. If you took this teflon rod and disks and the silastic facing, and you put a biofilm - I'm talking a beginner biofilm here, I'm talking 3 or 4 cells with a tiny bit of slime around them - into an animal (we put it into the peritoneum in rabbits), with the phagocytes and the white cells, will they be able to eat these beginner biofilms?

The next slide shows, in the red dots, that if we put in very small biofilms on day zero, they go right ahead to make huge bacterial biofilms, 5 x 7 to the 7^{th}, and last for about 42 days. Then Kathy did what I thought was the best experiment of all. She pre-immunized the rabbits against the exact of the same strain she used to make the biofilm, and that gives you the blue dots. When there were antibodies there, and phagocytes there, for some weird reason the bacterial biofilms did slightly better. As the New Yorkers would say, go figure. I mean, you've got antibodies and you've got phagocytes, and the biofilm just sails through and does wonderfully. So it's a really bad idea to put any biofilms into people - the host defenses don't work.

The next slide shows a summary diagram. You've got to sort of go slowly through this so we nail these ideas carefully. This was in Science in '99. If we take the left hand panel, here we've just installed a shiny metal material into a patient. The rest of the pink stuff is tissue. We have floating bacterial cells, they're white, and the green x's are antibiotics, and they work really well. The yellow y's are antibodies, and they work really well, and the Phagocytes take up any stray bacteria. That's the nice story. That's when the bacteria get cleared. Now, the unlucky: we're into panel B, and here the bacteria accreted onto the surface. I'm changing white bacteria to black, so we'll justify that later. They're surrounded by slime. The little blue x's aren't doing much. The yellow y's aren't doing much. The phagocyte is sort of sniffing around. Let's move to panel C. This is where we get into real trouble with these chronic infections. Here the bacteria are black and they produced a fairly large amount of this pink slime. Antibiotics aren't working. Antibodies aren't working at all, but they're attracting the phagocytes in. Then we get into what we call frustrated phagocytosis. In panel C we have a phagocyte there. It's trying to engulf the biofilm. It's not succeeding. Because it's frustrated and angry, it starts releasing these red dots, the degradive enzymes, and they have no effect on the biofilm at all, but they start to affect the surrounding tissue. It gets worse and worse, and we move into panel D. Now we have two phagocytes here, both try to digest the bacterial biofilm. They don't make any headway. They're actually producing a lot of collateral damage in the tissues.

This actually brings up a clinical point where we find that if we immune suppress in cystic fibrosis patients and prostatitis patients we do get some relief from this horrible collateral damage. So inflammation is our enemy. We've got bacterial in panels, everything from B on, and they're sort of going "nah, nah" at the immune system, and the immune system is coming on and producing the degradive enzymes, and we get a lot of collateral damage. The last point in the upper right hand side of panel D is that we can, at any time, have planktonic bacteria released again. This means that if we have a device related infection or prostatitis, we can get one of these exacerbations, and we can have acute symptoms, fever, malaise and so on.

The kicker on this particular diagram is that CDC figures show that we've almost finished with acute planktonic infections like typhoid and diphtheria. Sixty five percent of the infections seen by clinicians in the developed world are this kind of biofilm infection. Next is a list of things that might be on this list. They would be prostatitis, osteomyelitis, infections in cystic fibrosis, and most importantly, middle ear infections in kids. These chronic junky infections are of this type.

If we move to the next slide we see where I am now in the States - this is the Centre for Biofilm Engineering at Montana State. It was actually funded by the National Science Foundation - 22 million dollar funding, and an 11-year grant. And it wasn't from NIH and it wasn't from the science part of NSF. The funding was from the engineering part of NSF.

Engineers have problems with biofilms, and they wanted to solve them they built this building. This was an investment in engineering. The next slide shows the mix of graduate students that actually make the place work. There are 51 of them and only 8 of them that are trained microbiologists. Three of them are mathematical modelers and the rest are all engineers of one sort or another. We call them knuckle-draggers, but we don't do that in public usually. They aren't interested in little research projects, and just about everything else from here on is really their product. They're wonderful people, and they do great work, and they like to solve problems.

Now we're looking at an engineer's version of a biofilm. They were looking by confocal scanning laser microscopy, which has replaced electron microscopy pretty much completely. The dark sausages you see are bacteria. The slime shows up as a hazy blue matrix. And then there's a deep set of water channels. So we're able to actually look at bacterial biofilms directly. Engineers love to do this.

In the next slide we're looking at golden bacteria and red slimy material. We're looking the "Z" axis, which is perpendicular to the colonized surface. There's a mushroom right in the centre there. We're now looking at a real natural biofilm formed in a river. Here we see bacteria in large masses. These are filamentous bacteria. At 3:00 we see a free-living amoeba, which is coming down one of these filaments. It's picking up any loose bacteria that it can but it can't deal with the biofilm. I always think it's no wonder that phagocytes don't have much luck - free living amoeba have been trying for millions of years to eat biofilms and they didn't make it either.

Direct observation is our key, and so in the biofilm in the centre in this slide, there's a red nuclei in the green background. You see vaginal samples take from volunteers, and we have epithelial cells with lovely orange nuclei, a few bacteria kicking around. And on the right hand side you see the native state of the human vagina. We have bacterial cells, they're green, they're enclosed in huge amounts of pale green slime, and they overlay the actual human cells, or the orange nuclei so thickly that you can barely see the nuclei in many cases. So the engineering tact is to go in and look directly at biofilms on a surface. Here you can see the bacteria occupying maybe 5% of the volume, the green rods. About 95% of the volume is the green slime, and it's all sitting there above the tissues. So what engineers like to do is to look directly at things, and the slide we're looking at now shows an engineering marvel. This is a dissolved oxygen micro-electrode. It can be used to measure oxygen tension directly in a biofilm. Here we see on the orange arrows coming down where we've come and taken measurements using this dissolved oxygen electrode.

The next slide shows oxygen tension. That brown area in the centre of the slide is the cap of a mushroom, which we sort of made a glancing section through, it's called a cell cluster. This is *Pseudomonas* growing in the air. We have lots and lots of oxygen around the outside, and lots in these channels. The *Pseudomonas* are right in the cap of a mushroom where we have almost complete anaerobiosis. In that top isobar you'll see 0.010, meaning this is virtually anaerobic. *Pseudomonas* doesn't grow much as anaerobic. In the cluster of the head of the mushroom, we have very slow growing cells growing essentially anaerobically. If you think about going after a biofilm with an antibiotic, you could kill some fast growing aerobic cells, but slow growing anaerobic cells might not react to the antibiotic. After the antibiotic therapy was finished, they would re-grow and take over again. This sort of explains partly why the bacteria are so fantastically resistant.

Here's another summary slide, and I'd like to really lean on this because almost everybody listening to this teleconference will have had this experience. In the left hand panel we've got bacteria as they really grow. They have three different species there, brown, blue and light blue, and they're all growing on a surface surrounded in slime. The brown ones have given rise to a bright red planktonic cell. There are a few of these around, about 0.01% of what's there. Now you come by with a swab and we sample that. We pick up these planktonic bacteria, take them to a test tube shown on the right, and we grow them. I've had this experience with *Pseudomonas aeruginosa* directly from a CF patient. In the first transfer, it's got all kinds of slime around them, the walls of the tube and around the interface. It doesn't look good and you transfer it a couple more times until you get a nice turbid culture of planktonic bacteria. Because every time you transfer, you take the floating bacteria from the centre of the tube, you leave all the adherent bacteria behind, and finally you get a well-behaved bacterial suspension. But it's got almost nothing to do with the real population that we see on the left hand side of this image.

In the next slide you'll see these gray biofilm clusters with black cells inside. If we use a sterile scalpel blade to scrape a surface, we get a great big lump as we see on the second panel from the left. That's going to hit the surface of an agar plate and gives us one colony. So it's a CFU type situation. Even though, in the third panel over, these smaller aggregates are still going to give us one colony per aggregate. Now on the right hand side we have a bunch of planktonic bacteria looking like tadpoles, and they're going to give us one colony per cell. So there are some really,

really bad things happening in biofilm microbiology because techniques have not kept pace with concepts. What we tend to do now is use direct microscopy rather than using colony counts. Here's an excellent example from the vaginal study that we've done. On this slide we have a cell. The shiny things are red blood cells. That's an epithelial cell in the background with a nucleus about in the middle. There are some odd shaped cells and some cocci. All that we can do now with probes show off all the bacteria. In this slide we have rod shaped cells, they're lactobacillus. We have the nuclei shining nice and bright in the middle. And then we have a bunch of cocci in the upper right hand corner. Now if I use a staphylococcus specific probe, those cocci will light up like a flashlight. So we know that the cocci in that clump, in the upper right hand side, about 1:30 on this epithelial cell, are all *Staphylococcus aureus*. In fact what they're doing is making toxin and they're adding toxic shock in people.

We then go back to talking about direct observations. Here we have a red background and large green aggregate. This system is rather interesting because this is a dental population. What we were doing in this slide is using the live-dead stain, as it's called. We're looking at this population and rotating the image. We've killed bacteria in the system using an antibacterial agent. Here we have a red lawn with a few survivors, just a few percentage, and the big green towers are all surviving. Now the big green towers are really interesting bacteria, but looking at them this way, sideways, you can tell they're only going to give us one colony when they get plated out. If I take that dental population and kill it, and I do a count using a plate, I'll find a few survivors from the red background, a few of these large green balls. It'll look like I've got about a 90% kill but in fact I have lots of survivors. The survivors are those interesting ones in the towers, and while it looks pretty encouraging, a 99.9% kill, in fact I haven't done very much at all. I'd like to propose to everybody that we start looking directly at bacterial populations like the engineers do, and kind of abandon many of the old microbiological methods when it comes to biofilms.

Here we are looking at a kind of a puce background, and on the left hand side at 10:00 we have a mountain, sort of like a mesa, and the right hand side at around 4:00 we have a little ridge. This is another way of looking directly at biofilms in the modern way. Here we have made a reporter construct. That is we've put in a color reaction right behind the promoter of a gene that actually controls toxin production. The mountain on the left, that kind of mesa, is producing a certain amount of toxin. The ridge is really hot - you can see really bright yellow color there. And then over at 5:30 you can see a nice reaction on the ridge again. The point I'm making is that rather than using the old microbiological methods, we use the microscope, we use probes, live-dead probe, and we use reporter constructs. Then we can actually see what's happening in the biofilm. The traditional microbiological methods have not been particularly kind.

If we were going to take one image today of a biofilm and leave that pretty much with you, this is the image that I would use. We're looking down on the surface of a biofilm at about 6:00 we've got a mushroom top, we've got a bunch of towers in here, and a fairly deep channel. And this is the sort of English garden look at a biofilm on a surface. It's a fantastically complicated structure. It has flow operating through it that feeds it. This is what a biofilm really looks like. If you were looking at a biofilm in a sewage system it would look like that. If you were looking at one on a mechanical heart valve, it would look like that too.

The next slide shows that we can deform these mushrooms and towers in high flow. So we have tadpoles, three of them here, with their tails pointing up towards about 1:00. In a flowing system they should be oscillating back and forth. We can see how they operate, and in the next slide we can see what happens when we put these into extremely high flow. We have a wave that's moving through the biofilm, and pieces of the biofilm will flow off. It's a bit like a slimy, jelly-like mass on the surface, which is deformable, which is moving, and which is calving off little pieces. This is the reason that if you have endocarditis. Little pieces of biofilm are going to fly off and go off to various parts of your body. Clinicians who are treating you for endocarditis are going to be watching under your fingernails and in your retina to see where these pieces of biofilm land.

Let me leave an impression with you if I might. The biofilm is really a jelly-like mass, about 80% slime, and 20% cells. This jelly-like mass has certain characteristics that break off and behave in strange ways, and distribute themselves through the body.

Here we have a picture that should say Keith Kasnot in the bottom left hand side. This is the Scientific American article from July. The first panel shows bacteria coming into an area and settling on the surface. The second image shows them making large amounts of slime. The third image shows them starting to make a mushroom-like aggregate. The fourth image shows them recruiting bacteria from their surroundings. And the fifth image is your mature biofilm - it's got differentiated shape. It's got windows through which flow is happening. Bacteria are coming and other bacteria are leaving. This is probably the image that's the best to keeping in mind as far as a biofilm is concerned.

We come now to the most recent ideas on biofilms, two ideas. I think that these are the beginning of a new biofilm era. I mentioned that biofilms are fantastically resistant to antibiotics, and I mentioned that some are anaerobic and some are aerobic, and that's a problem. But this is something that really blew us away. Take lanes 5 and 6 on the right hand side of this gel. Lane 6 represents the membrane proteins of planktonic cells of *Pseudomonas aeruginosa*. Lane 5 represents the proteins of biofilm cells from the same vessel. Here you can see that almost none of these protein bands in lane 6 are seen in lane 5. What this is telling us is that the biofilm is making a whole new set of proteins. It is in fact a vastly different phenotype. The thing is that it's expressing very, very different sets of genes.

We weren't ready for this, and this was a terrible shock to us. And let me just tell you where this takes us, the discovery of the biofilm phenotype. We can watch bacteria develop, and we can watch them turn on genes, this panel which says there's gene activation. We can see on the left hand two panels where bacterium has settled. On the right hand side you can see from a probe we've developed, watch it turn on a particular gene, and it starts making slime. What this says is that these blue cells coming down onto a surface are changing the gene expression pattern to make the pink phenotype, making lots of polysaccharide - that bacteria we were pretty happy with.

The next slide is the one that gives us a real problem because we have planktonic bacteria on the left, sessile or biofilm bacteria on the right. Some proteins, shown by the red arrows on the left, are present in the planktonic cells and absent in the biofilm. Others shown by the blue are

virtually absent in the planktonic but present in the biofilm. Look at the next set of protein expression gels, and take the area maybe a little to the right of 12:00. Compare planktonic cells, which are called chemostat here, to the protein expression pattern in the biofilm at 1-day in the center. You can see that about 15 new genes have turned on in the biofilm. Let's look at the 6-day biofilm in the right hand panel. Most of these genes are sustained but at lower concentrations in older biofilms. So what this is saying to us is that there is a fantastically different gene expression pattern in biofilms from what we see in planktonic cells. And that's not just an academic curiosity. If your antibiotics which were all designed to kill planktonic bacteria were directed against those particular gene products or proteins - look over in the biofilm panel in the middle - they're totally missing. The targets that we've been aiming at in planktonic bacteria are missing in the biofilm. To a large extent that's why we're not having much luck with the antibiotics that we have.

This slide shows that there's a terrific amount of new messenger RNA synthesis. It just means that when bacteria get onto a surface, they make all kinds of new gene products, turning on all kinds of genes - at least 800 genes when they become biofilms. This is a large part of why they're resistant to antibiotics. But we can probably develop some new antibiotics that will address these new targets and give us some joy in chronic infection.

Here's an idea that we need to kind of pause on and take our time on. If bacteria of different species, shown here by different colours, are making these extremely elaborate structures, and if they're maintaining these channels like we see down amongst the stems of the mushrooms, then they must be talking to each other. This little cartoon has them talking to each other. The idea is that they have to have a pretty sophisticated way of talking to each other, or they couldn't possibly make these structures. This slowly occurred to us. We looked at quorum sensing because that's the only way that we've ever seen bacteria talk to each other. In the left hand panel you've got a scarce number of bacteria. They make signal molecules, and we know what these are - red and green molecules. They diffuse away from the cell, nothing much happens. Then on the right hand panel we have cells getting crowded. They're getting a quorum. They're getting all together. Now these signals, the red and green signals, are coming back into the cells and turning on certain activities by reacting with DNA which is that black circle you see in each cell. They turn on toxins, enzymes and surfactants. Quorum sensing is that bacteria there in small numbers kind of hunker down and try and survive, but they don't do much. And then when they get to a certain number, a quorum for obvious reasons, then they start to turn on their aggressive activity.

So what we did was to look at the chemicals that used in quorum sensing and here they are. In the next slide we have a lactone ring which is that ring shown on the right hand side. We have various structures leading off to the left, and these are the signals that actually tell bacteria when they have a quorum and when they don't. We took a bacterium - you can see normal architecture on the right hand side here. These bacteria are making 2 signals, a green one and a red one, on the right hand side. And they settle on a surface. They make lots of slime. They make the biofilm, and everything's copasetic. In the middle panel we've knocked out the red signal, the single mutant. And here they land, and they make slime, and make a nice biofilm. But in the left hand panel we've knocked out by mutagenesis the green signal. And we do that, it's really very amazing, so bacteria cannot make a biofilm. They can kind of accrete on the

surface, just kind of stack up. On the left hand side you see the wind type. Here are the bacteria, and the slime, and the towers, about 3 or 4 towers there. When we knock out that gene for quorum sensing, we just get, as shown on the right hand side here, a homogeneous bunch of bacteria, and no slime. The slightest little disturbance and pressure or anything, and they all wash off the surface. We found the signal that actually controls biofilms formation.

The signal is green at the top of this panel, the brown structure is a receptor protein and the multicoloured strand is DNA. This is called an R-protein or a receptor protein. It sits on the DNA, and without the signal in place it doesn't do anything. It just jams. But when the signal settles into its little slot up top there, then it activates the protein. It slides along the DNA and does its thing. Well this is sure to make any self-respecting biochemist go absolutely ape because, of course, what you want to do is to develop a blocker for this signal. The blocker here is shown in light blue. It would attach to the receptor protein. It would fit into the slot. Hopefully it would have those little hooks to hook itself, slide in right into the protein, and then that protein's not going anywhere. That one's not going to be doing anything. We were thinking that this had possibilities into the future, and there's about a billion dollars worth of companies now looking at these blockers.

The strategy is, in the next slide, you have a bacterium coming down, the natural signal molecule is the green dot there, it reacts with the receptor protein, turns on slime, makes a mushroom. Now in the lower panel you're going to have the blue molecules, the blockers. There are lots of them around - they can pivot every way. They compete for the slot on the brown receptor proteins. You're dooming those bacteria on the bottom channel to planktonic life. They can't make a biofilm. They aren't going to die or anything, they just can't make a biofilm. This is the strategy that we're pursuing at this point.

We were thinking, gosh you know, 10 years down the line we might have something. But this gets really exciting. We should be looking at a red marine algal species. This marine algal species live in Botany Bay in Australia. It does photosynthesis for a living, so it doesn't want biofilms on it. It produces a blocker for the signal for biofilm formation. It's called **a bromianted furanone :** but that doesn't really matter. The structure is shown on the right hand side. It makes this molecule, which blocks the molecule on the left: **an acetylated homoserine lactone.**. The algae make a blocker molecule that stops biofilm formation. Here we've taken the blocker molecule, put it into plastic, put it into this little structure on the bottom of Sydney harbour. It is in fact about 100 metres from a sewage outfall. You can see that we can stop biofilm formation and marine fouling. This is a spectacular discovery. I think it's really very exciting indeed.

Let's just go back and break it down into its essentials. If biofilms form (and they do), and if they have elaborate structures (and they do), there must be signals. Now if there are signals, you must be able to block those signals, and here we have blocked that signal and stopped biofilm formation for... it says 5 months here but it's actually around 11 months now. Now one more thing before we finish up here. There's another signal, remember the red one that wasn't doing much in the other reaction. It signals detachment. We can actually use the red signal to make bacteria get up and leave a surface. They call that induced detachment. You can have bacteria in a mushroom as we see here, they can start moving and then they can actually detach from a

surface. We can have a big mass of bacteria like this, embedded in slime, and they can either break off chunks and go somewhere else, or they can return to the swimming phase, or planktonic phase, and go somewhere else. Attachment is actually an activity that we can control. Biofilm signals have got us very exited indeed because we think we're going to be able to interfere with bacterial biofilm formation at two stages.

Now I'm going to flip through four slides and go to the last slide. Here you see the URL for our website. It's www.ercmontana.edu and that's a very interactive site. You'll find it very interesting. I think it's well maintained. Please stay in lots of touch with our work. The very last slide is a black slide with white lettering, and it says Biofilms 2003 - a big meeting on biofilms will be in Victoria, B.C., Canada in November 2003. You all come, and we'll talk about biofilms and thank you very much for attending this teleconference.

Paul:

Thank you Bill. We will have time for a couple questions. Before I open up the floor for questions, I wanted to ask one. You mentioned once before, and we've spoken often about whirlpool tubs and how biofilms affect the health of people who use them. Could you very briefly explain how that works?

Dr. Costerton:

I can. If you look at a whirlpool tub, it looks very innocent because it's nice and shiny and clean and wonderful. But you forget about the pipes that are back there that are actually feeding the jets that are actually giving you the joy with the tub. If you ever look at the biofilms back in those pipes that are feeding the jets, and feeding the pump and so on, there are huge amounts of biofilm down there. Those biofilms can give you lumps of biofilm in aerosol. When that happens, and you aspirate a lump of biofilm, you have bacteria in your lung. This was evidenced very recently where some people in a hospital setting that used a hot tub, passed an infection around in that way. I don't think it has a really big effect on really healthy people, but it's a real hazard if you were at all compromised.

Paul:

How do we get rid of those biofilms in the pipes?

Dr. Costerton:

I think we used bleach, but I think we go back in and clean them with bleach or some really good cleaning compound, some I trust and otherwise I don't.

Speaker:

Is there evidence of biofilm in hot water pipes, with water temperature of 180 degrees F and above.

Dr. Costerton:

They do, and we've looked at legionella in hospital systems, hot water systems and so on. They are in biofilms and they're there in fairly large numbers.

Speaker:

You made a comment on bleach being effective against biofilms. Is that because it's inate characteristic as a chlorine, or is it because the fact that it's an oxidizer?

Dr. Costerton:

It's the oxidizing function. With an oxidizer you're dissolving the matrix material and killing the bacteria. You go back to a totally squeaky clean surface. My favorite anti-biofilm agents of all time are bleach or any good oxidizer. If you put enough of it in there, you're right back to square one and you're right back to a clean surface.

Speaker:

So in that case, hydrogen peroxide would probably work as well, since it's a pretty decent oxidizer.

Dr. Costerton:

Raw hydrogen peroxide is pretty decent but not quite as strong as bleach.

Speaker:

Are there any studies on the recovery time for once you go back to ground zero?

Dr. Costerton:

Yes, entirely nutrient dependent. If you're in a real medium, if you've got a residue of bacteria, you'd be back to the original biofilm in around 12 to 14 hours. If you're in a ligotrophic situation, like ultra pure water, it's going to take you about a week to get back to the original.

Paul:

Folks, we're just about to lose our line. Bill, for providing such a fascinating presentation I want to thank you very much. Thank you as well to Johnson Wax Professional for their financial support, and to Dr. Syed Sattar for all of the assistance that he provides. Lastly, thanks to you folks for making this fun to do. You'll get an e-mail from me shortly, in fact it's probably waiting for you now. It will ask for your feedback, and I really would like that. I'm still learning how to do this well, and I'd like to hear from you. There's an e-mail address there for the Centre if you have some questions you'd like to ask. Ladies and gentlemen, I thank you very much, please enjoy your day.