

# Investigations into the Current Usage of Microorganisms in Medicine

Worcester Polytechnic Institute IQP Report

E Term 2011 – B Term 2011



Jared Guttmann

Professor Reeta Prusty Rao



## Abstract

The aim of this project was to research and understand the novel methods which microorganisms are used in medicine. Investigations led to the knowledge how bacteria, fungi, and viruses are used to treat ailments ranging from colon cancer to malaria. The advanced methods which microbes are used lead one to believe that the ailments which currently harm the human population will one day be preventable.

# Contents

Abstract.....	1
Chapter 1: History of Microbes in Medicine.....	3
Chapter 2: Bacteria in Modern Medicine .....	6
Figure 1: Liposome for Drug Delivery (Kosi, 2011) .....	7
Figure 2: Albert Einstein drawn in the bacteria <i>E.Coli</i> (Kickstarter, 2011).....	8
Chapter 3: Fungi in Modern Medicine.....	11
Figure 3: Fungi <i>Trametes Versicolor</i> (Emberger, 2008) .....	12
Chapter 4: Other Uses of Microorganisms in Modern Medicine.....	16
Chapter 5: Discussion.....	18
References .....	19

## Chapter 1: History of Microbes in Medicine

Microorganisms the small bacteria and fungi which inhabit just about every environment on Earth have become a large focus of the medical community in recent years. Though small (they can range from .15 micrometers to 700 micrometers), their impact and their potential impact is tremendous.

Microbes first came to human attention due to the work of the Dutch scientist Anton Van Leeuwenhoek. In 1676 using one of his homemade single-lens microscopes, Van Leeuwenhoek discovered what he called “animalcules,” and which are now what the science community currently refers to as bacteria (Dobell, 1960). It took around 200 years for the inclusion of microbes into preventative and palliative medicine. The advent of microorganisms in medicine, though seemingly a modern day application, actually began in the mid-19<sup>th</sup> century with the work of Louis Pasteur. The same century which science saw the advent of braille, Freudian psychoanalytics, and the Doppler Effect, came the rising use of microbes in medicine. However, a majority of civilization believed that disease was spontaneously generated. So before microbes could be used to benefit mankind, mankind had to prove they existed. Pasteur, through experiments with nutrient broths, rejected the common thought that microbes appeared spontaneously and that they traveled through the air causing diseases in silkworms as well as spoiling beverages such as wine, beer, and milk. Though not the first to propose germ theory of disease, Pasteur accepted the hypothesis of germ theory scientifically and was able to persuade much of Europe of the validity of his findings (Tiner, 1991). This understanding began to explain many historical phenomena, notably instances in India and China where people were vaccinated against the smallpox disease using powdered scabs of patients infected with smallpox (Temple, 1986). Over time, the development of vaccinations was used to help protect against a wide array of diseases such as measles, mumps, and hepatitis.

Currently, the use of microbes is not limited to the use in vaccinations. Microorganisms are widely used in modern medicine, and this is because microbes are an amazingly diverse population. Microbes consist of bacteria, fungi, archaea, protists, plants which are invisible to the naked eye, and plankton. Some scientists consider viruses to be microbes, though it is debatable due to the fact that viruses can't reproduce independently of a live host. Within this wide spectrum of microbes, there is an amazing amount of chemical and physical diversity among populations. A few examples of the diversity are that different microbes may or may not contain a nucleus, may contain DNA, RNA, whether single or double stranded, may be found in areas of extreme temperature, and may reproduce sexually or asexually. Though there is a large diversity of the potential of microbes in society, the main focus of this study will be on microbes in medicine.

Bacteria are unicellular organisms of the kingdom Monera, and typically contain a cell wall composed of peptidoglycan. Within the contents of the cell is the nucleoid region containing circular DNA, ribosomes, and plasmids. Bacteria are especially useful in medicine because of their short reproduction cycle (*Escherichia Coli* has been known to divide in 40 minutes) and the ease with which they can be genetically manipulated. Scientists have used bacterial genomic manipulation to understand more complex organisms through the understanding of bacterial gene and enzyme function, as well as metabolic pathway.

Fungi on the other hand are eukaryotic organisms of the kingdom Fungi, containing species such as mushrooms, yeasts, and molds. Fungi are distinct in character in that their cell wall is composed of the polysaccharide chitin, not peptidoglycan or cellulose which animals and plants, respectively, have. Fungi share many characteristics with other eukaryotes such as membrane bound organelles and have similar functional processes. In relation to animals fungi do not possess chloroplasts, however similarly to plants fungi maintain a cell wall, vacuoles, and may produce by both sexual and/or asexual means.

**Bibliography:**

Dobell, C. (ed.) 1960. *Antony van Leeuwenhoek and his "Little Animals."* Dover Publications, New York.

Temple, Robert (1986). *The Genius of China: 3,000 Years of Science, Discovery, and Invention.* New York: Simon and Schuster

Tiner, John Hudson, 1991. *Louis Pasteur: Founder of Modern Medicine.* Mott Media.

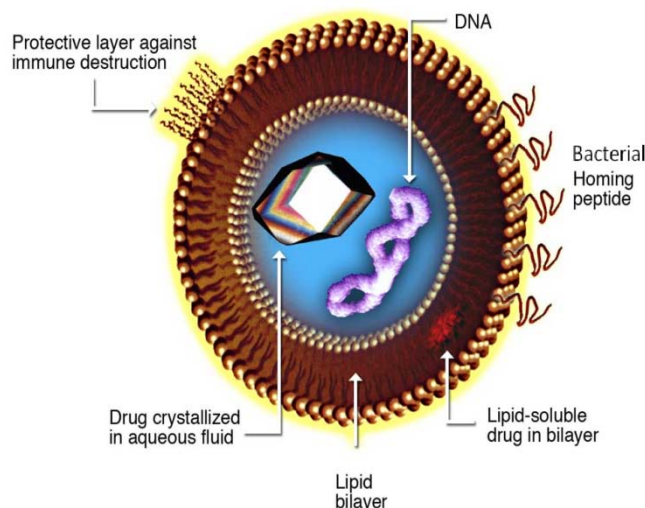
## Chapter 2: Bacteria in Modern Medicine

A common use of bacteria in modern medicine is as a delivery capsule for toxic drugs. The development of a delivery capsule was a necessary advancement in the treatment of cancer. Chemotherapy, the most common method of cancer treatment, is targeted not only for cancer cells but all rapidly dividing cells. This includes intestinal cells, bone marrow, mucosal cells, and stomach cells, among others. The use of chemotherapy as cancer treatment suppresses the immune system and may cause sickness and even other cancers, which is why site-specific delivery is so important in modern medicine. For example, the Cancer Research UK has modified the bacterium *Escherichia Coli* to transport an enzyme which destroys cancer cells. The bacterium was genetically altered in two ways by researchers. For one, a gene known as invasins was added allowing the bacteria to pass through the membrane of human cells, something it was previously unable to. Secondly, the researchers added a gene called listeriolysin O, a gene which turns the bacteria into a sort of ticking time bomb and causes the gene to spill its contents once it has entered the membrane of its destination cell. In this case, the cargo used was the enzyme purine nucleoside phosphorylase which activates an anti-cancer drug 6-MPDR. By use of testing in mouse tumors the combination was successful in killing more than 90% of cancer cells present, a very impressive amount. Site specific drugs such as this are of great importance for cancer drugs (Nitcheu-Tefit, 2007)

Alike the researchers at Cancer Center UK, researchers at InVitrogen Corporation in Grand Island, New York have discovered a bacteria *Clostridium Perfringens* that produces a protein *Clostridium Perfringens Enterotoxin* (CPE). The proteins act to specifically target the claudin-3 and claudin-4 epithelial receptors present in breast, prostate, lung, endometrial, thyroid, and pancreatic cancer tumors. The carboxy-terminal fragment of the CPE bacteria has a high-affinity to certain cell receptors, such as claudin-3 and claudin-4 cell receptors which are heavily present in the previously listed tumors

(Cocco et al. 2010). CPE could potentially be used in two ways in the future. For one, coupling radioactive isotopes to the CPE has potential to enable physicians to detect residual amounts of disease, a very effective way to locate minor metastasized portions of cancer throughout a patient's body. Another way CPE can be used is by coupling anticancer agents to it, which CPE would deliver directly to the tumor for therapeutic effect.

A slightly different bacteria *Clostridium novyi-NT* is tumor-specific and selectively infects the colorectal tumors which Johns Hopkins University researchers used to test the method (Gill, 2006). *Clostridium* is ideal for acting as a target inside of tumors since it is anaerobic and thrives in the hypoxic environment which is present on the interior of tumors. The drug which the researchers used was doxorubicin, a powerful DNA-damaging cancer agent. Doxorubicin, when injected encapsulated into mice with colorectal tumors caused the death of each mouse within two weeks. However when doxorubicin was encapsulated in liposomes (Figure 1) and *Clostridium* was present in the colorectal tumors, healthy cells were not targeted by the drug, and the anti-cancer agent was successful in eradicating a majority of the tumors. The bacteria act through a lipase which the research team named liposomase. Liposomase lyses the liposome once it enters the boundary of the tumor eradicating the tumor.



**Figure 1: Liposome for Drug Delivery (Kosi, 2011)**



In an almost surreal combination of computer programming and biology, researcher Christopher Voigt, a researcher at the University of California, has created computer programs which in time may be able to design bacterial circuits which produce biomolecules such as genes and proteins at the click of a mouse. So called “synthetic biology” may one day allow the easy manipulation of bacteria for medical use. These bacteria can be mix and matched with desired characteristics such as membrane signal sequences, macromolecule synthesis pathways, and chemical structure (Voigt, 2010). As Voigt describes the idea, they are looking to “create a programming language for cells so that you could write a program for a cell in the same way that you would for a computer or for a robot. That program is encoded on a piece of DNA and when you put that DNA into a cell it is able to run that program.” What is currently a time-consuming process of trial and error to produce specific bacteria may soon be an automated process. In a visually awe-inspiring example of the abilities of synthetic biology, Voigt’s lab has used variants of *E.coli* which change color in response to a projection of an image on it, to produce images of Albert Einstein and *E.coli* itself in culture (Figure 2).



**Figure 2: Albert Einstein drawn in the bacteria *E.Coli* (Kickstarter, 2011)**

Voigt's research will hopefully one day speed up the time genetic manipulation takes for researchers such as those at Cancer Center UK and thus increase the speed which medical breakthroughs are achieved.

Another application of synthetic biology in the medical field is the understanding of the human genome and using this to form personalized therapeutic recommendations for cancer patients. The company, Alacris Theranostics which was founded by renowned geneticist George Church, looks to use a computer system for modeling the biological network of a tumor and compare this to the normal tissues of the patient, displaying the genetic flaws in the tumor base pairs (Alacris Theranostics 2010). By identifying and characterizing somatic mutations, copy number variants, translocations, expression changes, and splice variants, Alacris will be able to recommend an appropriate therapy for the patient. Alacris is making it possible for individualized cancer treatment rather than continuing with the sometime ineffective treatments of cancer used today.

Bacterium, as stated before, can be replicated in large amounts in very short periods of time. Therefore when researchers find bacterium which provides medically important macromolecules such as proteins the production of a mass amount of said protein does not take an extensive amount of time. With current advances such as Voigt's synthetic biology this process may even take less time. One bacterial protein which is of use to the medical community is the *Botulinum* toxin. Produced by the bacterium *Clostridium Botulinum*, the neurotoxin is commonly used by physicians in Botox treatments for cosmetic procedure. *Botulinum* toxin type A, the first microbial toxin ever used for human medical treatment, serves as a treatment for a variety of strabismus (lazy-eye), blepharospasm (eyelid spasm), and hemifacial spasm (Erbguth,2004). By injecting the neurotoxin directly into the muscle, *Botulinum* toxin type A blocks the release of the neurotransmitter acetylcholine at myoneural junctions chemically suppressing hyperactive muscle disorders.

## **Bibliography:**

- Alacris Theranostics. "Unlocking the Power of Individualized Medicine." Company Presentation. Berlin, Germany. 18 Mar. 2010. Lecture.
- Cocco *et al.*, Clostridium perfringens enterotoxin carboxy-terminal fragment is a novel tumor-homing peptide for human ovarian cancer *BMC Cancer* 2010, 10:349
- Erbguth. Frank J. Historical notes on botulism, Clostridium botulinum, botulinum toxin, and the idea of the therapeutic use of the toxin. *Movement Disorders*. Volume 19, Issue S8, pages S2-S6, March 2004.
- Gill, Victoria. "Bacteria That Help Fight Cancer." Royal Society of Chemistry n/a (2006): n/a. Print.
- Kickstarter. "A Documentary Film about Synthetic Biology by Field Test Film Corps » Images in E. Coli from Voigt Lab UCSF — Kickstarter." *Kickstarter*. N.p., 15 Feb. 2011. Web. 21 July 2011. <<http://www.kickstarter.com/projects/637230479/a-documentary-film-about-synthetic-biology/posts/54791>>.
- Kosi, Gramatikoff. *Liposome. Liposome for Drug Delivery*. Web. 13 Aug. 2011.
- Nitcheu-Tefit J, Dai MS, Critchley-Thorne RJ, et al. Listeriolysin O expressed in a bacterial vaccine suppresses CD4<sub>+</sub>CD25<sup>high</sup> regulatory T cell function in vivo. *Journal of Immunology*. 2007;179(3):1532-1541
- Voigt, Christopher A. Clancy, Kevin. Programming cells: towards an automated 'Genetic Compiler', *Current Opinion in Biotechnology*, Volume 21, Issue 4, August 2010, Pages 572-581

## Chapter 3: Fungi in Modern Medicine

Everyone has heard of penicillin, the fungal mold which can treat all sorts of infections, but this is simply a snowflake on the tip of the iceberg that is the modern day use of fungi in medicine. Though not as useful as bacteria in regards to modification of structural characteristics and their use as entire organisms, the large focus of Fungi in medicine is the molecules which fungi naturally produce are vast and effective in fighting diseases. Molecules such as polysaccharides and complexes formed with polysaccharides have been found to exhibit antitumor and immunostimulating properties.

One derivative, known as lovastatin, is naturally created by oyster mushrooms which acts to lower LDL cholesterol levels through inhibition of the enzyme HMG-CoA reductase. HMG-CoA reductase is an important enzyme in the liver and is a central role in the production of cholesterol in the organ. Statins, the family of drugs which lovastatin is a member of, act to lower LDL cholesterol and has been shown to decrease the number of heart attacks and sudden cardiac death by 60% and reducing the risk of stroke of 17% (Law, 2003).

The mushroom *Trametes versicolor* (Figure 3) produces a polysaccharide called polysaccharide-K and is known to act in a variety of anti-cancer mechanisms (Suto, 1994). Polysaccharide-K acts by a variety of mechanisms including the suppression of tumor detachment, cell matrix degrading enzymes, tumor growth by inhibition of angiogenesis, expression of oncogenes, and the reduction of free radicals. Animal research conducted with polysaccharide-K has shown that it has the ability to increase the survival time in test subjects with spontaneous metastasis lung cancer, and it suppresses lesion growth of liver cancer in test subjects.



**Figure 3: Fungi *Trametes Versicolor* (Emberger, 2008)**

The drug paclitaxel is produced by the fungi *Nodulisporium sylviforme*, and is used as a means of disrupting the mechanism of reproduction in ovarian, breast, and lung cancers. Paclitaxel acts by stabilizing the microtubule of the mitotic spindle and prevents the movement of chromosomes to the metaphase plate which occurs in normal mitosis (Zhao, 2004). The mitotic block caused by Paclitaxel eventually leads to the apoptosis of the cell and thus the eradication of cancer cells.

*Isaria Sincliarii*, is a fungi which produces the compound myriocin. Through chemical synthesis researchers were able to produce the immunosuppressive drug fingolimod. Fingolimod was a milestone drug because it was the first disease modifying drug designed to be taken orally which was approved by the Food and Drug Administration (Horga, 2008). Fingolimod is used to reduce relapse and delay the progression of relapsing multiple sclerosis. The synthetic drug acts by building up stores of lymphocytes in lymph nodes and preventing the lymphocytes from interacting with the central nervous system and causing the auto-immune responses present in patients with multiple sclerosis. Patients taking Fingolimod was found to reduce patient relapse rates to less than 50% of those taking the placebo.

Researchers at the University of Westminster have created a possible method of preventing the passage of malaria from mosquitos to humans. According to the Center for Disease Control malaria infects between 300-500 million people each and every year, resulting in more than 1 million fatalities. Professor Angray Kang uses the fungus *Metarhizium Anisopliae* to kill the malaria parasite in the mosquito before it can be passed on to humans (Fang, 2011). Contact between the mosquito and the fungus causes the fungus to bore into the mosquito through the cuticle. Once inside the mosquito, the fungus multiplies in the circulatory system. Through genetic engineering, the fungus releases an antibody into the hemolymph which attacks the malarial parasite, causing the malarial spores clump together preventing them from entering the mosquito's salivary glands. If the spores cannot reach the salivary glands they cannot be passed on to infect humans. In laboratory testing, mosquitos exposed to the mutated *Metarhizium Anisopliae* were found to have levels of malarial infection 85% lower than normal. Even more promising results were found when the fungus was mixed with a scorpion toxin, with infection levels 97% lower than normal. Since the method of transmission between the fungus and the mosquito results from a simple touch by the mosquito, a mixture of the fungus and the scorpion toxin can be spread on mosquito nets and will protect the user from malarial infection. This approach may also offer solutions to a variety of other vector-borne diseases such as lyme disease, dengue infection, and yellow fever to name a few.

*Boletus Edulis*, a mushroom found throughout the northern hemisphere shows promise in inhibiting the spread of the human immunodeficiency virus-1 (Zheng, 2007). The mushroom produces a lectin (a sugar binding protein), which has a large impact on the activity of the HIV-1 reverse transcriptase. Since the first step of retroviral infection following the injection and uncoating of viral nucleic RNA into the host cell is the action of reverse transcriptase in reading the RNA and writing a complimentary DNA sequence, the best place to halt HIV-1 infection other than preventing the entrance into the host would be at the reverse transcriptase. Researchers at the China Agricultural University in

Beijing were able to show that through a protein-protein interaction, the lectin produced by *Boletus Edulis* inhibited HIV-1 reverse transcriptase activity in a level of potency higher than that of other natural products. The mechanism whereby *Boletus Edulis* works may be very useful in the fight against HIV. For both recently infected patients and long-term patients the reverse transcriptase inhibition will inhibit the spread of the infection providing a means of early treatment of the infection.

The comb tooth mushroom *Hericium coralloides* is a colorless fungi which grows on dead hardwood trees and creates a substance which erinacin E can be isolated from. At the department of Neuropsychopharmacology and Hospital Pharmacy at Nagoya University School of Medicine, research shows that erinacin E acts as an effective nerve growth factor stimulator (Yamada, 1997). This ability is especially useful as a potential suppressor of the effects of degenerative diseases such as Alzheimer's disease. Patients suffering from Alzheimer's have degenerating cholinergic neurons in their central nervous system. Cholinergic neurons are those which have acetylcholine neurotransmitters, and include neuromuscular junctions, preganglionic neurons, and brain stem complexes. By increasing the amount of nerve growth factors in the patients' circulatory system, researchers restored the amount of nerve growth factors in the frontal cortex and the parietal cortex using erinacin E.

### **Bibliography:**

- Emberger, Gary. "Trametes Versicolor." *Messiah College*. N.p., n.d. Web. 2 Oct. 2011.  
<[http://www.messiah.edu/Oakes/fungi\\_on\\_wood/poroid%20fungi/species%20pages/Trametes%20versicolor.htm](http://www.messiah.edu/Oakes/fungi_on_wood/poroid%20fungi/species%20pages/Trametes%20versicolor.htm)>.
- Fang, Weiguo. Vega-Rodriguez, Joel. "Development of transgenic fungi that kill human Malaria parasites in mosquitos". 25 Feb 2011 *Science*. 2011, Volume 331 pg 1074-77
- Horga, Alejandro. Montalban, Xavier. FTY720 (Fingolimod) for Relapsing Multiple Sclerosis, *Expert Review of Neurotherapeutics*, 4 Jun 2008; *Expert Review Neurotherapy*. 2008;8(5):699-714

- Law MR, Wald NJ, Rudnicka AR (June 2003). "Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis". *BMJ* **326** (7404): 1423.
- Suto T, et al. Clinical study of biological response modifiers as maintenance therapy for hepatocellular carcinoma. *Cancer Chemotherapy and Pharmacology* 1994; 33: S145-8.
- Yamada K, Nitta A, Hasegawa T, Fuji K, Hiramatsu M, Kameyama T, Furukawa Y, Hayashi K, Nabeshima T. Orally active NGF synthesis stimulators: potential therapeutic agents in Alzheimer's disease. *Behav Brain Res.* 1997 83(1-2):117-22.
- Zhao K. *et al.* (2004). "Study on the Preparation and Regeneration of Protoplast from Taxol-producing Fungus *Nodulisporium sylviforme*" (PDF). *Nature and Science* **2** (2): 52–9.
- Zheng S, Li C, Ng TB, Wang HX. (2007). "A lectin with mitogenic activity from the edible wild mushroom *Boletus edulis*". *Process Biochemistry* **42** (12): 1620–24



## Chapter 4: Other Uses of Microorganisms in Modern Medicine

Though not necessarily a microbe, virus-like particles have become very popular in the formation of modern vaccines. Virus-like particles are non-infectious cells because they lack any form of viral genetic material, but may contain the envelope, capsid, or both. In a 2009 randomized, blind, placebo-controlled trial the virus-like particle vaccine groups displayed statistically higher immune responses than the placebo immune response (Lopez-Macias, 2011). The reason why virus-like particles are so effective in their use as vaccines is because the human body responds to the virus-like particle as if it were a pathogenic virus. By presenting viral antigens on virus-like particles, the individual's immune response is boosted against a specific antigen, leading to a stronger immune response if infection were to occur. This same process is used against norovirus, which is the most common cause of acute gastroenteritis within the United States. Ligocyte Pharmaceutical Inc, is currently testing a virus-like particle which is aimed at enhancing the immune response against the norovirus (Ligocyte, 2011).

Beta-glucans, which are not specifically tied to one type of microbe but rather are the polysaccharides of D-glucose monomers linked by Beta-glycosidic bonds, are used in medicine. Whole glycan particles, when ingested give immune enhancement to the user and has even been shown to decrease the infectivity of infectious organisms such as anthrax (Ostroff 2004). By stimulating cytokine release and increasing CR3 receptor activation, Beta-glucans can increase the resistance to infection whether the intake occurred before or after infection. Even more promising, Beta-glucans seem to increase the efficacy of antibiotics and vaccines through similar mechanisms.

Lastly, Dr. Ifat Rubin-Bejerano started a company named ImmuneXcite which seeks to develop a cancer therapy. By creating cancer-specific antibodies which conjugate to polysaccharides, Rubin-Bejerano plans to trick the immune system's neutrophils into believing that the tumor cells are actually invading fungi. Since neutrophils aggressively attack bacteria and fungi, target cells such as cancer cells

or simply pathogenic cells will be more aggressively attacked by the immune system than without the conjugate (Dedesma, 2010). The model has already proven effective against resistant cancer cells in mouse model, and the future of ImmuneXcite looks very promising.

### **Bibliography:**

Dedesma, Carlos. "Reporting From The Early Life Science Conference." *MIT Entrepreneurship Review*. Massachusetts Institute of Technology, 21 May 2010. Web. 1 Oct. 2011.  
<<http://miter.mit.edu/article/reporting-early-life-science-conference>>.

Ligocyte. "Norovirus Vaccine" Ligocyte Web 1 Sep 2011.  
<<http://www.ligocyte.com/downloads/Noro.pdf>>

Lopez-Macias, Constantino. "Safety and immunogenicity of a virus-like particle pandemic influenza A H1N1) 2009 vaccine in a blinded, randomized, placebo-controlled trial of adults in Mexico." *Vaccine*, 7.99 (2011): 1 Sep. 2011

Ostroff, Gary R. 2004. Use of beta-glucans against biological warfare weapons and pathogens including anthrax. US Patent Number 20040014715 Filed October 9 2002, and issued January 22 2004

## Chapter 5: Discussion

It can be seen that the application of microorganisms such as bacteria and viruses, as well as virus-like particles, has the potential to advance the medical field at an even faster rate than it is currently progressing. By combining the continuing understanding of microbiology with the increased knowledge about illnesses and cancers as well as the diversity in research throughout the world, many diseases and other ailments seem to be on the verge of being relieved, cured, or even eradicated. One main hindrance to the progression in the medical field is the waiting time for approval of each drug or process. To understand the drawback of the waiting period for an approved drug, consider the average of 400 million cases of malaria per year. When it takes the 12 years to approve of an effective drug, 4.8 billion more cases of malaria will have been diagnosed. The liability of companies and the side effects of the patients is the driving force for this waiting period, and that's the way it's going to be. Regardless of the politics of it all, and as important as the development of drugs such as Lovastatin has been, the largest breakthroughs which microbes appear to be able to affect is the treatment of cancer and the spread of malaria. Previously, cancer was either surgically removed or the all of the patient's dividing cells would be destroyed for the duration of chemotherapy. By finding mechanisms to deliver the potentially deadly chemotherapeutic drugs directly to the tumor cells, the application of bacteria as drug vehicles looks to have a stunning impact on the oncology field in the 21<sup>st</sup> century. Even more importantly for lower socioeconomic countries, the use of fungus to decrease Malarial infection could be crucial to the positive development of these nations. If these countries were to decrease the number of Malarial cases from 300-500 million per year to 9-15 million per year, these nations would be giving their children a future. Medicine, which is always looking to make strides in its care of patients, looks to have found very promising partners, but it takes a microscope to see them.

## References

Cover Image: Image of Human Invasin Gene taken from Howard Hughes Medical Institute at the California Institute of Technology. October 29, 2011.

<http://www.cco.caltech.edu/~bjorker/structures.html>