

## FY2010 Appropriation Request Form

Office of Congresswoman Jackie Speier  
211 Cannon House Office building  
Washington D.C. 20515  
Phone: 202/225-3531  
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Website: [www.speier.house.gov](http://www.speier.house.gov)

**Date Submitted:** February 26, 2009

**Project Name:** Novel Bactericidal Proteins for Food Safety

**Organization:** AvidBiotics Corp. South San Francisco, CA Yes, the organization is in the 12<sup>th</sup> District.

Submitted by James L. Knighton, President, AvidBiotics

**Amount Requested:** \$6,000,000

**Appropriations Bill:** Agriculture; USDA; CREES

**Local Contact:** No contact in Washington D.C.; Jim Knighton (President) [jim@avidbiotics.com](mailto:jim@avidbiotics.com); 650.873.1141 (office); 650.799.5415 (mobile)

David W. Martin, MD (CEO); [dmartin@avidbiotics.com](mailto:dmartin@avidbiotics.com); 650.873.1115 (office); 650.706.1046 (mobile)

**Organizations Main Activity:** AvidBiotics, a young, private, non-venture funded company in South San Francisco, CA has discovered a method of altering a *naturally* occurring set of proteins to kill virtually any targeted bacterial pathogen without harming surrounding beneficial bacteria. These proteins are safe for human consumption and benign to the environment unlike traditional, chemical-based antibiotics. The Company has shown efficacy against a wide array of targeted bacteria in the laboratory and is pursuing the technology, with a partner, against a deadly bacteria commonly found in Intensive Care Units, *C. difficile*. However, the technology has far more expeditious utility against food borne pathogens that continually plague our food supply. Unless solved in an environmentally safe and commercially viable way (which excludes irradiation or viral treatment) food borne pathogens will continue to be an ever increasing threat to the general population and threaten the California agriculture industry. This base technology also has significant opportunity in a broad array of non-food applications (e.g., against methane producing bacteria in livestock which is a primary greenhouse gas; diagnosis and treatment of weaponized pathogens for biodefense, and potential treatment of drug-resistant bacterial infections in the human disease.). The USDA and FDA requested and received a review of the technology and both organizations expressed enthusiasm and support for its development. Numerous food-related organizations have expressed similar enthusiasm. Over the last two years the company has invested in advancing the technology and it is ready for

extensive pilot studies and development funding which is available only through significant federal government support. Normal funding mechanisms are not open for such applications and without significant government aid the application will not be pursued. Importantly, federal funding of the project in food safety is directly “leveragable” in all other applications where bacteria are implicated – biodefense, environment and human disease.

**Project Cost Breakdown:** The project consists of three phases the latter two of which focuses on one target pathogen Phase 1: \$1,000,000 to design and engineer pyocins to target *E. coli* O157:H7, *Salmonella enterica*; and *Campylobacter jejuni*, Phase 2: \$2,000,000 to conduct safety study, develop manufacturing process and Quality Control assays for *E. coli* O157:H7 only; Phase 3: \$3,000,000 to manufacture GLP material at scale (*E. coli* O157:H7 only) and conduct suitability testing (effectiveness) in a food processing facility and submit to the regulatory agencies for approval as a food additive.

**Project Description:** Controlling food borne pathogens, especially *E. coli* O157:H7 has been a high priority in the food industry. Much effort has been placed at eliminating them from farm animals, which are often carriers, but still the best protection is proper cooking by the consumer to kill the bacteria. Fresh produce and juices present a much more difficult problem. These products are often consumed without cooking, and controlling the source of contamination is difficult because it is often unpredictable, deadly examples include recent *E. coli* contaminations of spinach and apple juice and the most recent *Salmonella* contamination of peanuts. A mild, safe, and effective method of eliminating possible bacterial contaminants from fresh vegetables and other products would greatly reduce the occurrence of these serious outbreaks.

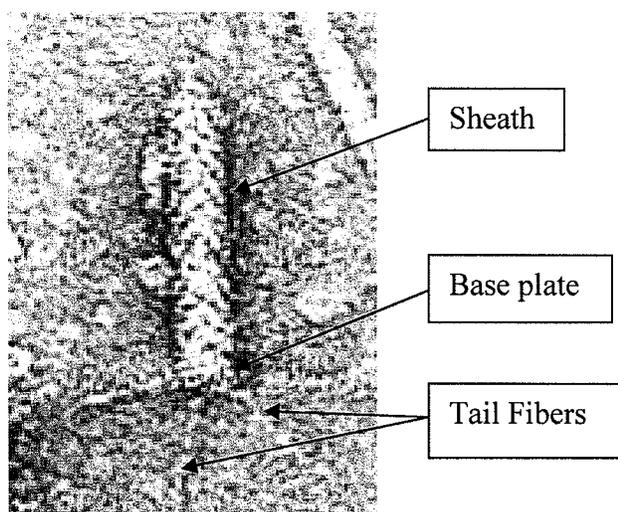
The bactericidal proteins we would like to develop are engineered R-type pyocins. These pyocins are large protein structures encoded by the genome of a bacterium, such as *Pseudomonas aeruginosa*, and serve as natural defenses, usually against other, related strains of bacteria. A target bacterium can be killed by a single pyocin molecule. **Avidocin™ proteins** are pyocins that have been designed and made to kill specific bacteria by attacking via certain of the latter’s exposed surface molecules that convey pathogenicity. We have generated pyocins that target and kill *E. coli* and the important, potential biowarfare pathogen, *Yersinia pestis*. After binding tightly via their tail fibers to the bacterium’s surface, pyocins plunge their needle-like “core” into the target bacterium. The penetration results in bacterial membrane depolarization and prompt cell death. **Avidocin™ proteins** are made to specifically target and kill only select bacteria, leaving other, useful bacteria unscathed. Neither a traditional antibiotic nor a bacteriophage, R-type pyocins are pure protein, targeted antibacterials.

Engineered R-type pyocins have potential applications as antibacterial agents in a variety of situations where antibiotics are ineffective or undesirable. The most interesting and relevant applications include:

- Food Safety – Animal Carcass and Meat Processing (e.g. *E. coli* O157:H7 in cattle and beef processing) and Fresh Produce Farming and Processing (e.g. *E. coli* O157:H7, *Campylobacter*, *Salmonella*)
- Greenhouse Gas Control – methane gas control from livestock (various methanogens)

- Biodefense – (e.g. *Y. pestis*)
- Human Health – both prophylactic and therapeutic applications (e.g. *P. aeruginosa*, *E. coli*, *C. difficile*; MRSA)

R-type pyocins resemble the non-flexible, contractile, tail structures of bacteriophages (see Figure 1). Pyocins rapidly and specifically kill target cells by first binding to the surface lipopolysaccharide (LPS) via the tail fibers (the main spectrum determinant, followed by sheath contraction and core penetration through the outer membrane, resulting in depolarization of the cytoplasmic membrane potential and cell death (Uratani and Hoshino, 1984). In some respects pyocins can be viewed as defective prophages that produce noninfectious particles that have no capsid and contain no DNA, but consist only of the tail apparatus.



**Figure 1.** Scanning electron micrograph of an R-type pyocin showing four of the six tail fibers. The tail fibers are a major spectra determinant and can be engineered to change target specificity.

Non-modified naturally occurring pyocins are specific for *P. aeruginosa* strains, with a few examples of bactericidal activity against certain strains of *Hemophilus*, *Neisseria* and *Campylobacter* species (Filiatrault et al., 2001; Morse et al, 1976; Morse et al, 1980; Blackwell et al., 1981, 1982). However we have developed the technology to engineer the killing spectra to different bacterial species by altering the pyocin tail fibers.

We propose to engineer pyocins to target *E. coli* O157:H7, *Campylobacter jejuni*, and *Salmonella* species and to develop them as agents to kill the bacteria during food processing. Our main approach will be to engineer pyocins by fusing tail fiber from related *Myoviridae* phage that target the pathogen to the tail fiber of the R-type pyocin.

*Technological Rationale.* In both the R-type pyocins and the *Myoviridae* phages, the long tail fibers are a major component that determines target specificity. The tail fibers form homotrimers, of which 6 copies are attached to the base plate. A chaperone is likely to be required for proper

folding of the tail fibers into trimers and/or assembly of the tail fibers onto the base plate. Similar chaperones also seem to be required among phages of the *Myoviridae* and are likely to act specifically on their own tail fibers. The N-terminus of the tail fiber is the portion that binds to the base plate, whereas the C-terminus, or the tip, is the end that contacts the bacteria through an interaction with the LPS, and is the region that confers target specificity. The phage P2 tail fiber and the R2 pyocin tail fiber share sequence similarity in the N-terminal region whereas the C-terminal region has little sequence similarity, not surprising since they have completely different target spectra. We were able to manipulate these target spectra by fusing the C-terminal portion of the P2 tail fiber to the N-terminal portion of the R2 pyocin, generating a chimeric pyocin that can kill *E. coli*. The host for phage P2 is *E. coli* C and some K12 strains and predictably, the hybrid R2-P2 pyocin now has that target spectrum. Similarly, we have developed a pyocin to kill an important biodefense agent, *Yersinia pestis*, by fusing the C-terminus of the tail fiber from yersiniophage L-413C to the pyocin tail fiber. Therefore we have developed the technology to switch the target specificity of pyocins even to different bacterial species. We propose to extend this technology to target food pathogens by generating novel pyocins using parts of tail fibers of *Myoviridae* phages specific for this important pathogen.

**How will this earmark expand AvidBiotics' capacity and how will we sustain the work beyond federal funding? :** As an emerging biotech company focusing on novel antibacterial discovery and development progress the technical knowledge gained against food borne pathogens will be directly applicable to numerous other applications where bacteria cause considerable damage, for example in human, animal and environmental situations. Additionally the expertise and infrastructure used to develop the technology for food can be leveraged for those other applications. If we are successful in proving the approach in food safety two sources of post-government financial support become feasible: 1) from potential vendors and customers of the technology and 2) private funding sources (which are not available at this stage of development in the arena of food safety).

**Local Significance:** The significance is twofold: 1) locally, if AvidBiotics is successful in developing the protein against food borne pathogens, it could become a significant entity in the biotech arena and will need to expand significantly over the next two years, all in District 12; and 2) given the magnitude of importance of the agricultural industry to the California economy and the threat food borne pathogens pose it is of critical importance to solve the problem in a way that protects the public and the environment.

**How many residents of the 12<sup>th</sup> CD benefit?** If this protein-based approach proves to be viable on a large, production scale all residents of the 12<sup>th</sup> CD benefit as well as the state-wide and nationwide population through safer food.

**Other supportive organizations or state/local elected officials:** Dole Fresh Vegetables has written a letter of support of our efforts. Many food processors have expressed verbal enthusiasm.

**Other Funding Requests:** An FY10 Appropriation request was submitted through Senator Feinstein's office. A grant request has been made through the NIH. These grants tend to be very small (\$275k) in relation to the work that needs to be done in order to bring a product to the public. These are designed to be research grants, not for development.

**Previous receipt of federal funds for this project:** None.

**Organizational Staff and Board Members:**

**David W. Martin, MD Co-founder, CEO and Board member;** a scientist/clinician from Duke Medical School and a Howard Hughes Investigator at UCSF prior to being Senior VP of Research and Development at Genentech, DuPont Merck, etc. has accumulated over 25 years of drug discovery and development expertise.

**Mr. James L. Knighton Co-founder, President and Board member;** has graduate degrees in Genetics and Business from the University of Pennsylvania, has 20 years of pharma/biotech experience with large (DuPont Merck) and small (Sugen, Caliper Life Sciences) companies in both the public and private sectors.

**Jeffery F. Miller, Ph.D. Board member;** AvidBiotics' third co-founder; is a consultant and chairman of the company's Scientific Advisory Board, Currently, Dr. Miller is M. Philip Davis Chair of the Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine at UCLA. His Ph.D. is from Tufts University School of Medicine, and his postdoctoral experience was at Stanford University School of Medicine with Professors Stanley Falkow and Lucy Tompkins.

**Kevin Cameron, JD, Board member;** brings a strong entrepreneurial, legal and transactional background. JD, University of Chicago; General Counsel for Moxidigital and North Pointe Communications; Founder and President of Glass, Lewis and Company, a significant worldwide shareholder services company. He also brings to AvidBiotics noteworthy experience in both building a company *de novo* as well as maintaining excellent corporate governance.

**Other relevant materials:** Letter of support from Dole Fresh Vegetables is attached.



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February 3, 2009

Senator Dianne Feinstein  
United States Senate  
331 Hart Senate Building  
Washington, DC 20510

Dear Senator Feinstein:

On behalf of Dole Foods, Inc., I want to express the importance of funding AvidBiotics' development of a novel protein-based bactericide for a bacteria-specific "kill step", a step in food processing critical to ensuring food safety.

As everyone worldwide is becoming acutely aware our food supply is in constant danger of contamination by deadly pathogens. Modern methods of control revolve around less than ideal options that include irradiation, chemical treatment, and more recently, the ability to treat with live viruses (bacteriophages). Each of these methods has unique utility but each is burdened with technical, environmental, cost and/or marketing challenges.

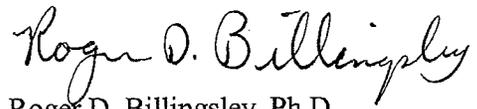
AvidBiotics, small biotech company from California, has discovered and partially developed a novel, environmentally safe and effective alternative to existing options. I have reviewed AvidBiotics' technology and believe it to be a viable and desperately needed "kill step" for the fresh produce and meat industries.

The technology has several distinct advantages over other potential "kill step processes" such as irradiation, bacteria phages or chemical treatment. They include:

- Naturally occurring pure protein (likely non-toxic)
- Non-corrosive
- No DNA
- Effective at 4°C (processing temperature)
- Kills vegetative and non-vegetative bacteria
- Kills only the targeted bacteria
- Biodegradable
- Does not compromise food quality (colorless, tasteless)

I urge you to support funding of this critical program to improve food safety.

Sincerely,

A handwritten signature in cursive script that reads "Roger D. Billingsley". The signature is written in black ink and is positioned above the printed name.

Roger D. Billingsley, Ph.D.  
Sr. Vice President, Research and Development