SAN DIEGO STATE UNIVERSITY Research Foundation | TECHNOLOGY TRANSFER OFFICE

INFO SHEET

Genetically engineered antibacterial, and protective, microbe-specific bacteriophage formulations for the treatment of obesity and other conditions

Background

Mucosal surfaces provide both, critical immunological protection against invading bacterial pathogens and support of large communities of commensal microorganisms. Being exposed to the environment, mucosal surfaces are also the infection sites for many bacterial diseases in humans. Modulation of a patient's microbiome e.g., by reducing the population of *Firmicutes* (which are associated with weight gain and the inability to lose it) and supplementing with "good" bacteria is an attractive possibility for obesity treatment. Antibiotics are not particularly useful for treating obesity and some other pathologies because they act indiscriminately (e.g., killing not only the *Firmicutes* but also normal flora). Targeted approaches like endotoxins, antibodies, etc., tend to be too narrow to decrease the relative abundance of a phylum like *Firmicutes*.

Bacteriophages (phages) defend mucosal surfaces against bacterial infections. Mucus-adherent phages reduce bacterial infection of life-like mucosal surfaces more effectively than non-adherent phages. Developing mucosaltargeting phage to selectively remove invasive pathobiont species from mucosal surfaces has been reported by others. For example, a lytic bacteriophage that can localize to the epithelial surface by binding heparan sulfated glycans, positioning it near its host *Escherichia coli* can potentially be used to selectively remove invasive pathobionts from the gastrointestinal tract and to prevent the development of disease.

The gut microbiota changes are implicated in the development of obesity and type 2 diabetes (T2D). The gut harbors viral community that is predominated by bacteriophages that attack bacteria in a host-specific manner and have the potential to alter the gut microbiota. Fecal virome transplantation from mice with a lean phenotype into mice with an obese phenotype has been reported to result in reduced weight gain and normalized blood glucose parameters in the latter relative to lean mice, and this is thought to be mediated via fecal virome transplantation -induced gut microbiota changes.

The problem

While mucus-adherent phages reduce bacterial infection, the i*n vivo* mucosal surfaces are more complex than simulated mucus environments. *In vivo* diversity of bacterial host and even greater diversity of phage strains reduce the probability of a successful phage-host encounter. In addition, the mucus layer contains a mix of macromolecules, all of which may affect phage diffusion. Mucosal surfaces, being exposed to the surrounding *milieu*, experience environmental fluxes that may affect mucus structure and phage particle diffusion. In essence, phages effective *in vitro* do not consistently reduce mucosal bacterial host levels *in vivo*.

SDSU Solution

The Rohwer Lab has genetically engineered bacteriophages (synthetic bacteriophages or phagemids) that have chemical/structural modifications. Among these, the exterior surface of the bacteriophage comprises at least one heterologous carbohydrate binding domain (CBD), a moiety/domain that can bind a component of a mucus or mammalian/environmental mucus or a mucin. Such moiety/domain can target the phage to and concentrate it in a specific region of a mucosal surface that may overlap with a bacterial host range.

Additionally, the phage may be adapted to a physico-chemical environment of the mucus/specific region of a mucosal surface. At least one moiety or domain capable of binding to a component of a mucus comprises or is derived from phage(s). The bacteriophages are screened and selected for lysogenic/bactericidal/bacteriostatic activity against bacteria of interest.

Rohwer Lab is developing an exemplary microfluidic device design with chip made of poly(dimethylsiloxane) (PDMS). The device emulates the microenvironment of a mucus-producing human lung epithelial surface experiencing constant fluid-flow across its surface and turnover dynamics. Using these chip microenvironments, the team has investigated trilateral interactions among bacteria, phages, and mucosal epithelial cells. The team has employed two phages; conducted phage therapy experiments to estimate the titer of both, phages and bacteria present in phage-infected mucosal epithelium inoculated by bacteria, and phage detachment experiments for the two phages across a range of physiological mucin concentrations.

Value Proposition:

- **Bacteriophage adherence to mucus (BAM) model -** BAM domain (specialized proteins on the virions) identification and utilization for a targeted approach to specific mucosal surfaces **for concentrating a bacteriophage or phagemid** in an epithelial mucus zone or increasing adherence of a bacteriophage or phagemid on a mucus/in an epithelial mucus zone
- Enrichment for or selection for virus and/or a bacteriophage that can target, bind to, change the phenotype of, make non-pathogenic or kill a microbe (e.g., specific binding and targeting of *Fermicutes* – bacteria which are associated with weight gain and the inability to lose it)
- Administration of certain bacteria (probiotic or therapeutic or CRISPR-modified bacteria targeted for killing by the chemically or structurally modified bacteriophage) and establishment/generation of a desired microbial niche or microenvironment, or the manipulation of a switch between one state/condition of the niche or microenvironment to another one
- Delivery of payload (a label, a coat, a drug, an antibiotic, a bacteriostatic agent, a cytotoxic agent, or other molecules) by chemically/structurally modified bacteriophage/genetically engineered bacteriophage/a synthetic bacteriophage or phagemid

Applications:

- Use as nutraceutical, a pharmaceutical or a pharmaceutical preparation comprising/formulated with bacteriophages to sculpt a patient's microbiome to treat obesity; treat bacterial infections (e.g., by MSRA, *Clostridium*, *Escherichia coli*, *Shigella*, *Salmonella*), and genetically predisposed and chronic disorders and conditions via personalized therapy
- Kits and methods to enrich for and/or isolate viruses and/or phages interacting with the bacteria of interest
- BAM-Domains to enrich for and/or isolate phages that attach or adhere to natural/artificial surfaces

Stage of Development

Prototype

Intellectual Property

Issued US patent (11,214,773), a pending US continuation (17/534,372), a pending EU application (16849443.3) and a pending US application (16/761,037).

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