Beneficial bacteria may help ward off infection

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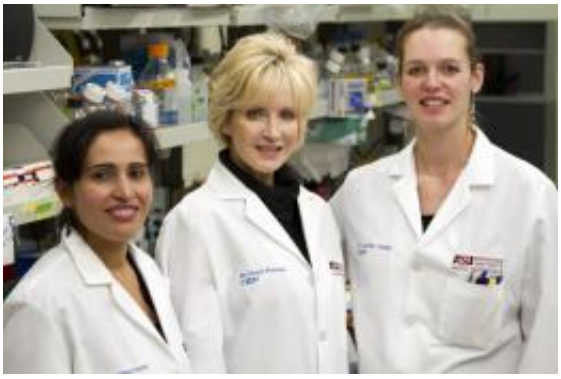

Cheryl Nickerson (center) led Biodesign's research group, including Shameema Sarker (left) and Aurélie Crabbé (right). They were joined by an international team. Credit: The Biodesign Institute at Arizona State University

(Medical Xpress) -- While many bacteria exist as aggressive pathogens, causing diseases ranging from tuberculosis and cholera, to plague, diphtheria and toxic shock syndrome, others play a less malevolent role and some are critical for human health.

In a new study, Cheryl Nickerson and her group at ASU's Biodesign Institute, in collaboration with an international team including Tom Van de Wiele and lead author Rosemarie De Weirdt at Ghent University, Belgium, explore the role of Lactobaccilus reuteri – a natural resident of the human gut – to protect against foodborne infection.

Their results demonstrate that this beneficial or probiotic organism, which produces an antimicrobial substance known as reuterin, may protect intestinal epithelial cells from infection by the foodborne bacterial pathogen Salmonella.

The study examines for the first time the effect of reuterin during the infection process of mammalian intestinal cells and suggests the efficacy of using probiotic bacteria or their derivatives in future therapies aimed at thwarting Salmonella infection.

Members of the Nickerson lab at the Biodesign Institute's Center for Infectious Diseases and Vaccinology involved in this study were Shameema Sarker and Aurélie Crabbé.

Results of the new study recently appeared in the journal *PloS ONE.*

Cell cultures: now in 3-D!

Over the past decade, the Nickerson group and their colleagues have developed organotypic three-dimensional (3-D) tissue culture models of the small and large intestine, lung, placenta, bladder, neuronal tissue and vaginal epithelium that mimic key characteristics of the parental tissue, and applied them to study the infectious disease process. Such models offer exciting new insights into host-pathogen interactions, cell proliferation, differentiation and immune function, and are providing a platform to understand normal tissue homeostasis and transition to disease.

For the current study, 3-D colon epithelial cells were used. Nickerson explains that cells derived for study through this technique more faithfully approximate key in vivo responses to S. Typhimurium infection, compared with the traditional monolayer methods, making such cells an ideal model to observe infection processes.

3-D cell culture models are cultured in a special environment within a device known as a Rotating Wall Vessel bioreactor – a cylindrical, rotating apparatus, filled with a culture medium supplying essential nutrients, oxygen and physical forces to the cells. Within the reactor, the natural sedimentation of cells due to gravity is balanced by the bioreactor's rotation, resulting in a gentle tumbling of cells within the media in the chamber.

During the culturing phase, cells attach themselves to tiny porous beads, termed microcarriers, or other scaffolding. Under these conditions, cells are able to respond to molecular and chemical gradients in three-dimensions in a way that approximates their behavior under in vivo conditions, causing the cells to aggregate based on natural cellular affinities and form 3-D tissue-like structures

“In previous studies, we applied our 3-D intestinal cell cultures as human surrogates to further our understanding of how Salmonella interacts with the intestinal epithelium to cause gastrointestinal disease,” Nickerson explains. “We found that these models were able to respond to infection in key ways that mimicked the parental tissue in vivo and which conventional models could not recapitulate. We are excited to advance the use of our 3-D models in the current work to study how commensal intestinal microbes and their products can protect against Salmonella-induced foodborne infection. The results of this study may provide fundamental knowledge for development of new probiotics and other functional food based strategies.”

Bacterial blizzard

A swarm of some hundred trillion bacteria occupies the human body, outnumbering human cells by about 10 to 1. Among these are members of the genus Lactobacilli, some of which have been associated with therapeutic, probiotic properties, including anti-inflammatory and anti-

cancer activity.

The current study zeros in on Lactobacillus reuteri – one of the more than 180 species of Lactobacilli. The group investigated the potential of this bacterium to inhibit the early stages of Salmonella infection, seeking to identify plausible mechanisms for such inhibitory effects.

Intestinal infections by non-typhoidal Salmonella strains induce diarrhea and gastroenteritis, and remain a leading source of foodborne illness worldwide. Such infections are acutely unpleasant but self-limiting in healthy individuals. For those with compromised immunity however, they can be deadly and the alarming incidence of multi-drug resistant Salmonella strains has underlined the necessity of more effective therapeutics.

The use of benign microorganisms offers a promising new approach to treating infection from pathogens like Salmonella and indeed, L. reuteri has been shown to help protect against gastrointestinal infection and reduce diarrhea in children.

Safeguarding cells

The origin of L. reuteri's protective role still remains unclear, and the present study investigated whether reuterin, a metabolite produced by L. reuteri during the process of reducing glycerol in the gut, could be one of the keys to protection. While it has been speculated that reuterin acts by regulating immune responses or competing with Salmonella for key binding sites, the current study represents the first in vitro examination of host-pathogen interactions using human intestinal epithelium in the presence of reuterin-producing L. reuteri.

Two approaches were used to study host-pathogen interactions. In the first, 3-D intestinal epithelial cell aggregates were seeded into 24-well

plates. Salmonella was added to these intestinal cells along with supernatant of L. reuteri – that is, cell-free culture medium in which the Lactobacillus grew and produced reuterin (obtained by filtering out the bacteria).

In the second approach, L. reuteri was first allowed to produce reuterin in the presence of the 3-D colon cells (seeded into the wells), after which the cells were exposed to Salmonella. Here, the L. reuteri bacteria (in the presence of glycerol) produced reuterin in situ. In both approaches, non-reuterin exposed controls were also tested, and the effect of reuterin on a Salmonella population in the absence of host cells was assessed as well.
L. reuteri regulates response to infection

The results showed a reduction in the Salmonella population (without host cells) after one hour of exposure to a diluted supernatant containing reuterin. Further, the reuterin-containing ferment of L. reuteri was shown to significantly reduce adhesion, invasion and intracellular survival of Salmonella to 3-D colon cells, compared with an untreated control.

In an unexpected twist, the application of L. reuteri supernatant lacking glycerol actually stimulated adhesion, invasion and intracellular survival of Salmonella. The authors speculate that the stimulatory effect observed may have been due to low concentrations of acetic acid, previously shown to stimulate expression of Salmonella virulence-related genes.

Applying the second approach, live L. reuteri were incubated with 3-D epithelial cells and the medium supplemented with glycerol, allowing for in situ production of reuterin. The presence of L. reuteri was shown to reduce the population of Salmonella by diminishing their capacity for adhesion, invasion and intracellular survival and this effect increased when L. reuteri were producing reuterin.

Another interesting detail uncovered in the study is that the effects of reuterin on Salmonella's infectious capacity are increased in the presence of host cells, suggesting that some type of synergistic protection occurs during epithelial infection, potentially involving the combined activity of reuterin and host cell gene-related responses.

Prolonged exposure (of 24 hours or more) to the reuterin-containing supernatant solutions caused a loss of viability in host cells, though shorter exposure times did not appear to adversely affect them. Importantly, the introduction of L. reuteri strains in vivo have been safely carried out in infants and even immuno-compromised adults, indicating that other cell types, host factors or the complex gut microbiota in vivo could counteract the observed cytotoxic effects of reuterin in vitro.

While the authors stress that much work remains, particularly in terms of understanding reuterin's role in the context of a complex gut microbiome, the results are encouraging and suggest a new avenue for fighting Salmonella infection, through the process of glycerol conversion to reuterin by L. reuteri.

*The international research team for this project also included Stefan Roos, Department of Microbiology, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden, Sabine Vollenweider & Christophe Lacroix, of the Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland, and Jan Peter van Pijkeren & Robert A. Britton, Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, Michigan.

Provided by Arizona State University