Dealing with stress: New research highlights the survival skills of disease-causing E. coli

January 30 2012, by Richard Harth

This is Professor Mellata (left) with Roy Curtiss, director of the Center for Infectious Diseases and Vaccinology at the Biodesign Institute. Credit: The Biodesign Institute, Arizona State University

Escherichia coli bacteria thrive in the lower intestine of humans and other animals, including birds. Most are vital constituents of the healthy gut flora, but certain forms of *E. coli* cause a range of diseases in both humans and poultry.

In this month's issue of the journal PLoS ONE, a team of researchers at Arizona State University's Biodesign Institute investigates disease-causing *E. coli* strains known as APEC (for Avian Pathogenic *E. coli*). By studying circular segments of bacterial DNA known as plasmids, the group uncovered some of the tricks used by these highly adaptive organisms to survive, even in the face of daunting environmental

challenges.

According to assistant research professor Melha Mellata, lead author of the current study, the research is an important step toward a more thorough understanding of the genetic underpinnings of pathogenic *E. coli*: "*E. coli* bacteria that are able to persist and cause diseases have developed multiple strategies to achieve this," she says. "It is important to elucidate the genetic mechanisms used by these bacteria so that we can turn their own weapons against them."

Birds, including chickens can become infected with APEC, causing colibacillosis—an acute and often fatal disease, resulting in significant economic loss for the poultry industry. Further, because APEC bacteria bear a genetic blueprint similar to that of other members of the group of Extra-intestinal pathogenic *E. coli* or ExPEC, to which they belong, the danger exists for such avian bacterial strains to cross the genetic barrier to infect humans, causing so-called zoonotic diseases.

Retail chicken products are also believed to act as reservoirs for existing *E. coli* strains responsible for human ExPEC infections. As their name implies, these extra-intestinal bacterial pathogens cause infections outside their customary habitat in the gut. They are responsible for illnesses including septicemia, newborn meningitis and urinary tract infections. ExPEC infections result in significant loss of life and cost the U.S. health care industry billions of dollars.

While the genetic kinship of human and avian pathogenic *E. coli* strains is cause for concern, it may also provide an opportunity for the development of a vaccine capable of cross-protecting humans and birds, if a group of genes common to all extra-intestinal *E. coli* can be identified and targeted. Roy Curtiss, director of Biodesign's Center for Infectious Diseases and Vaccinology, oversees a project aimed at achieving this goal.

In the current study, the team—including undergraduate researchers, Jacob Maddux and Timothy Nam—investigated the genetic sequence of several large plasmids in a strain of APEC commonly used for research purposes. The presence of multiple large plasmids is characteristic of ExPEC bacteria, particularly APEC. Previously, the first of three large plasmids had been sequenced and analyzed by the group and found to code for virulence factors, which help the bacterium infect its host. The two other large plasmids were sequenced for the first time in the present study, as well as a smaller APEC plasmid, whose significance remains obscure.

Unlike the first of the three large plasmids examined, the second and third do not encode for common virulence factors and appear to play only a minor role in the actual infection process of APEC bacteria. The team hypothesized that these plasmids instead conferred heightened survival potential during stressful environmental situations, including bacterial subsistence soils, poultry litter or under acidic conditions.

In order to test the hypothesis, the group began by fully sequencing these two large plasmids as well as a smaller plasmid. They next examined the contribution of all four plasmids, both individually and in combination, as the APEC bacteria colonized human intestinal epithelial cells. The APEC strains, with their complement of plasmids, were studied under varying environmental conditions to assess their resistance to acid and bile in the human GI tract; growth under iron-poor conditions and varying carbon sources; and ability to clump together to form biofilms—a critical component of the infection process.

In order to study the role of plasmids on APEC interaction with enteric cells, the group used a 3-D cell culture model of human intestinal epithelium, which has been shown to more accurately mimic the structure-function of the in vivo tissue than traditional monolayer cultures. Cheryl Nickerson's research group at the Biodesign

Institute®—participants in the current study—have worked extensively in the development and application of 3-D cell cultures as human surrogate infection models. The application of these advanced enteric models to dissect the molecular mechanisms of APEC pathogenesis was a logical choice for these studies.

The large plasmids under investigation did not appear to have a significant effect on the ability of APEC-derived strains to associate with and invade human intestinal epithelial cells. Further experiments however implicated large plasmids—for the first time—in APEC's ability to resist acid and bile, two critically important tools for *E. coli* survival, particularly under the low pH conditions found in certain foods and in the stomach.

Many pathogenic bacteria, APEC included, form aggregates of material known as biofilms. Biofilms are implicated in 65-80 percent of human infections. Their mechanisms of formation are therefore a matter of considerable medical concern. ExPEC cells form biofilm concentrations in both the gastrointestinal and urinary tracts. By examining the function of three large plasmids in biofilm formation (separately and in combination) at varying temperatures, the group was able to tease out some of the key features of ExPEC biofilm formation. They found that 4 distinct kinds of biofilm formed under the influence of the large plasmids, depending on temperature conditions.

Plasmid-driven biofilm formation may play an essential role in the virulence of APEC and other ExPEC forms, by conveying survival advantages in various environmental niches found in the host. Likewise, the means by which ExPEC bacteria are able to modify their metabolism to make use of available nutrients is an important factor in their pathogenesis. A gene cluster located on one of the large plasmids was found to code for an alternate sugar pathway, again improving the pathogen's prospects for survival under changing nutrient conditions.

(Intriguingly, the gene cluster does not occur in other forms of *E. coli*, though it is present in another important pathogen—Salmonella.)

The combined results are a significant advance toward a comprehensive understanding of extra-intestinal *E. coli* pathogens and their mechanisms of survival. In earlier work, assistant research professor Mellata generated vaccine candidates specifically targeting APEC infection. The current research improves the prospects for a new range of vaccine candidates conveying cross protection from ExPEC infections in both human and avian populations. "We are very confident that our strategy in designing a much broader vaccine targeting multiple subgroups of pathogenic *E. coli* will result in positive health and economic impacts," Melha says.

Provided by Arizona State University