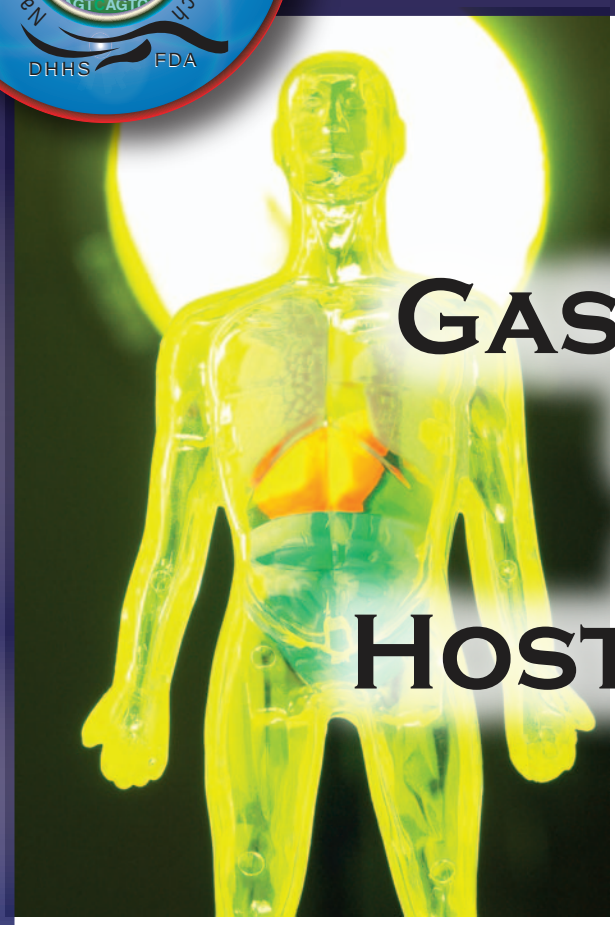
Microbiology

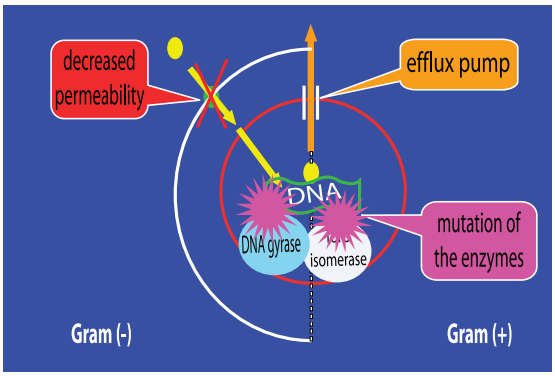


Gastrointestinal Microbiology and Host Interactions

We have the capability:

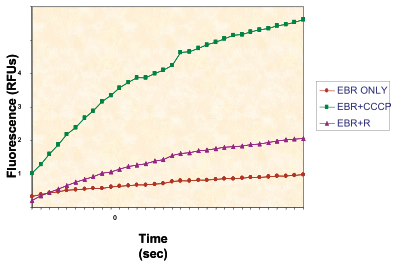
- to examine how exogenous compounds, such as antimicrobial residues, probiotics, and food additives affect the intestinal microbiota, including changes in bacterial populations, antimicrobial resistance, and colonization-barrier effects.
- to determine how endogenous host-derived factors, such as steroid hormones, bile acids, complex carbohydrates, and host immunity impact the microbiota and potential transient pathogens.
- to use traditional cultures, biochemical techniques, molecular-based methods, membrane arrays and microarray methods to detect the human intestinal microbiota.
- to use an *in vitro* culture system which simulates the human colonic environment, to determine changes to the normal intestinal microbiota due to dietary exposure to food contaminants.
- to investigate xenobiotic metabolism, such as that of phytoestrogens (plant hormones), food additives and supplements, by the intestinal microbiota and to determine the impact these substances have on the microbiota in the human gastrointestinal tract.
- to purify and characterize metabolites from xenobiotics, including azo dyes, food additives, plant hormones and food supplements formed by human intestinal and skin microbiota and determine their potential as useful or harmful.
- to evaluate the composition and efficiency of probiotic products and their impact on the human intestinal microbiota.

Determination of the mechanism of resistance to different FDA approved fluoroquinolones



Evaluation of presence of efflux pump

Comparison of mutations in gyrase and topoisomerase induced by fluoroquinolones of different structures in bacteria

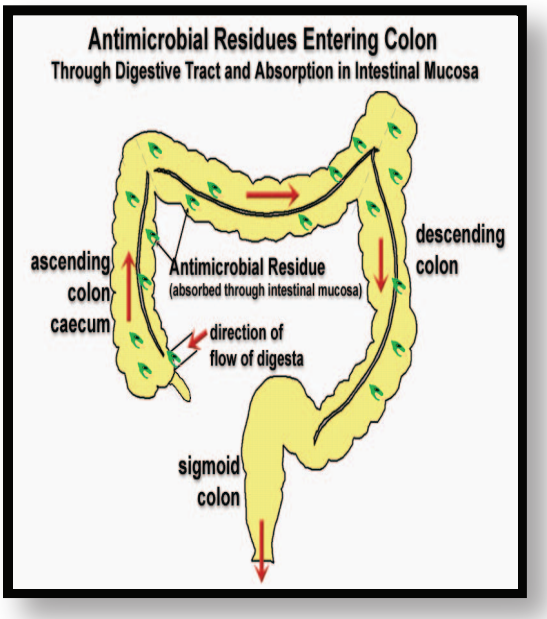


- **Multiple drug efflux systems are encoded in virtually all bacteria investigated or sequenced to date and supply the largest degree of intrinsic resistance of any single genetically-encoded factor therein.** For example, one prototypical pump is sufficient for resistance to several structurally unrelated classes of molecules, including chloramphenicol, novobiocin, macrolides, β-lactams, fluoroquinolones, tetracyclines, synthetic and natural detergents (bile acids), steroid antibiotics, organic dyes and solvents, and biocides. We have determined that steroid hormones are strong substrates for these systems. Such host-derived molecules (which also include bile acids) may either induce expression of resistance mechanisms or compete as substrates for their function, thereby altering antimicrobial resistance phenotypes. We are currently developing model indicator organisms that can be used to assess efficacy for the development and future analysis of efflux pump inhibitors.

- **We have developed a biological assay for the measurement of the concentrations of antimicrobial drug residues in food that cause failure of the intestinal microflora to prevent infection by *Salmonella*.** The assay provides a means to directly observe detrimental effects of antibiotics on the intestinal barrier against food poisoning. The highlight of this work is a model of the human intestinal microflora that provides protection against *Salmonella* invasion of a human intestinal cell line and uses a protection assay to measure concentrations of antimicrobial drugs that break down the protective intestinal barrier. These results help us calculate more accurately the levels of Acceptable Daily Intake of residues of these drugs in foods.

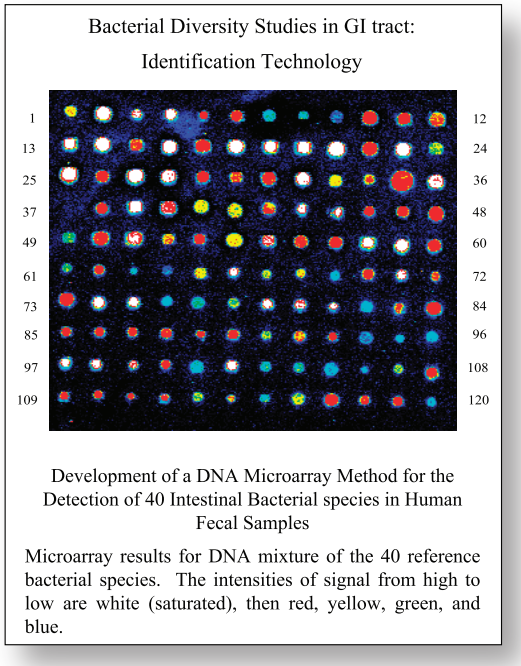
- **The Division of Microbiology has a specialized animal facility to maintain mice without any bacteria in their intestinal tracts.** Studies using this facility give us a better understanding of how changing bacterial populations in the intestinal tract though the use of probiotics affect the mechanisms that protect us from food poisoning. Breeding pairs of germfree normal mice and immune-deficient mice are colonized with a model human intestinal flora. Some of these mice are further colonized with a commercial mixture of probiotic bacteria considered to be helpful for protection against food-poisoning bacteria and others are colonized with *Salmonella* or *Campylobacter*, bacteria that cause food poisoning in humans. The effects of these bacteria on the immune systems of the mice and on the populations of the bacteria in their intestinal tracts have been measured.

The human gastrointestinal tract is colonized with a complex and diverse population of anaerobic bacteria, which play an important role in human health. They contribute to the digestion and absorption of dietary nutrients and provide a barrier to protect the GI tract from colonization by pathogenic bacteria. Therefore, they contribute significantly to health and well-being and, hence, any qualitative or quantitative shifts in the composition of the intestinal microbiota may contribute to increased susceptibility to infection. We have applied basic science approaches to advance this area of research from several perspectives, which include (i.) developing methods to monitor microbial changes within the human gut, particularly when exposed to residual levels of antimicrobial compounds, (ii.) examining antibiotic and drug-mediated effects on indigenous bacteria in the overall stability of the GI tract microbial ecosystem, including resistance development, mechanisms of resistance and identification of commensal resistance genes (with the potential for transmission to pathogens), and (iii.) identifying factors contributing to the maintenance of the microbiota or, alternatively, the exclusion of common pathogens, which will advance our capacity to evaluate food contaminants and additives, dietary supplements, and probiotic effectiveness. Our laboratories are designed for the study of the interactions between mammalian hosts, commensal bacteria, probiotics and enteric pathogens.



Some examples of the expertise of the team are listed below:

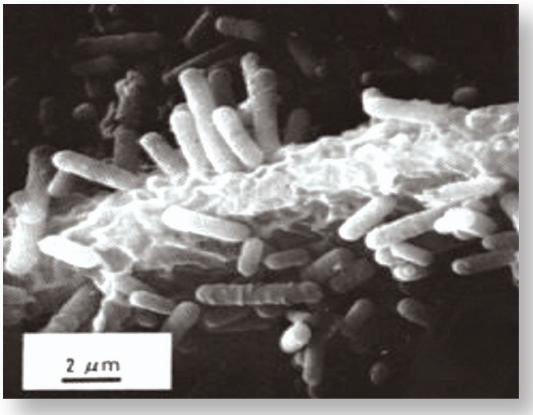
- **There are special food safety concerns for the residues of antimicrobial drugs, since therapeutic doses of antimicrobials can cause adverse effects on the human intestinal microflora.** Assessing the safety of drugs and other compounds involves understanding their effects on the gastrointestinal tract microbiota. We have been instrumental in the development of a decision tree for determining the limits on Acceptable Daily intake of antimicrobials in food, which was adopted by the WHO and used in the FDA/CVM Guidance for Industry #52.



Development of a DNA Microarray Method for the Detection of 40 Intestinal Bacterial species in Human Fecal Samples

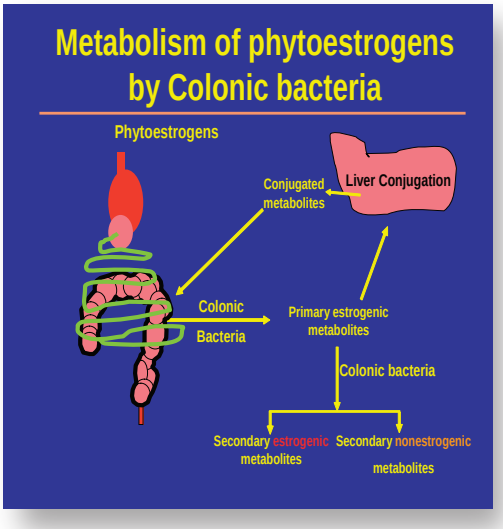
Microarray results for DNA mixture of the 40 reference bacterial species. The intensities of signal from high to low are white (saturated), then red, yellow, green, and blue.

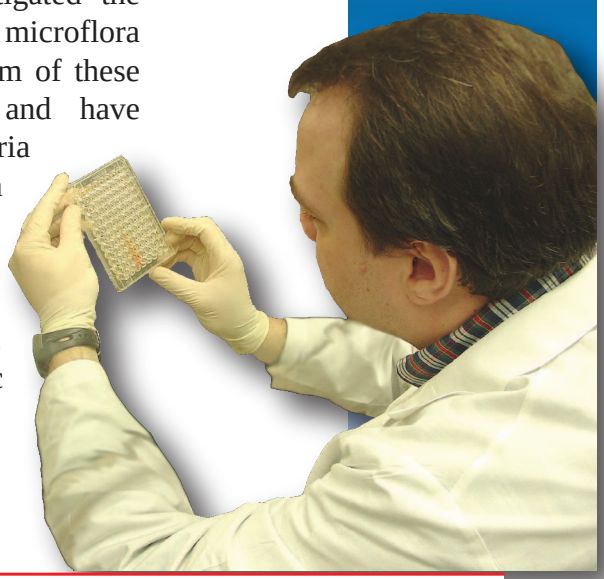
- ***Lactobacillus* species are currently being studied in the Division of Microbiology because they serve as important indicators of gastrointestinal and vaginal tract health and are heavily used by consumers intentionally, as probiotic supplements and in microbially fortified foods.** Little is known about their antibiotic resistance profiles or how these commensal organisms respond to antibiotics ingested by the consumer. We found that endogenous steroids produced by the liver and released into the intestine tend to reverse the inherent resistance to aminoglycoside antibiotics.



- **The pigments used in tattoos and topically applied colorants (cosmetics) are subject to FDA regulation.** The metabolism of the colorants by the skin and intestinal microflora and the potential toxicity of the reaction products to the human body are currently being studied in the Division. We have designed genomic approaches to determine the role of skin microflora in the metabolism of tattoo dyes. This investigation provides valuable information on the toxicity of colorants metabolized by bacteria and their enzymes. We have identified, cloned, and over-expressed azoreductases from a skin bacterium, *Staphylococcus aureus*, and an intestinal bacterium, *Enterococcus faecalis*. The properties of the azoreductases indicate that the enzymes have a broad spectrum of substrate specificity and are capable of degrading a wide variety of azo dyes.

- **There is considerable interest in human consumption of soy isoflavonoids, which have the potential to decrease the risk for cardiovascular diseases and hormone-related cancers.** We have investigated the role of intestinal microflora in the metabolism of these phytoestrogens and have detected bacteria from the human intestinal tract that convert isoflavonoids to estrogenic and non-estrogenic metabolites.





www.fda.gov/nctr/science/divisions/micro.htm

March 13, 2006

Carl E. Cerniglia, Ph.D.
Director, Division of Microbiology
3900 NCTR Road
Jefferson, AR 72079
Tel: 870-543-7341 Fax: 870-543-7307
E-mail: carl.cerniglia@fda.hhs.gov

William Slikker, Jr., Ph.D.
Acting Director, NCTR
3900 NCTR Road
Jefferson, AR 72079
Tel: 870-543-7950 Fax: 870-543-7576
E-mail: william.slikker@fda.hhs.gov