Life after death: The role and composition of the thanatomicrobiome in the decomposition of mammalian organs

The proposed project is aimed at systematically evaluating the role of the thanatomicrobiome (i.e., death-microbiome) in the decomposition of human organs. We focus on humans because much more is known about its microbiome through the Human Microbiome Project (HMP) than the microbiomes of any other mammal. Hence, we will be able to relate knowledge from the HMP to the new knowledge gained by studying the thanatomicrobiome. It is important to note that before humans are born, they are essentially "microbial-cell free". Once they pass through the uterus, consume food, and interact with parents/siblings and the environment, they become inoculated with microbes that live and thrive in their bodies. Recent breakthroughs in DNA sequencing technologies by the HMP have resulted in a paradigm shift in our understanding of cells in humans because we now know that 90% of the cells in adult humans are microbial. When a human dies, however, the percent of microbial cells in a human body proliferate due to the release of nutrients from necrotic human cells. The microbial species involved in this proliferation and their potential roles in decomposing organs and tissues is not known.

Intellectual Merit: The goals of the proposed study are threefold: (i) to establish a working baseline of the thanatomicrobiome (blood, liver, spleen, heart and brain) of human cadavers that have known post-mortem intervals (PMIs) (i.e., the elapse time since actual death), (ii) to narrow down the number of sampling sites (blood, liver, spleen, heart and brain) to one/three in order to provide an in-depth assessment of the thanatomicrobiome in selected organs in many cadavers, (iii) identify the microbial community signatures that could be used to accurately determine PMI. The microbial community signatures refer to the abundance of 16S rRNA genes in specific organs at certain PMI and temperature. The DNA will be extracted from the organs and the blood, amplified using PCR using universal primers targeting 16S rRNA genes, and then sequenced. The DNA sequences will be uploaded to the MG-RAST server for annotation and the microbial abundance will be revealed at multiple levels of taxonomic resolution (i.e., Domain to Species). We intend on determining if microbial communities found in specific organs provide a time-dependent microbial signature that is related to the PMI. This proposal builds upon our ongoing study involving the thanatomicrobiome in the heart, liver, spleen, and brain of three cadavers.

Broader Impacts: Alabama State University (ASU) is a historically black university (HBCU) that offers BS degree in forensic biology and forensic chemistry and MS degree in forensic science. During the two year project, four undergraduate and one master student, funded by the proposal, will be actively involved in extracting and sequencing DNA, blasting the 454 sequences on the NCBI web site, interpreting the sequence annotations from MG-RAST, summarizing the results, writing abstracts, presenting their research at local and national meetings, and submitting manuscripts to peer-reviewed journals. Students will also be involved in autopsies, which will deepen their understanding of biology. This project provides hands-on experience to [redacted], who will be exposed to, and acquire experience in, theoretical and practical methods in molecular biology and genetics. These experiences will give them an edge in applying to graduate programs. We assert that this project will reveal new information, in terms of basic research, on how microbial populations change in organs as human bodies decompose - which is currently not known - and will help a junior STEM faculty member build research capability and effectiveness by improving research/teaching/training of undergraduates in “hands-on” research experiences. Funding of this project would go a long way towards the development of *inquiring minds* and *inquiring attitudes* and enhancing scientific collaborations at local (ASU), regional ([redacted]), national (University of Washington) levels.