Response to Reviewer Comments – S_temp1077

Reviewer 1 (Gurtler):

Comment: Within the risk mitigation section of Objective 2, reviewer suggested adding language to search for novel approaches, technologies, or antimicrobial compounds to our approach for enhanced prevention and control foodborne pathogens.

Response: We have added the following language to the proposal:

"Aside from continued investigation and evaluation of known mitigation strategies, we will also seek out novel approaches for the prevention and control of foodborne pathogens including 1) the optimization or development of transformative technologies and 2) the discovery or synthesis of new antimicrobial compounds with potential GRAS status. Emerging technologies may include high-intensity ultrasound, microwave heating, and pulsed electric fields for the inactivation of primarily vegetative bacteria, yeast, and molds with potential to also inactive viruses (Jermann et al. 2015). With respect to new antimicrobial compounds, there is the possibility to continue optimizing the application of biologically-derived antimicrobials including peptides and essential oils via integration of nanotechnologies. Moreover, novel delivery systems for established and newly discovered antimicrobials will be explored further to increase applicability."

Reviewer 2 (Ma):

Comment: The reviewer suggested to provide better clarification of the overall approach to Objectives 1 and 2.

Response: We have added additional language in Objective 1 to specify the potential approaches that can be taken to achieve our goals. The bulk of the addition is related to the application of emerging "omics" technologies for enhancing our ability to provide better hazard characterization for risk assessment inputs. The primary text is shown below with additional minor revisions throughout the text (see track changed document as well):

Page 9: "One specific novel approach to investigating the relationship between indicators and pathogens will be to apply some of the emerging "omics" technologies to identify better indicators or predictors of pathogen presence under varying environmental conditions—patterns that remain undetected by classical methods. For instance, one could apply metagenomics for enhanced characterization of the microbial communities in food and food manufacturing environment and determine how these communities may change over time in relation to the environmental conditions associated with processing, preservation, sanitation, and storage. There has already been some progress in identifying markers that can allow for qualitative or even quantitative relationships to be drawn between specific processes and/or formulation conditions and strain level microbial responses. The use of strain level response data will introduce added value to quantitative microbial risk

assessment (QMRA) and associated modeling tools. As outlined by den Besten et al. (2018), instead of relying on phenotypic data such as a minimal temperature where growth is expected, the model inputs could look at strain level response intensity (i.e. a biomarker identified via metagenomics) over a continuous variable (i.e. temperature range). To further enhance and address some of the shortcomings of metagenomics (i.e. poor sensitivity for low level contamination, amplification bias, and inability to distinguish between genetic material originating from viable and non-viable cells), metatranscriptomic sequencing may be used as a complimentary tool. Basically, metatranscriptomic sequencing detects cDNA created from RNA extracted from a given microbial community and thus identifying genes in a population that are being transcribed and maybe even translated."

With respect to Objective 2, we have already addressed the need for additional clarity in our response to Reviewer 1. Additional minor revisions have been included within the Objective 2 and some are shown below (see track changed document as well):

Page 11: "This will also be a critical point to integrate microbial community data as well as strain-level data using metagenomics and metatranscriptomics as discussed in Objective 1."

Page 11: "In addition, there are emerging statistical and probabilistic techniques that have been developed to support the inclusion of "omics" data within QMRA. As highlighted by Membré and Guillou (2016), partial least square regression is being optimized for interpretation of omic data to aid in subsequent quantification of the probability of exposure to a given pathogen in food. Another useful technique is the use of Bayesian networks and inference to build gene regulatory networks that could help, for example, 1) predict the duration of lag growth phase of a given foodborne pathogen or 2) overcome lack of biological data which drives the uncertainty in the most QMRA models. By integrating these new techniques into QMRA, the data generated in these emerging "omics" technologies will become more applicable."

Reviewer 3 (Neal):

No comments provided with respect to revision.