A. Bio. Brian P. Hedlund earned his Ph.D. from the University of Washington, Seattle, in 2000, and received his postdoctoral training with Karl Stetter at the University of Regensburg, Germany. He returned to the U.S. in 2003 as an assistant professor in the School of Life Sciences at the University of Nevada Las Vegas (UNLV) and has since been promoted to professor.

He and his collaborators have made significant contributions to our understanding of the structure and function of microbial communities in geothermal springs, the function of the nitrogen cycle at high temperature and the discovery of deep microbial lineages through cultivation, environmental genomics and experiments probing the activities of uncultivated microorganisms in nature. He has also contributed to microbial systematics as a researcher, an author of the SeqCode, a former member of Bergey's Manual Trust and former editor for "Bergey's Manual of Systematics of Archaea and Bacteria and Antonie van Leewenhoek."

B. Title. Role of glycoside hydrolases and allantoinases in the diversification of predominant subsurface bacteria

C. Summary of Project. The bacterial phylum *Atribacterota* evolved over a billion years ago and currently inhabits anaerobic terrestrial and marine subsurface environments where some of them can grow over millions of years with little or no exchange with the surface. The phylum is divided into two classes: (i) *Atribacteria*, members of which rely on glycoside hydrolase (GH) enzymes to ferment polysaccharides and (ii) JS1, members of which are thought to ferment polysaccharides, peptides, hydrocarbons, and allantoin, the latter relying on allantoinase enzymes. *This proposal seeks to test the hypothesis that the evolution of GHs and allantoinases over ~1 billion years led to the diversification of Atribacterota into distinct niches in the Earth's subsurface.* In **Specific Aim 1**, we will use a bioinformatics approach to trace the evolution of GHs and allantoinases over ~1 billion years of evolution. This aim seeks to identify the evolutionary origins and forces responsible for the distribution of these enzymes in extant *Atribacterota* and determine whether the evolution of these enzymes corresponds to habitat or niche changes. In **Specific Aim 2**, we will combine synthetic biology, biochemistry, and bioinformatics approaches to test the functions of GH and allantoinase enzymes. This aim seeks to determine the activities and basic biochemical properties of these enzymes, which will provide mechanistic links between the enzymes, the ecophysiology of extant *Atribacterota*, and evolutionary events uncovered by Specific Aim 1. Together, these aims will illuminate the evolutionary history and ecophysiology of a major inhabitant of the marine and terrestrial subsurface and provide insights into low-energy subsurface ecosystems that may guide interpretations of potential microbial life in subsurface environments on other planets and moons. If funded, this work would be done by postdoctoral fellow Alex Lai and two undergraduate students. This project does not augment any existing funded research, and resulting data would be published and leveraged for proposals to NASA or NSF.