

Diverse microbes from the human gut microbiome secrete extracellular purines

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Introduction: Extracellular purines, including ATP and ADP, serve as important signaling molecules to maintain homeostasis. Several studies have identified that microbial and mammalian cells release purines into their environment. While purinergic signaling, via specific receptors, have been well characterized in immune and neural responses, less is known within the gastrointestinal tract especially about which microbiome communities are capable of secreting extracellular purines. To address this, we sought to identify human gut microbes capable of ATP production. **Methods & Results:** We examined >100,000 annotated genomes using the Integrated Microbiome Genome Database, as well as the enzymes involved in ATP production and clearance in bacteria. Genomes were sorted based on those identified as part of the human microbiome using the IBD TAMMA database. Interestingly, we found that >70% of the human gut microbiota had the enzymes involved in ATP and ADP production, suggesting that ATP/ADP production is conserved amongst bacterial taxa. To assess ATP production, we grew a representative cohort of bacteria and fungi in a chemically defined medium for 6 and 24 hrs and performed a luciferin-based ATP assay on microbial supernatant. In general, we found that ATP concentrations peaked at 6 hours and were sustained at the 24-hour time point. We identified that *Streptococcus mitis*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* generated similar levels of ATP, while *Streptococcus pyogenes* secreted the highest amount (~500nM) of ATP. We also found that fungal strains *Candida glabrata* and *C. tropicalis* produced ~150 nM ATP in supernatant. We did not observe striking differences of ATP production between commensal and pathogenic microbes. To assess whether microbial ATP could activate host purinergic receptors, we performed agonist assays on cells expressing the calcium indicator GCaMP6s and observed increased intracellular calcium signaling from bacteria supernatant containing ATP. **Conclusions:** Collectively, our findings demonstrate that several microbes are capable of secreting exogenous ATP, specifically Gram-positive and fungal species, and that this ATP can activate host purinergic signaling. This work reveals unique patterns of exogenous ATP production among diverse bacterial groups.