

Members of bacterial species share a common set of genes which encode identical cellular functions. Outside this core gene set, subspecies (strains) carry genes shared among a smaller segment of the species, some of which may have been transferred from another bacterial species. These strain-level differences are known to produce within-species variations in characteristics such as antibiotic resistance and human cell receptor binding. Much remains to be understood concerning such intraspecific variation, particularly among species isolated from natural conditions. Until recently, few diverse and natural single-species strain collections existed by which researchers could study characteristics such as resistance to ionizing radiation and predict their underlying genetic causes. Here we report the generation of three such strain collections (*Lactobacillus fermentum*, *L. gasseri*, and *L. salivarius*) sampled from 700 human donors. We isolated a wide range of bacterial species from donor stool and identified them at the species level. To ensure that each strain within the collections is unique, we obtained an average of one strain per species per donor (243 strains of *L. fermentum*, 164 strains of *L. gasseri*, and 105 strains of *L. salivarius*). Furthermore, we demonstrate the diversity of each strain collection through assessments of their common vs. distinct gene content. The assembly of our strain collections open the door to studies on natural variation of gamma radiation resistance in bacterial species and potential genetic markers identifying bacteria exposed to contaminating levels of gamma radiation.