

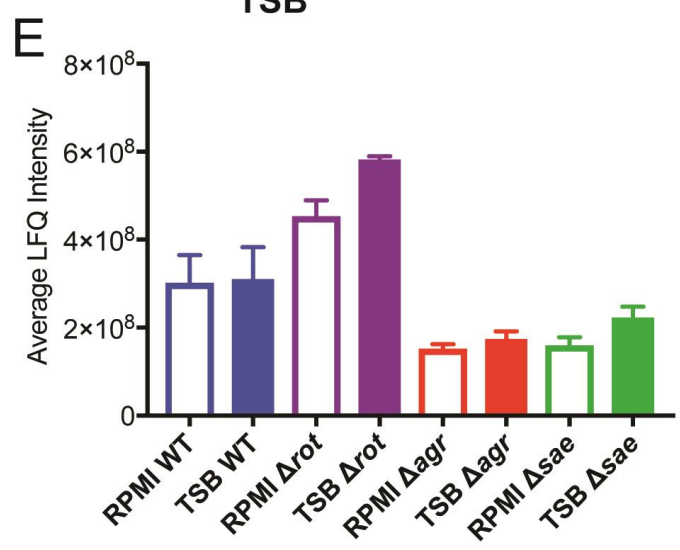
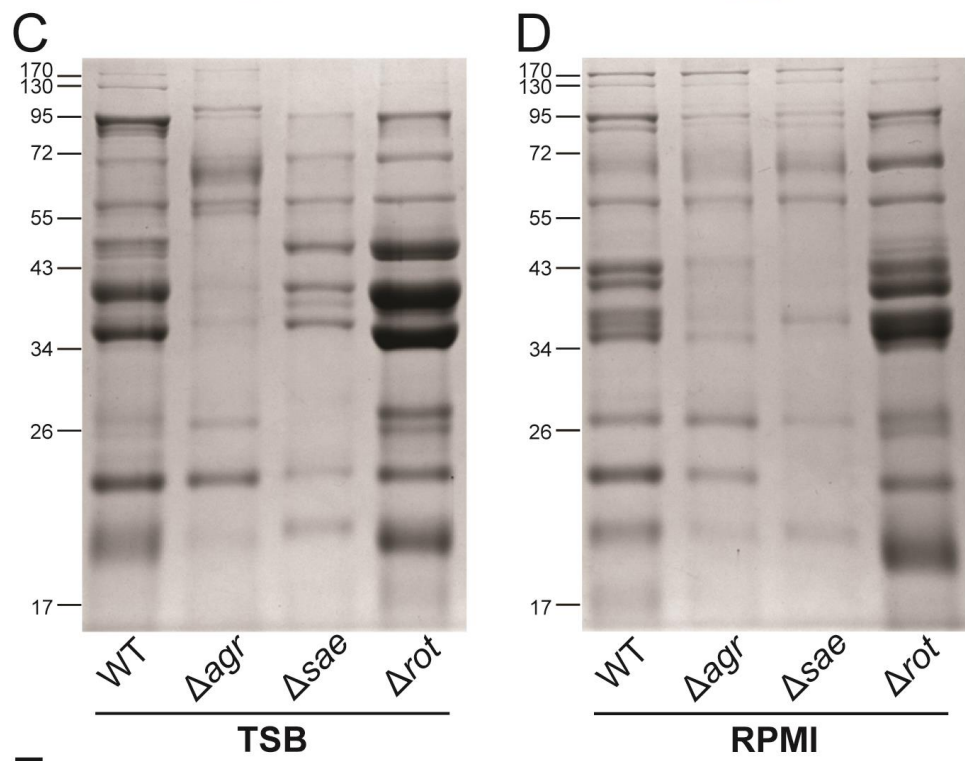
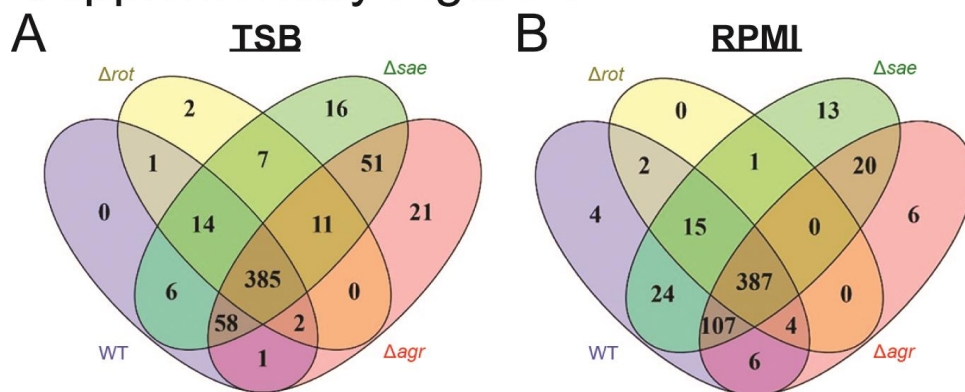
**Figure S1. The exoproteins quantified from WT and isogenic mutant *Sa* do not vary widely, but the abundance of these proteins does.** (A) Approximately 65% of the quantified proteins are identified in all exoprotein samples. This is clearly shown in the venn diagrams comparing the proteins identified in the WT and isogenic mutants per growth condition. (B) The major difference between the strains is the abundance of subsets of proteins within the exoproteome. A silver stained gel of 5% of the exoproteome samples shows a greater abundance of proteins in particular molecular weight ranges in the  $\Delta rot$  mutants regardless of the growth condition. (C) The overall protein level in the exoproteomes is slightly greater when *Sa* is grown in nutrient rich media as seen in the bar graph of the average LFQ intensity for all proteins per strain type.

**Figure S2. The composition of the exoproteome at different growth stages minimally varies, but the intensity of certain proteins do change over the colonies lifetime.** (A) Venn diagrams display the high overlap of proteins between the three time points. (B) Additionally, the exoproteome composition is not widely effected by the growth conditions. (C) The overall protein level in the exoproteomes is slightly greater when *Sa* is grown in nutrient rich media. This can be seen on the silver stained gel of 5% of the exoproteome samples. (D) A heatmap of the protein quantitation data shows that the exoproteomes are more similar within each growth condition than within a growth stage. LFQ intensity values were log<sub>2</sub> transformed, missing values were imputed from the normal data distribution, and then z-scores were calculated. A z-score indicates how many standard deviations a value is from the mean [ $z = (X - \mu)/\sigma$ ],  $X$  = value,  $\mu$  = population mean,  $\sigma$  = standard deviation]. Unsupervised hierarchical

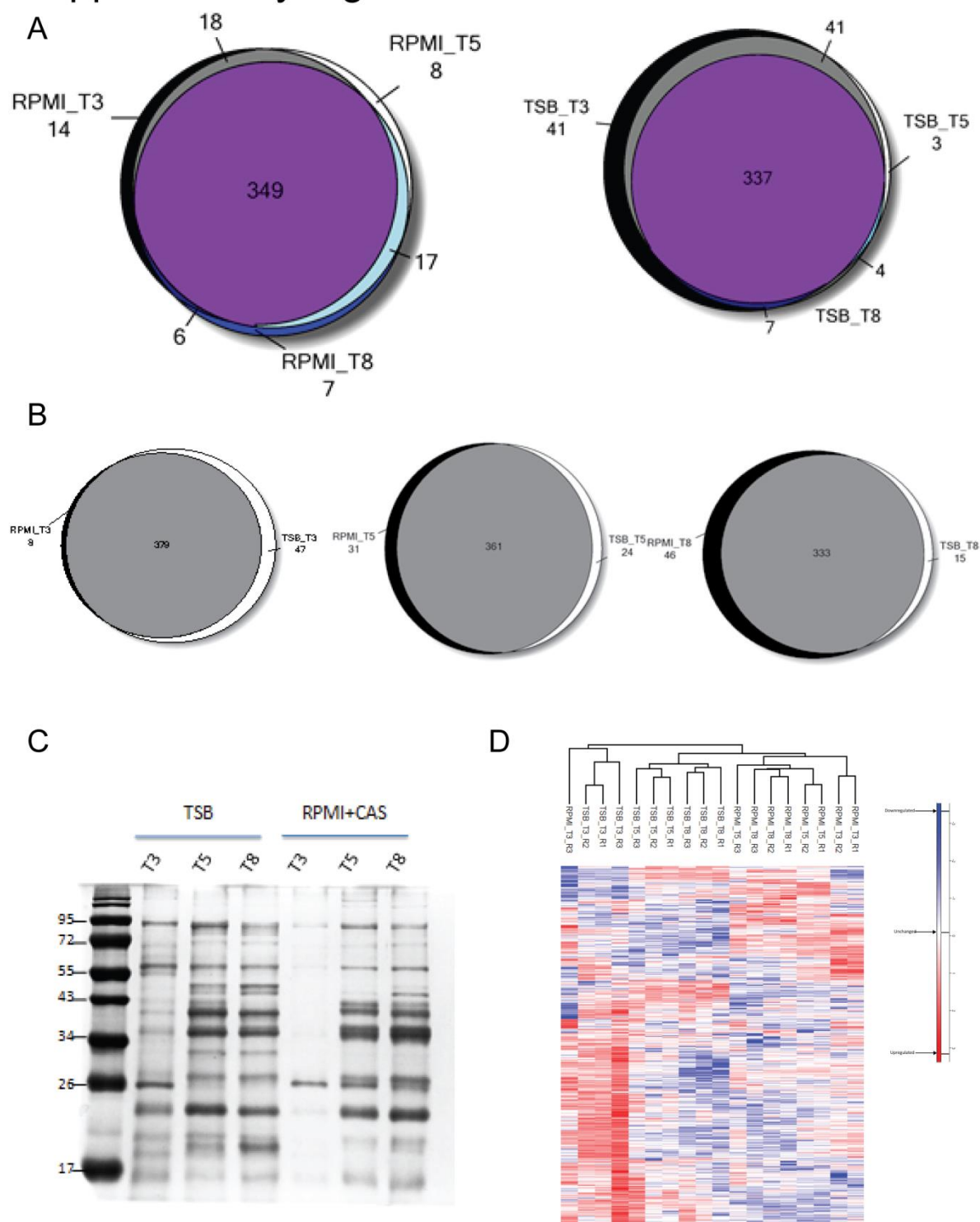
clustering is used to generate a heat map from the z-scores representing protein groups in the matrix as colors. Each row in the heat map is a different protein group and each column is an individual sample. The clustering at the top indicates which samples are most closely related based on the relative intensity of the quantified protein groups.

**Figure S3. Exoproteomes from a selection of 13 reference strains show a range of diversity.** The exoproteome composition, complexity and intensity varies across the 13 reference strains analyzed. This can be seen on the silver stained gel of 5% of the exoproteome samples. Colonies were normalized to the same optical density prior to culture filtrate collection so these differences in exoproteome are not a result of difference in colony size.

# Supplementary Figure 1



# Supplementary Figure 2



# Supplementary Figure 3.

