

Bile acids are intestinal metabolites that are biotransformed into diverse secondary bile acids to aid with digestion and absorption. However, once modified by the gut microbiome, they can produce serious health implications including colorectal cancer risk. We hypothesized that this bile acid metabolism is reflected in bacterial cell morphologic changes. To test this hypothesis, we anaerobically cultured and generated light microscopy images of *Clostridium scindens* in media containing 100 μ M cholic acid (a known substrate in the production of the carcinogenic bile acid deoxycholic acid), *C. scindens* in media containing NaCl (a positive control; 1% NaCl is shown to cause shrinkage through osmosis in bacterial cells), and *C. scindens* in media alone (negative control) (8 images per group; 500 bacterial cells per image). We developed an image-based model using MicrobeJ (an ImageJ plug-in developed for analysis of bacterial images) by using smoothed particle contours and a skeletonization algorithm adjusting area, length, width, and circularity parameters to accurately detect cells. We observed a significant difference in shape descriptor analysis between curvature of the end points and center of the medial axes, width of the medial axes, ratio between the major and minor axes of the cells, ratio between area and convex area, angularity, roundness ($4 \times \text{area} / \pi \times \text{major axis}^2$), length of the medial axes, circularity ($4\pi \times \text{area} / \text{perimeter}^2$), and perimeter of the outside boundary between *Clostridium scindens* with and without the presence of cholic acid ($p < 10^{-5}$ for all comparisons; Welch's two-tailed t-test). Of note, this represented a larger difference than the delta between the two controls. Our data demonstrate that image-based analysis can enable detection of cellular morphologic differences of *C. scindens* based on metabolic profile with respect to bile acids. In principle, this approach could be expanded to other bile acids and/or beyond bile acid metabolism to identify bacterial metabolic behaviors of interest (e.g. assessing or predicting effects of clinically relevant compounds targeting the microbiome) or aid with statistical modeling screening for colorectal cancer.