Stickland amino acid availability alters the expression of the bile acid inducible (*bai*) operon in commensal *Clostridia*

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Clostridium scindens is a commensal that metabolizes primary bile acid cholate (CA) into secondary bile acid deoxycholate (DCA), which are inhibitory to *Clostridioides difficile*. Recent work showed that the *C. scindens bai* operon decreased expression in the presence of the proline precursor hydroxyproline. Proline and glycine are important for Stickland fermentation in both commensal *Clostridia* and the pathogen *C. difficile*. The consumption of these amino acids by *C. scindens* provides competition, further contributing to the inhibition of *C. difficile* by an intact gut microbiota. We hypothesize that the availability of amino acids used for Stickland fermentation alters the expression of the *bai* operon in commensal *Clostridia,* ultimately controlling secondary bile acid metabolism. To test this, we grew *C. scindens* in excess proline or glycine, in the presence and absence of CA or DCA. At mid-log growth, supernatant was collected for RNAseq and metabolomic analysis. Supplementation of CA significantly increased expression of the *bai* operon in *C. scindens*. Supplementing proline and CA maintained the same magnitude of expression, while the addition of glycine significantly decreased expression. Proline and CA altered the global transcriptome with 270 genes being differentially expressed, compared to only 50 genes with glycine. Genes important for carbohydrate metabolism, proline usage (*prd*), and cellular energetics in the form of molybdenum transfer and biosynthesis increased, while genes involved in the synthesis of proline and arginine metabolism (*pro* and *arg*) decreased. These findings suggest an avenue to modulate secondary bile acid production in commensal *Clostridia* with amino acid supplementation via diet.