Additional file 1 — Biofilm Model Description

Model equations

The biomass equation for each species had the form,

$$\frac{\partial X_i}{\partial t} = \mu_i X_i + D_{X,i}\frac{\partial^2 X_i}{\partial z^2}, \tag{1}$$

where $X_i(z,t)$ is the local biomass concentration (g/L) of species i, $\mu_i(z,t)$ is the local growth rate (h^{-1}) calculated from the metabolic reconstruction and $D_{X,i}$ is a biomass diffusion coefficient. Boundary conditions were imposed to represent zero biomass flux at the intestine-biofilm boundary ($z = L$) and biomass removal by continuous erosion [1] at the biofilm-stool interface ($z = 0$),

$$\frac{\partial X_i(L,t)}{\partial z} = 0, \qquad -D_{X,i}\frac{\partial X_i(0,t)}{\partial z} = k_{X,i}[X_{b,i}(0) - X_i(0,t)], \tag{2}$$

where $k_{X,i}$ is a]the biomass mass transfer coefficient and $X_{b,i}(0)$ is the bulk planktonic concentration of species i in the stool, which was assumed to be zero for simplicity. The nutrient transport equations had the form,

$$\frac{\partial N_i}{\partial t} = \sum_{j=1}^{n} v_{i,j}X_j + D_{N,i}\frac{\partial^2 N_i}{\partial z^2}, \tag{3}$$

where $N_i(z,t)$ is the local concentration of nutrient i (glucose, cysteine, isoleucine, leucine, methionine, proline serine, tryptophan, valine), $v_{i,j}(z,t)$ is the uptake flux (mmol/gDW/h) of nutrient i by species j calculated from the metabolic reconstruction, $D_{N,i}$ is a nutrient diffusion coefficient and $n = 4$ is the number of species. Boundary conditions were imposed such that unconsumed nutrients could be removed from either boundary,

$$-D_{N,i}\frac{\partial N_i(0,t)}{\partial z} = k_{N,i}[N_{b,i}(0) - N_i(0,t)] \tag{4}$$

$$-D_{N,i}\frac{\partial N_i(L,t)}{\partial z} = k_{N,i}[N_{b,i}(L) - N_i(L,t)], \tag{5}$$

where $k_{N,i}$ is the nutrient mass transfer coefficient, and $N_{b,i}(0)$ and $N_{b,i}(L)$ are bulk concentrations of nutrient i. The bulk nutrient concentrations at the biofilm-stool interface were set as $N_{b,i}(0)$, while bulk concentrations at the intestine-biofilm interface $N_{b,i}(L)$ were assumed to be zero for simplicity. The byproduct transport equations and boundary conditions had a similar form,

$$\frac{\partial P_i}{\partial t} = \sum_{j=1}^{n} v_{i,j}X_j + D_{P,i}\frac{\partial^2 P_i}{\partial z^2} \tag{6}$$

$$-D_{P,i}\frac{\partial P_i(0,t)}{\partial z} = k_{P,i}[P_{b,i}(0) - P_i(0,t)] \tag{7}$$

$$-D_{P,i}\frac{\partial P_i(L,t)}{\partial z} = k_{P,i}[P_{b,i}(L) - P_i(L,t)], \tag{8}$$

where $P_i(z,t)$ is the local concentration (mmol/L) of byproduct i (acetate, butyrate, CO_2, ethanol, formate, lactate, propionate, succinate), $D_{P,i}$ is the byproduct diffusion coefficient, $k_{P,i}$ is the byproduct mass transfer coefficient, $P_{b,i}(0)$ and $P_{b,i}(L)$ are bulk concentrations of byproduct i, which were assumed to be zero at both boundaries for simplicity. The exchange flux $v_{i,j}(z,t)$ (mmol/gDW/h) of product i from species j was positive if the byproduct was secreted and negative if the byproduct was consumed.

Each species was allowed to consume all the supplied nutrients, which included glucose, eight amino acids, nitrate and the primary bile acid taurocholate. Uptake kinetics for each nutrient and byproduct were assumed to follow Michaelis-Menten kinetics:

$$v_i = \frac{v_{max,i} S_i}{K_{m,i} + S_i} \tag{9}$$

where $S_i(z,t)$ is the local extracellular concentration (mmol/L) of substrate i (nutrients and byproducts), $v_{max,i}$ is the maximum uptake rate (mmol/gDW/h) and $K_{m,i}$ is Michaelis-Menten constant (mmol/L). The calculated local uptake rates $v_i(z,t)$ (mmol/gDW/h) were imposed as transport bounds in the linear program used to solve the metabolic reconstructions.

Model solution

The multispecies biofilm model was solved using DFBAlab [2], which required the specification of lexicographic optimization objectives to overcome the problem of alternative optima in the FBA problems. Because the biofilm model included four species and $N = 25$ spatial node points, a total of 100 FBA problems were solved at each time point. Following the procedure established in our previous study [3], we ordered the lexicographic optimization objectives into the following tiers (Table S1): (1) growth; (2) secretion of experimentally observed byproducts: *B. thetaiotaomicron* (acetate, succinate, propionate, CO_2) [4], *F. prausnitzii* (lactate, butyrate, formate, CO_2) [5], *E. coli* (acetate, ethanol, formate, lactate, succinate, CO_2) [6]; *C. difficile* (acetate, butyrate, propionate, CO_2) [7]; (3) uptake of possible cross-fed byproducts: *B. thetaiotaomicron* (lactate, ethanol, formate), *F. prausnitzii* (acetate, succinate), *E. coli* (butyrate, propionate) , *C. difficile* (succinate, formate, ethanol, lactate); (4) uptake of glucose; (5) uptake of supplied amino acids (cysteine, isoleucine, leucine, methionine, proline, serine, tryptophan, valine); (6) uptake of nitrate by *E. coli* and (7) uptake of taurocholate by *C. difficile*.

All objectives were maximized, which translated to maximization of secretion fluxes due to their positivity and minimization of uptake fluxes due to their negativity. The *B. thetaiotaomicron* model had 17 objectives due to the lack of butyrate, nitrate and taurocholate exchange fluxes, the *F. prausnitzii* model had 16 objectives due to the lack of ethanol, propionate, nitrate and taurocholate exchange fluxes, the *E. coli* model had 19 objectives due to the lack of taurocholate exchange flux, and *C. difficile* model had 19 objectives due to the lack of nitrate exchange flux.

Table 1: Lexicographic optimization objectives.

Objective	***B. thetaiotaomicron***	***F. prausnitzii***	***E. coli***	***C. difficile***
1	Biomass	Biomass	Biomass	Biomass
2	Acetate	Lactate	Acetate	Acetate
3	Succinate	Butyrate	Ethanol	Butyrate
4	Propionate	Formate	Formate	Propionate
5	CO_2	CO_2	Lactate	CO_2
6	Lactate	Acetate	Succinate	Succinate
7	Ethanol	Succinate	CO_2	Formate
8	Formate	Glucose	Butyrate	Ethanol
9	Glucose	Cysteine	Propionate	Lactate
10	Cysteine	Isoleucine	Glucose	Glucose
11	Isoleucine	Leucine	Cysteine	Cysteine
12	Leucine	Methionine	Isoleucine	Isoleucine
13	Methionine	Proline	Leucine	Leucine
14	Proline	Serine	Methionine	Methionine
15	Serine	Tryptophan	Proline	Proline
16	Tryptophan	Valine	Serine	Serine
17	Valine	Not specified	Tryptophan	Tryptophan
18	Not specified	Not specified	Valine	Valine
19	Not specified	Not specified	Nitrate	Taurocholate

References

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