Effect of proximity on mutually cross-feeding lactic acid bacteria in yogurt fermentation using Flux Balance Analysis

1. Auxotrophic metabolites

a. *L.bulgaricus* auxotrophies

Table 2: Auxotrophic metabolites for Lactobacillus bulgaricus

Lactobaccilus bulgaricus auxotrophies	
Metabolite name	COBRA reaction name
L-Alanine exchange	EX_ala__L_e
L-Arginine exchange	EX_arg__L_e
Calcium exchange	EX_ca2_e
Cadmium exchange	EX_cbl1_e
Chloride exchange	EX_cl_e
Co2+ exchange	EX_cobalt2_e
L-Cysteine exchange	EX_cys__L_e
Fe2+ exchange	EX_fe2_e
Fe3+ exchange	EX_fe3_e
Folate pseudo-reaction	EX_fol_e
Glycine exchange	EX_gly_e
L-Histidine exchange	EX_his__L_e
L-Isoleucine exchange	EX_ile__L_e
K+ exchange	EX_k_e
Lactose exchange	EX_lcts_e
L-Leucine exchange	EX_leu__L_e
L-Lysine exchange	EX_lys__L_e
L-Methionine exchange	EX_met__L_e
Magnesium exchange	EX_mg2_e
Manganese exchange	EX_mn2_e
Molybdate exchange	EX_mobd_e
Nicotinate exchange	EX_nac_e
L-Phenylalanine exchange	EX_phe__L_e
Phosphate exchange	EX_pi_e
(R)-Pantothenate exchange	EX_pnto__R_e
L-Proline exchange	EX_pro__L_e
Riboflavin exchange	EX_ribflv_e
L-Serine exchange	EX_ser__L_e
L-Tryptophan exchange	EX_trp__L_e
L-Tyrosine exchange	EX_tyr__L_e
L-Valine exchange	EX_val__L_e
Zinc exchange	EX_zn2_e
Pyridoxal exchange	EX_pydx_e

b. *S. thermophilus* auxotrophies

Table 1: Auxotrophic metabolites for Streptococcus thermophilus

Streptococcus thermophilus autotrophies	
Metabolite name	COBRA reaction name
Phosphate exchange	EX_pi_e
Nicotinate exchange	EX_nac_e
Peptides	EX_mpept_e
Lactose exchange	EX_lcts_e
Riboflavin exchange	EX_ribflv_e
(R)-Pantothenate exchange	EX_pnto__R_e
Thiamin exchange	EX_thm_e

2. Viscosity of milk at different temperatures (Reference)

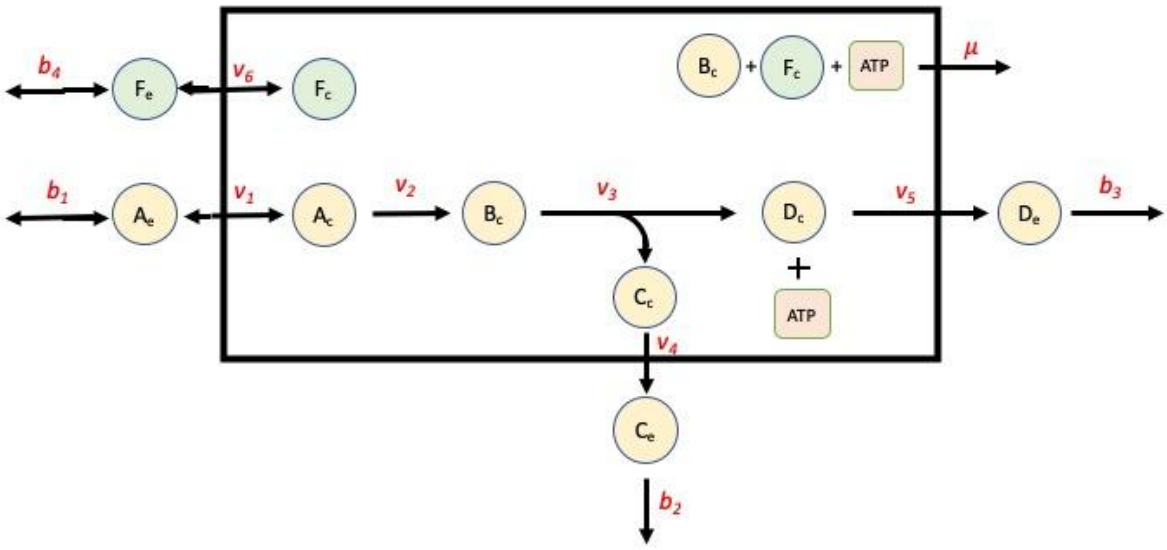
Table.10.2 Viscosity of milk, skim milk and lactose at various temperatures

Temperature (°C)	Viscosity (cp)			
	Whole milk	Skim milk	5% lactose	Water
5	3.254	3.96	1.76	1.519
10	2.809	2.47	1.50	1.308
15	2.463	2.10	..	1.140
20	2.127	1.79	1.15	1.005
25	1.857	1.54	1.03	0.894
30	1.640	1.33	0.91	0.894
35	--	1.17	. --	0.723
40	--	1.04	0.74	0.656

3. Toy Models

Toy Model for *L. bulgaricus*

In this Toy Model for L. bulgaricus, we consider the case where an auxotrophic metabolite is being consumed along with a substrate that allows for the growth of the bacteria. The following toy model is employed for carrying out Flux Balance analysis.



In this toy model, we have a cell that consumes substrate A at rate b_1, secretes C at rate b_2, it grows at the rate μ and it has four transporters (v_1 , v_4, v_5, v_6) and two intracellular reaction (v_2, v_3). It also has a cross-feeding compound, F which is consumed at rate b_3. The units of rates b and v are [mmol/gDW*h]

In this toy model, there is only one solution instead of multiple solutions that exist in real cases, when the growth rate is maximized.

The mass balances at steady state for this network are

A_e	$-1\ b_1 - 1\ v_1 = 0$
A_c	$1\ v_1 - 1\ v_2 = 0$
B_c	$1\ v_2 - 1\ v_3 - 1\ \mu = 0$
C_c	$1\ v_3 - 1\ v_4 = 0$
D_c	$1\ v_3 - 1\ v_5 = 0$
F_c	$1\ v_6 - 1\ \mu = 0$
C_e	$1\ v_4 - 1\ b_2 = 0$
D_e	$1\ v_5 - 1\ b_3 = 0$
F_e	$-1\ b_4 - 1\ v_6 = 0$
ATP	$1\ v_3 - 1\ \mu = 0$

The matrix form of these equations is:

S.v =

A_e
A_c
B_c
C_c
D_c
F_c
C_e
D_e
F_e
ATP

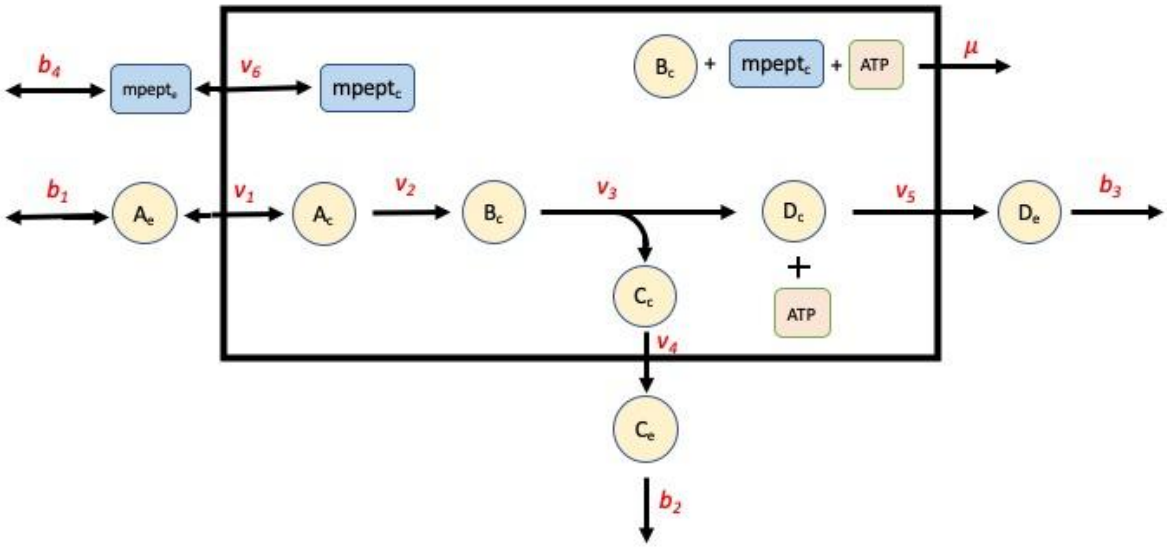
$$\begin{pmatrix} -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{pmatrix} * \begin{pmatrix} b1 \\ v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ b2 \\ b3 \\ b4 \\ \mu \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

0 LB

In the second case, we will consider a Toy model for when a cross-feeding compound is not auxotrophic, however it does improve the growth rate to increase.

Toy Model for *S. thermophilus*

Similar to the toy model above, for S. thermophilus, we make a toy model wherein A is the subsutrate that is being taken up by the cell along with another cross feeding compound - mpept, which it receives from L. bulgaricus in order to have it's second exponential growth phase during yogurt fermentation.



In this toy model, we have a cell (S. thermophilus) that consumes substrate A at rate b_1, secretes C at rate b_2, it grows at the rate μ and it has four transporters (v_1, v_4, v_5, v_6) and two intracellular reaction (v_2, v_3). It also has a cross-feeding compound, mpept which is consumed at rate b_3. The units of rates b and v are [mmol/gDW*h] and the rate of μ [1/h].

In this toy model, there is only one solution instead of multiple solutions that exist in real cases, when the growth rate is maximized.

The mass balances at steady state for this network are

A_e	$-1\ b_1 - 1\ v_1 = 0$
A_c	$1\ v_1 - 1\ v_2 = 0$
B_c	$1\ v_2 - 1\ v_3 - 1\ \mu = 0$
C_c	$1\ v_3 - 1\ v_4 = 0$
D_c	$1\ v_3 - 1\ v_5 = 0$
$mpept_c$	$1\ v_6 - 1\ \mu = 0$
C_e	$1\ v_4 - 1\ b_2 = 0$
D_e	$1\ v_5 - 1\ b_3 = 0$
$mpept_e$	$-1\ b_4 - 1\ v_6 = 0$
ATP	$1\ v_3 - 1\ \mu = 0$

S.v = 0 LB

A_e
A_c
B_c
C_c
D_c
mpept_c
C_e
D_e
mpept_e
ATP

$$\begin{array}{c} \begin{matrix} b1 & v1 & v2 & v3 & v4 & v5 & v6 & b2 & b3 & b4 & \mu \end{matrix} \\ \begin{pmatrix} -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{pmatrix} \end{array} * \begin{pmatrix} b1 \\ v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ b2 \\ b3 \\ b4 \\ \mu \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

4. Initial nutrient concentrations for ST and LB media

The initial concentration of all products of ST and LB were set to 0. In initial metabolite concentrations of the media components (nutrients) were set to 0.1 mmol/L.gDW. However a few values had to be increased in order to allow the cells to grow properly. The nutrients whose values were more than 0.1 can be found in the code, in CrossFeeding.m. The values of the initial concentrations can be modified easily.

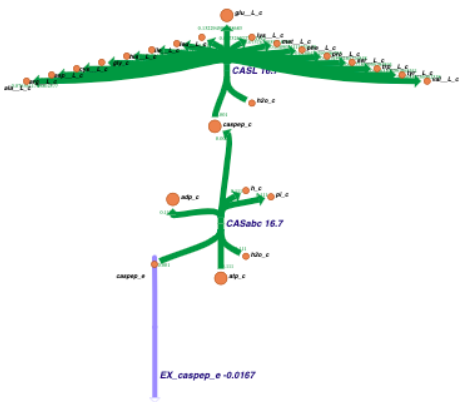
5. Sensitivity analysis for *S.thermophilus* final biomass

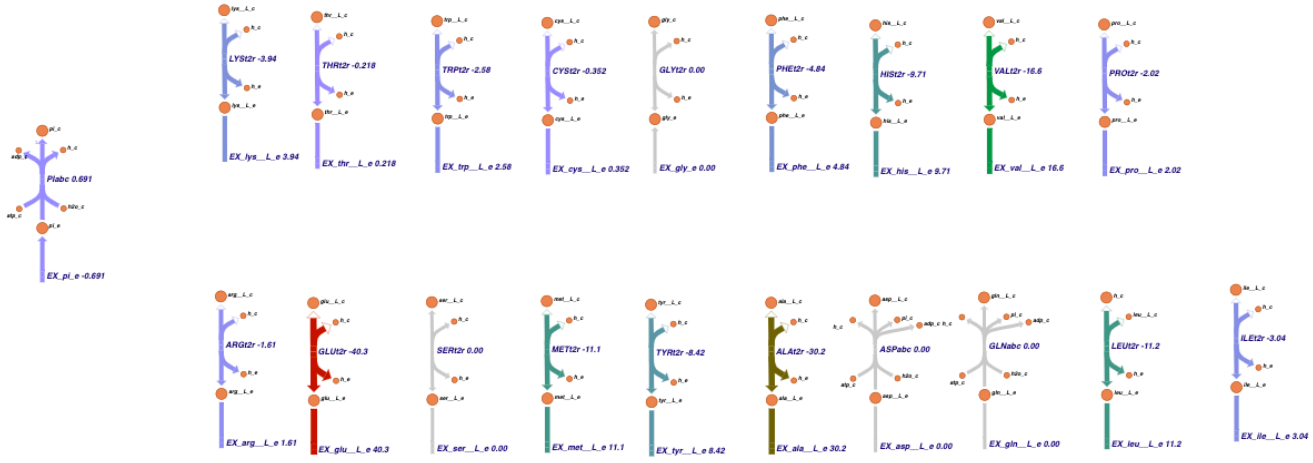
Final Biomass value at 30h noted for simulations with different distances between ST and LB

Distance between ST and LB	Final biomass after 30h	Absolute change in Biomass
μm	gDW	$\frac{\Delta gDW}{\Delta \mu m}$
0	4.364	Reference
1	4.377	0.013
2	4.404	0.013
3	4.447	0.014
4	4.454	0.002
5	4.464	0.002
6	4.401	-0.010
7	4.379	-0.003
8	4.350	-0.004
9	4.273	-0.009
10	4.184	-0.009
11	4.064	-0.011
12	4.015	-0.004

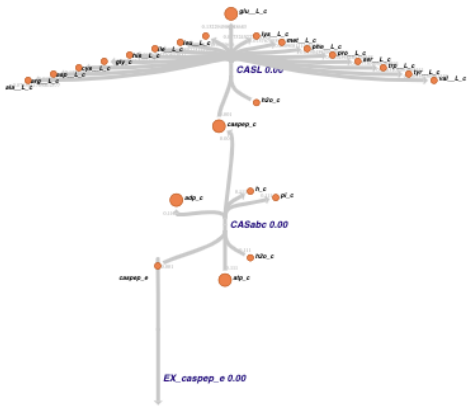
6. ESCHER visualizations - Full maps can be shared on request

When the distance between cells is 10 µm, at the flux through caspep and amino acids is shown. At at the 5th time step when Peptides are still available in media, the flux through caspep is very high and the cell is moving amino acids in the extra cellular space





However when peptides run out in the media, the flux through caspep is 0 and the amino acids



are consumed from the media.