Appendix A: mathematical model of growth of two species of *Lactobacillus* in the mouse forestomach.

A mathematical model is presented that describes competition between two strains (100-23 and 100-33) in the presence of two substrates (maltose and glucose). For simplicity it is assumed that the mouse forestomach acts as a continuous fermentor, so that explicit expressions for the steady state concentrations of substrate and bacteria can be obtained. These are then be used to derive conditions for which the two strains can co-exist.

Concentrations of substrate and bacteria in the vessel are denoted by C and B, respectively (mg/mL). The turn-over rate is denoted by k (h^{-1}). Substrate is assumed to enter the forestomach continuously at a rate of $k\ C_{in}$ where C_{in} denotes the substrate concentration (mg/mL) in the medium entering the vessel. Absorption of water and substrates is assumed to be absent. Gain in bacterial mass is modelled as the product of the observed fractional growth rate (G_{obs}, h^{-1}) multiplied by B. The substrate required to achieve this growth is given by $G_{obs}\ B/E$, where E (mg biomass/mg substrate) is the efficiency of converting substrate into biomass (E is assumed less than 1). Bacterial death is assumed negligible with loss of biomass occurring only via the outlet (modelled as $k\ B$). Superscripts 'malt' and 'gluc' refer to maltose and glucose respectively, and subscripts '1' and '2' refer to strains 100-23 and 100-33. The change in substrate concentration is given by inflow minus outflow minus use for bacterial growth. The change in bacteria is given by bacterial growth on maltose and glucose minus outflow:

$$\frac{dC^{malt}}{dt} = k\,C_{in}^{malt} - k\,C^{malt} - B_1 G_{1obs}^{malt}/E_1^{malt} - B_2 G_{2obs}^{malt}/E_2^{malt} \tag{1}$$

$$\frac{dC^{gluc}}{dt} = k\,C_{in}^{gluc} - k\,C^{gluc} - B_1 G_{1obs}^{gluc}/E_1^{gluc} - B_2 G_{2obs}^{gluc}/E_2^{gluc} \tag{2}$$

$$\frac{dB_1}{dt} = B_1 G_{1obs}^{malt} + B_1 G_{1obs}^{gluc} - kB_1 \tag{3}$$

$$\frac{dB_2}{dt} = B_2 G_{2obs}^{malt} + B_2 G_{2obs}^{gluc} - kB_2 \tag{4}$$

The observed fractional growth rates on maltose and glucose for strain 1 are modelled as (Ballyk & Wolkowics, 1993)

$$G_{1obs}^{malt} = G_1^{malt}\frac{C^{malt}/M_1^{malt}}{1 + C^{malt}/M_1^{malt} + C^{gluc}/M_1^{gluc}} \tag{5}$$

$$G_{1obs}^{gluc} = G_1^{gluc}\frac{C^{gluc}/M_1^{gluc}}{1 + C^{malt}/M_1^{malt} + C^{gluc}/M_1^{gluc}} \tag{6}$$

where G_1^{malt} (h^{-1}) is the maximum fractional growth rate on maltose and M_1^{malt} (mg/mL) the half saturation constant (concentration at which half the maximum growth rate is achieved). G_1^{gluc} and M_1^{gluc} are similarly defined for growth on glucose. This model formulation assumes that strain 1 is able to feed on both substrates simultaneously. The concentrations of each of the substrates with respect to the values of M determine whether feeding will occur mainly on maltose (when $C^{malt}/M_1^{malt} > C^{gluc}/M_1^{gluc}$) or glucose (when $C^{malt}/M_1^{malt} < C^{gluc}/M_1^{gluc}$). Note that when one of the substrates is absent, glucose say, growth on maltose simplifies to the standard Monod model: $G_{1obs}^{malt} = G_1^{malt}C^{malt}/(M_1^{malt} + C^{malt})$. Growth of species 2 on maltose and glucose is defined analogously to that of species 1:

$$G_{2obs}^{malt} = G_2^{malt}\frac{C^{malt}/M_2^{malt}}{1 + C^{malt}/M_2^{malt} + C^{gluc}/M_2^{gluc}} \tag{7}$$

$$G_{2obs}^{gluc} = G_2^{gluc} \frac{C^{gluc}/M_2^{gluc}}{1 + C^{malt}/M_2^{malt} + C^{gluc}/M_2^{gluc}} \tag{8}$$

Requirements for co-existence of two species

In steady state the concentrations of substrates and bacteria are constant, so that equations (1)-(4) will be equal to zero. It can be shown that for B_1 and B_2 to co-exist in steady state, the following two requirements need to be met (derivations given in Appendix C):

$$\frac{G_1^{malt} - k}{M_1^{malt}} > \frac{G_2^{malt} - k}{M_2^{malt}} \quad \text{and} \quad \frac{G_2^{gluc} - k}{M_2^{gluc}} > \frac{G_1^{gluc} - k}{M_1^{gluc}} \tag{9}$$

and

$$\frac{G_1^{gluc}/M_1^{gluc}}{G_1^{malt}/M_1^{malt}} \; \frac{E_1^{malt}}{E_1^{gluc}} < \frac{(C_{in}^{gluc} - C^{gluc}) / C^{gluc}}{(C_{in}^{malt} - C^{malt}) / C^{malt}} < \frac{G_2^{gluc}/M_2^{gluc}}{G_2^{malt}/M_2^{malt}} \; \frac{E_2^{malt}}{E_2^{gluc}} \tag{10}$$

Equation (9) states that species 1 should have a relative advantage on maltose compared to species 2 (i.e. G_1^{malt} relatively large and M_1^{malt} relatively small compared to G_2^{malt} and M_2^{malt}, respectively), whilst at the same time species 2 should have a relative advantage on glucose compared to species 1. The right hand side of Equation (10) implies that if species 2 has an advantage on glucose compared to maltose ($G_2^{gluc}/M_2^{gluc} > G_2^{malt}/M_2^{malt}$), then, for the two species to co-exist, this relative advantage should not be too large (as species 2 would outcompete species 1), i.e. the efficiency E_2^{gluc} should not be too large compared to E_2^{malt}. Similar reasoning holds for the left hand side, namely that for species 1 the relative advantage on maltose (compared to glucose) should not be too large, i.e. the efficiency E_1^{malt} should not be too large compared to E_1^{gluc}. In addition, the middle section of equation (10) implies that the glucose input should not be too small compared to the maltose input (as otherwise species 2 will go extinct), and vice versa.

Appendix B: Forestomach parameter values

Assume a mouse of 20 g, and let the forestomach be 2% of BW = 0.4 ml. Let water intake be 10% of body weight (BW) per day, so that k = 2/0.4 d^{-1} = 0.2/h. Let dry matter intake be 1% of BW/d, of which 1% is assumed to be as glucose and 1% as maltose. Then glucose and maltose intakes are 2 mg/d each, and combined with water intake of 2 mL/d, the substrate concentration C_{in} in the medium entering the vessel is 1 mg/mL for both maltose and glucose. The maximum growth rates are set equal to LN(2)/doubling time, with doubling times as reported in the Results section, so that G_1^{malt}=0.87/h, G_1^{gluc}=0.47/h, G_2^{malt}= 0.78/h and G_2^{gluc} = 1.09/h. The affinities (1/M) are set such that 100-33 shows a very strong affinity for glucose and 100-23 shows a strong affinity for maltose, whilst its affinity for glucose is assumed half that of maltose. The affinity of 100-33 for maltose is assumed weakest. The values used are $M_2^{gluc} = 0.01$, M_1^{malt} = 0.1, M_1^{gluc}=0.2 and $M_2^{malt} = 0.4$ mg/mL, and loosely agree with the observed transport rates into the cell (Figure 5). The efficiency E of converting substrate into biomass was set to 0.5 g biomass/g substrate for both species and both substrates. This is an arbitrary choice due to lack of data.

With these parameter settings the requirements for co-existence have been met; namely equation (9) becomes 6.7 > 1.5 and 89 > 1.4, whilst equation (10) becomes 0.3 < 17.2 < 55.9. The substrate concentrations in steady state are 0.03 mg/mL for maltose and 0.002mg/mL for glucose. The biomass concentrations for 100-23 and 100-33 are 0.34 and 0.64 mg/mL respectively, indicating that under the above conditions the two species co-exist in numbers of similar magnitude. For 100-23 98% of its biomass gain is derived from maltose (with the remaining 2% from glucose), whilst for 100-33 77% of its growth is derived from glucose (with the remaining 23% from maltose).

Appendix C: Steady state solution

Without loss of generality it has been assumed that species 1 has a relative advantage on maltose. In steady state, equations (1)-(4) are equal to zero. Then

$$k = G_{1obs}^{malt} + G_{1obs}^{gluc} \tag{11}$$

$$k = G_{2obs}^{malt} + G_{2obs}^{gluc} \tag{12}$$

Substituting equations (5)-(6) into (11) and (7)-(8) into (12) gives:

$$k = \frac{\frac{G_1^{malt}}{M_1^{malt}} C^{malt} + \frac{G_1^{gluc}}{M_1^{gluc}} C^{gluc}}{1 + \frac{1}{M_1^{malt}} C^{malt} + \frac{1}{M_1^{gluc}} C^{gluc}}$$

$$k = \frac{\frac{G_2^{malt}}{M_2^{malt}} C^{malt} + \frac{G_2^{gluc}}{M_2^{gluc}} C^{gluc}}{1 + \frac{1}{M_2^{malt}} C^{malt} + \frac{1}{M_2^{gluc}} C^{gluc}}$$

Write $a = G_1^{malt}/M_1^{malt}$, $b = G_1^{gluc}/M_1^{gluc}$, $c = 1/M_1^{malt}$, $d = 1/M_1^{gluc}$, $e = G_2^{malt}/M_2^{malt}$, $f = G_2^{gluc}/M_2^{gluc}$, $g = 1/M_2^{malt}$, $h = 1/M_2^{gluc}$:

$$k = \frac{a\,C^{malt} + b\,C^{gluc}}{1 + c\,C^{malt} + d\,C^{gluc}}$$

$$k = \frac{e\,C^{malt} + f\,C^{gluc}}{1 + g\,C^{malt} + h\,C^{gluc}}$$

These can be rewritten into

$$k = (a - kc)C^{malt} + (b - kd)C^{gluc}$$

$$k = (e - kg)C^{malt} + (f - kh)C^{gluc}$$

These solve for C^{malt} and C^{gluc} as

$$C^{malt} = \frac{[\,(f - kh) - (b - kd)]\,k}{(a - kc)(f - kh) - (b - kd)(e - kg)}$$

$$C^{gluc} = \frac{[(a - kc) - (e - kg)]\,k}{(a - kc)(f - kh) - (b - kd)(e - kg)}$$

For a valid steady state solution (i.e. $C^{malt} > 0$ and $C^{gluc} > 0$) we require

$(a - kc) > (e - kg)$ and $(f - kh) > (b - kd)$, i.e.:

$$\frac{G_1^{malt} - k}{M_1^{malt}} > \frac{G_2^{malt} - k}{M_2^{malt}} \quad \text{and} \quad \frac{G_2^{gluc} - k}{M_2^{gluc}} > \frac{G_1^{gluc} - k}{M_1^{gluc}}$$

In steady state, equations (1)-(2) are also equal to zero and this gives

$$B_1\,G_{1obs}^{malt}\,/E_1^{malt} + B_2\,G_{2obs}^{malt}\,/E_2^{malt} = k(C_{in}^{malt} - C^{malt})$$

$$B_1\,G_{1obs}^{gluc}/\,E_1^{gluc} + B_2\,G_{2obs}^{gluc}\,/E_2^{gluc} = k(C_{in}^{gluc} - C^{gluc})$$

Writing $l = G_{1obs}^{malt}/\,E_1^{malt}$, $m = G_{2obs}^{malt}\,/E_2^{malt}$, $n = k(C_{in}^{malt} - C^{malt})$, $p = G_{1obs}^{gluc}\,/E_1^{gluc}$, $q = G_{2obs}^{gluc}\,/E_2^{gluc}$, and $r = k(\,C_{in}^{gluc} - C^{gluc})$:

$$l\,B_1 + m\,B_2 = n$$

$$p\,B_1 + q\,B_2 = r$$

which gives $B_1 = (nq - mr)/(lq - mp)$ and $B_2 = (lr - np)/(lq - mp)$. For $B_1 > 0$ and $B_2 > 0$ we need $(nq - mr) > 0$ and $(lq - mp) > 0$ and $(lr - np) > 0$. This can be rearranged into $p/l < r/n < q/m$, i.e.

$$\frac{G_{1obs}^{gluc}\ E_1^{malt}}{G_{1obs}^{malt}\ E_1^{gluc}} < \frac{C_{in}^{gluc} - C^{gluc}}{C_{in}^{malt} - C^{malt}} < \frac{G_{2obs}^{gluc}\ E_2^{malt}}{G_{2obs}^{malt}\ E_2^{gluc}}$$

Replacing G_{1obs}^{gluc}, G_{1obs}^{malt}, G_{2obs}^{gluc} and G_{2obs}^{malt} by equations (5) –(8), and multiplication by C^{malt}/C^{gluc} gives:

$$\frac{G_1^{gluc}/M_1^{gluc}}{G_1^{malt}/M_1^{malt}}\ \frac{E_1^{malt}}{E_1^{gluc}} < \frac{(C_{in}^{gluc} - \ C^{gluc})\ /\ C^{gluc}}{(C_{in}^{malt} - \ C^{malt})/\ C^{malt}} < \frac{G_2^{gluc}/M_2^{gluc}}{G_2^{,malt}/M_2^{malt}}\ \frac{E_2^{malt}}{E_2^{gluc}}$$