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pH feedback and phenotypic diversity within bacterial functional groups of the human gut

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HIGHLIGHTS

- Microbial diversity in the human colon is very high with apparently large redundancy (and hence diversity) within functional groups.
- Using computer simulation we propose and analyse a fluctuation dependent mechanism for the promotion of diversity.
- pH fluctuations follow from microbial growth and interact with small differences in acid tolerance between strains to promote microbial diversity.

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ABSTRACT

Microbial diversity in the human colon is very high with apparently large functional redundancy such that within each bacterial functional group there are many coexisting strains. Modelling this mathematically is problematic since strains within a functional group are often competing for the same limited number of resources and therefore competitive exclusion theory predicts a loss of diversity over time. Here we investigate, through computer simulation, a fluctuation dependent mechanism for the promotion of diversity. A variable pH environment caused by acidic by-products of bacterial growth on a fluctuating substrate coupled with small differences in acid tolerance between strains promotes diversity under both equilibrium and far-from-equilibrium conditions. Under equilibrium conditions pH fluctuations and relative nonlinearity in pH limitation among strains combine to prevent complete competitive exclusion. Under far-from-equilibrium conditions, loss of diversity through extinctions is made more difficult because pH cycling leads to fluctuations in the competitive ranking of strains, thereby helping to equalise fitness. We assume a trade-off between acid tolerance and maximum growth rate so that our microbial system consists of strains ranging from specialists to generalists. By altering the magnitude of the effect of the system on its pH environment (e.g. the buffering capacity of the colon) and the pattern of incoming resource we explore the conditions that promote diversity.

1. Introduction

The attempt to explain the mechanisms behind the abundant bio-diversity seen in many resource competition systems, e.g. Hutchinson's 'paradox of the plankton' (Hutchinson, 1961), has generated many years of rich debate. The paradox is that although basic resource competition theory predicts the competitive exclusion of competing species by the most successful species, with only the same number of species co-existing as there are distinct resources, observations show that in fact far more species than resources coexist in most ecosystems (Tilman, 1977, 1981; Rothhaupt, 1988; Sommer, 1985). Hutchinson himself believed that the paradox was best explained by the prevention of the system from reaching equilibrium as a result of changing external conditions. However, Huisman and Weissing Huisman and Weissing (1999, 2001, 2002) showed that chaotic dynamics can result purely from species competition for abiotic resources, thus internal mechanisms can also keep the system away from equilibrium, and hence from competitive exclusion.

Generally in microbial systems, a few abundant taxa dominate the community but also present is a long tail of low abundance taxa which can flourish under favourable environmental conditions (Sogin et al., 2006). The microbial system in the human gut is one of the most diverse and heavily populated in existence (Whitman et al., 1998) with up to 10^{12} bacteria for every gram of gut contents (Gibson et al., 2004) and several hundred different

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bacterial strains co-existing at any one time (Eckberg et al., 2005; Qin et al., 2010). However, functional redundancy (and hence diversity) of colonic microbiota is high (Egert et al., 2006), and indeed such diversity is thought to be critical to stability since key services will be robust to the extinction of any particular species (Yachi and Loreau, 1999).

The process of fermentation in the human gut can provide up to 10% of the energy the human body extracts from food (Macfarlane and Macfarlane, 2007) and it is also widely recognised that the colonic microbial community has a very important role for human health with connections to immunity, obesity, cancer and conditions such as inflammatory bowel disease (Danese et al., 2004; Frank et al., 2007). Fermentation occurs when the colonic microbial system grows on undigested food entering from the small intestine. This produces metabolic waste-products which are principally gases (carbon dioxide, hydrogen and methane) or short chain fatty acids such as acetate, butyrate and propionate (Macfarlane and Gibson, 1997) which can be absorbed by the host through the intestinal wall. In addition to this, some bacteria can also consume the fermentation products of others (cross-feeding).

From undigested food types (substrates) to metabolites used in cross-feeding, the system is driven by a range of resources, leading to a number of coexisting bacterial functional groups (Flint et al., 2007). However, assuming that within a functional group the strains are competing for the same resources, then the observed within-group diversity cannot be solely explained by differing resource use. Existing simulation models of colonic fermentation include (Muñoz-Tamayo et al., 2010) which can qualitatively reproduce fermentation patterns but does not address the issue of diversity and Gudelj et al. (2007) which takes an evolutionary approach where diversity is generated through mutations and biochemical trade-offs. In this work we investigate the novel idea that small differences in acid tolerance, coupled with acid production from bacterial growth on a fluctuating resource promotes diversity. We show that coexistence becomes possible under equilibrium conditions. Under far from equilibrium conditions pH fluctuations make it harder for any particular strain to outcompete another.

The growth of bacteria is affected by the acidity of their environment, with groups having a preferred range of pH which may vary slightly between strains within the group – e.g. within the Bacteroides group there are measured variations in pH response (Walker et al., 2005; Duncan et al., 2009). However, the environmental pH also changes in response to bacterial growth since the by-products of growth are often acidic, e.g. short chain fatty acids, SCFA, (Bowns et al., 1974; Macfarlane et al., 1992; Nugent et al., 2001). Environmental conditions are therefore influenced by the transit of substrates leading to temporal as well as spatial heterogeneity in pH. Within the area at the entrance to the large intestine known as the cecum there is likely to be only temporal fluctuations as it is well-mixed. However proceeding from the cecum towards the rectum there is a gradient in average pH with lower average values at the proximal end where substrate and hence SCFA concentrations are high (Cummings, 1997). Thus, we postulate that, in the presence of a time-varying incoming resource, the system is kept away from a single species fixed point equilibrium through an internal feedback mechanism to its environmental pH. To test this we simulate the growth of a single functional group which contains a large number of bacterial strains that differ slightly in their pH response. This involves the numerical solution of a system of ordinary differential equations (ODEs) describing bacterial growth, incoming resource concentration, SCFA production and environmental pH. Using this approach we consider the effect that pH feedback and patterns of incoming resource (in terms of frequency and magnitude of oscillation) have on intra-functional-group phenotypic diversity.

2. Methods

To explore the effect of environmental feedback on diversity we choose a simple theoretical situation analogous to a single-resource chemostat experiment. We consider a single bacterial group which uses one substrate and as it grows produces one metabolite. The group consists of many (N) different bacterial strains which differ slightly in their phenotypic trait regarding pH preference but, for simplicity, are identical in their other traits. If environmental pH is constant, then the strain whose preferred pH is the same as the environmental pH dominates the system to the exclusion of all others. However, we assume the metabolite produced by the strain is acidic and that the maximum growth rate of each strain is dependent on the environmental pH so that as the amount of metabolite changes so too does the environmental pH and hence bacterial growth rates. The model consists of a system of ODEs which describe the changing concentrations of a large number of bacterial strains – each strain is modelled individually but they are coupled via competition for incoming substrate and the effect of their growth by-products on the environmental pH. The details are described in the following sections.

2.1. System dynamics

We define the growth rate, f_i, of bacterial strain i with concentration b_i (g per volume) at time t, using a Michaelis-Menten function such that,

$$f_i(R_1, H_i, \mathrm{pH}, t) = G^m H_i(\mathrm{pH(t)}) \frac{R_1(t)}{k + R_1(t)}, \tag{1}$$

where k is the half saturation constant, G^m is the maximum growth rate (Table 1), R_1 is the concentration of dietary substrate (g per volume) and $H_i(\mathrm{pH})$ is a pH limitation function which is described later. The rate of change of concentration of strain i is then determined by,

$$\dot{b}_i(t) = b_i(t)[f_i(R_1, H_i, \mathrm{pH}, t) - W], \tag{2}$$

where W is the washout rate from the system (the inverse of the transit time through the volume). For simplicity we assume that this is constant. The uptake of resource R_1 by a given strain is determined by the efficiency of the bacteria, u, which is the number of grams of resource needed for 1 g of bacterial growth. For simplicity we assume u does not vary between strains, thus the rate of change of resource is given by

$$\dot{R}_1(t) = R_1^{in}(t)W - u \sum_{i=1}^{N} b_i(t) f_i(R_1, H_i, \mathrm{pH}, t) - R_1(t)W \tag{3}$$

where N is the number of bacterial strains and R_1^{in} is defined by a sinusoidal function to represent ingestion patterns, such that,

$$R_1^{in}(t) = R_1^{ref}\left[1 + F_m \sin\left(\frac{2\pi t}{F_p}\right)\right], \tag{4}$$

where $0 \le F_m \le 1$ and $F_p > 0$ represent the magnitude and the time period of fluctuations respectively, t represents time in days and R_1^{ref} is the average incoming resource concentration. Bacterial growth is not completely efficient (i.e. $u > 1$) and the substrate that is taken up but not converted to bacterial biomass is converted to metabolite, R_2, such that,

$$\dot{R}_2(t) = [u - 1] \sum_{i=1}^{N} f_i(R_1, H_i, \mathrm{pH}, t) b_i(t) - R_2(t)W. \tag{5}$$

Table 1
Parameters (top) and variables (middle) and initial conditions (bottom). The system has been simplified such that most values are defined in terms of R_1^{ref}, u and W.

Variable	Description	Default value	Units
R_1^{ref}	Average incoming substrate concentration	10	g L^{-1}
u	Bacterial usage of R_1	5	g R_1 (g b)$^{-1}$
W	Wash out rate	1	d^{-1}
γ^{ref}	Reference pH feedback factor	$-\log_{10}(0.5)(\approx 0.3)$	
F_m	Magnitude of fluctuation in incoming resource (fraction of R_1^{ref})	1	
F_p	Period of fluctuation of incoming resource	1	d
G^m	Maximum specific growth rate of all strains	10 W	d^{-1}
k	Half saturation constant	$R_1^{ref}/1000$	g L^{-1}
N	Number of bacterial strains	91	
pH_{opt}	Optimum pH value	6.25	pH units
R_2^{ref}	Maximum metabolite concentration given R_1^{ref}	$\frac{(u-1)}{u}R_1^{ref}(=8)$	g L^{-1}
T	Simulation time		d
b_i	Concentration of strain i		g L^{-1}
γ	pH feedback factor		
f_i	Specific growth rate of strain i		d^{-1}
H	pH limitation on bacterial growth		None
H_i^m	$H_i(\mathrm{pH}_{opt})$		None
H_a^m	Average value of H_i^m trait		None
R_1	Substrate concentration		g L^{-1}
R_1^{in}	Incoming substrate concentration		g L^{-1}
R_2	Metabolite concentration		g L^{-1}
t	Time		d
x	Variance of pH limitation function		pH units
$b_i(0)$	Concentration of strain i at time $t=0$	$\frac{R_1^{ref}}{Nu}$	g L^{-1}
$R_1(0)$	Concentration of substrate at time $t=0$	$\frac{k}{u}$	g L^{-1}
$R_2(0)$	Concentration of metabolite at time $t=0$	$\frac{(u-1)}{u}R_1^{ref}$	g L^{-1}

2.2. Steady state solutions

The bulk properties of the system reach a periodic steady state determined by the incoming resource. Defining b_T as the total bacterial concentration (i.e. $b_T=\sum_{i=1}^{N} b_i$ for a total of N strains) and f_T as the average growth rate over all the strains, at steady state ($t=t_s$), Eq. (2) states $b_T(t_s)[f_T(t_s)-W]=0$, which assuming $b_T(t_s)>0$, implies $f_T(t_s)=W$. Substituting this into Eq. (3) and assuming that all of the resource is consumed by the bacteria i.e. $R_1(t_s)\approx 0$, then the average total bacterial concentration over one cycle is given by

$$\overline{b_T}(t_s)\approx\frac{R_1^{ref}}{u}. \tag{6}$$

Similarly setting Eq. (5) to zero and substituting the above results gives the metabolite concentrations over one cycle as

$$\overline{R_2}(t_s)\approx R_1^{ref}\frac{u-1}{u}=R_2^{ref}, \tag{7}$$

such that R_2^{ref} represents the maximum period-averaged concentration of metabolite that can be sustained in the system given the average rate of resource supply characterised by R_1^{ref}. In our simulations we use these approximations to determine the initial conditions for bacteria and metabolite concentrations and to ensure the bulk properties of the system are in a periodic steady state when we compare different model experiments.

2.3. The effect of pH on growth rate

Bacteria are known to have preferred pH ranges (Duncan et al., 2009; Walker et al., 2005) but some can grow under unfavourable pH conditions by employing a costly 'Acid Tolerance Response' (Foster, 1991). Whether or not bacteria use this response is likely to depend on intracellular pH tolerance. Broad intracellular pH tolerance may be offset by lower maximal intracellular activity. In the case of enzymes, for example, broad tolerance is thought to be associated with lower catalytic efficiency at the optimal pH (the 3D structure giving maximal activity at a specific pH is likely to be different to that which confers wide pH tolerance).

If a bacterial strain has a broad intracellular pH tolerance then they can allow their internal pH to fall with the external pH. In our model such strains correspond to pH generalists. However in the case of a narrow intracellular pH tolerance bacteria are forced to use energy to expel hydrogen ions to preserve their intracellular environment within tight limits (the acid tolerance response). This requires increasing energy to be expended by bacteria as the pH goes further from the optimal, causing growth rates to drop off sharply. In our model such strains correspond to pH specialists. Relevant arguments on this topic, though not specific to pH, are given by Kacser and Beeby (1984).

In this study we focus on the continuum of specialist to generalists where bacteria vary in both their ability to grow at some optimal pH value (identified as the mean pH level) and in their ability to tolerate deviations from this optimal pH. A more complete model would also allow variation in the optimum pH between different bacterial strains but this is omitted from our model to allow us to concentrate on the effect of the acid tolerance response.

Based on these ideas, we assume a trade-off between the width of a strain's preferred pH range and its maximum growth rate, such that specialist strains can grow quickly over a narrow range of pH and generalists grow more slowly on a wide range of pH. We define a dimensionless pH limitation function for strain i as $H_i=H(x_i,\mathrm{pH})\in[0,1]$ where x_i varies between strains. This is then used to modify the maximum growth rate (Eq. (1)). For simplicity we consider H to be a Gaussian distribution shape where the mean corresponds to the optimal pH for bacterial growth, pH_{opt}, which we assume to be the same for each strain.

Different values for the variance, x_i, of a strain's H_i function naturally provide a trade-off between maximum growth at the

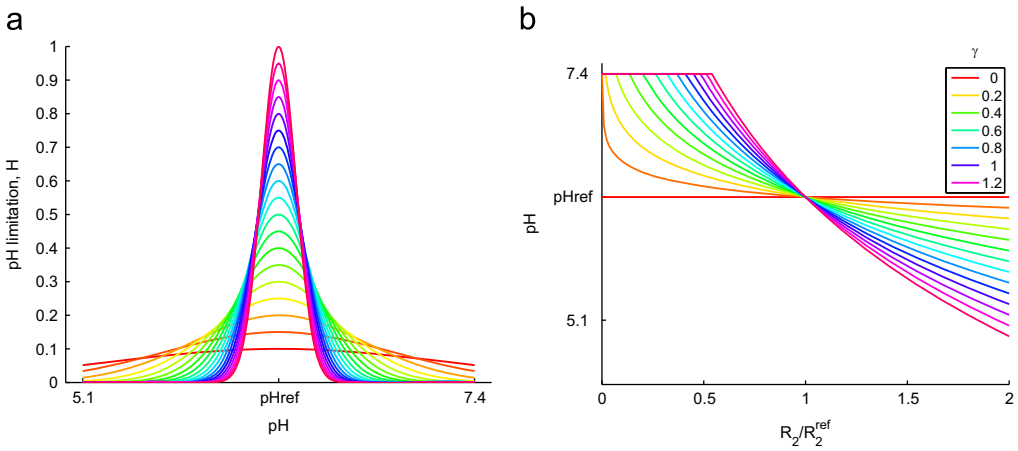

Fig. 1. (a) The pH limitation on bacterial growth, H_i, for strains ranging from generalists (red) to specialists (pink) (Eq. (8)) and (b) effect of metabolite concentration, R_2, on gut pH for different feedback factors, γ (Eq. (9)). Note we restrict the pH to values below the pH of plasma (i.e. pH 7.4). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

optimal pH and growth rates at nonoptimal values in the form of a generalist/specialist trade-off (Fig. 1a). Thus we are essentially studying the community dynamics of a group of strains with different values of a single phenotypic trait, H_i, that controls the degree of pH specialism, which is given by,

$$H_i(\mathrm{pH}) = H_i^m \exp\left(-\frac{[\mathrm{pH}-\mathrm{pH}_{opt}]^2}{2x_i}\right). \tag{8}$$

Here H_i^m is the maximum value of H_i, which occurs at pH_{opt}, and is shown by the peaks in Fig. 1a. From our definition of H_i the largest value this can take is 1 and for bacterial growth to occur, it must be greater than W/G^m (derived by combining Eqs. (2) and (1)). To preserve the Gaussian shape, achieve a trade-off between growth rate and pH tolerance, and maintain the range specified above, we define $H_i^m = (W/G)(1/\sqrt{x_i})$. Thus the values of x_i are chosen so that at the generalist extreme of the trait interval, $H_i^m = W/G^m$, representing a cost to acid tolerance but allowing growth over a wide range of pH values, and at the specialist extreme $H_i^m = 1$ so there is no cost but the range of pH values over which growth can occur is much narrower (Fig. 1a). Thus we define the pH trait of each strain to be represented by its value of H_i^m. Note this effectively means that we have reparameterised Eq. (8) in terms of H_i^m using the identity $x_i = (GH_i^m/W)^2$.

2.4. The effect of metabolite concentration on pH

Increasing concentrations of acidic metabolite will lower the pH; however the exact nature of this relationship is not known due to possible buffering by secretions from the gut wall. When the system has settled to periodic equilibrium, the average concentration of metabolite produced over one period of a fully utilised incoming substrate is R_2^{ref} (we call this reference metabolite concentration). We expect metabolite concentration to fluctuate around R_2^{ref}. Since metabolite concentration determines pH there is a corresponding reference pH, for simplicity this is pH_{opt}, around which pH will also fluctuate (Eq. (8)). As R_2 falls below R_2^{ref} pH increases above the reference pH and similarly R_2 increasing above R_2^{ref} leads to pH falling below the reference value. The extent to which pH fluctuates around the reference value is determined by the strength of the pH feedback, γ. Since pH is on a log scale we assume gut pH as a function of R_2 is given by

$$\mathrm{pH}(\gamma, R_2) = \min\left(\mathrm{pH}_{opt} - \frac{\gamma}{\gamma^{ref}} \log_{10}\left(\frac{R_2}{R_2^{ref}}\right), \mathrm{pH}_{\max}\right) \tag{9}$$

γ^{ref} is a scaling factor chosen such that when $\gamma = 1$, if the metabolite concentration is half of its reference value, then the change in pH is one pH unit (Table 1). The magnitude of γ controls the sensitivity of the environmental pH to the presence of metabolite and therefore measures the extent to which pH fluctuations are buffered by secretions from the gut wall. We refer to γ as the feedback factor and by altering its value we investigate the effect of environmental feedback on phenotypic diversity. Fig. 1b demonstrates how pH changes with R_2 for a range of γ values. Since the pH of the colon should not exceed that of plasma (Macfarlane et al., 1992) we choose $\mathrm{pH}_{\max} = 7.4$.

2.5. Measuring phenotypic diversity

In order to examine the phenotypic (trait) diversity we consider the bacterial population in terms of its trait values, H_i^m. Our simulations are initialised with N strains, each strain i has equal biomass b_i, and is assigned a trait value of H_i^m at N equally spaced intervals over $[W/G^m, 1]$. The community can then be characterised in terms of measures of the trait value given the biomass distribution e.g. Norberg et al. (2001). Thus, the average trait, H_a^m, for the population at time t is given by

$$H_a^m(t) = \frac{\sum_{i=1}^{N} H_i^m b_i(t)}{\sum_{i=1}^{N} b_i(t)}. \tag{10}$$

and the trait variance, V, is given by

$$V(t) = \frac{\sum_{i=1}^{N} [H_i^m - H_a^m(t)]^2 b_i(t)}{\sum_{i=1}^{N} b_i(t)}. \tag{11}$$

The trait variance is representative of the phenotypic diversity. Due to the sinusoidal nature of the incoming resource we take the diversity measure $\overline{V}$ to be the mean of the trait variance over the time for one full cycle of incoming resource, i.e.

$$\overline{V}(T) = \frac{1}{F_p} \int_{T-F_p}^{T} V(t)\, dt \tag{12}$$

where T is a given point in time.

2.6. Simulation experiments

The ODEs are solved numerically with the parameter values and initial conditions given in Table 1. For greater generality most of the parameters and initial variables are expressed in terms of the values of R_1^{ref}, u and W which are based on values used in

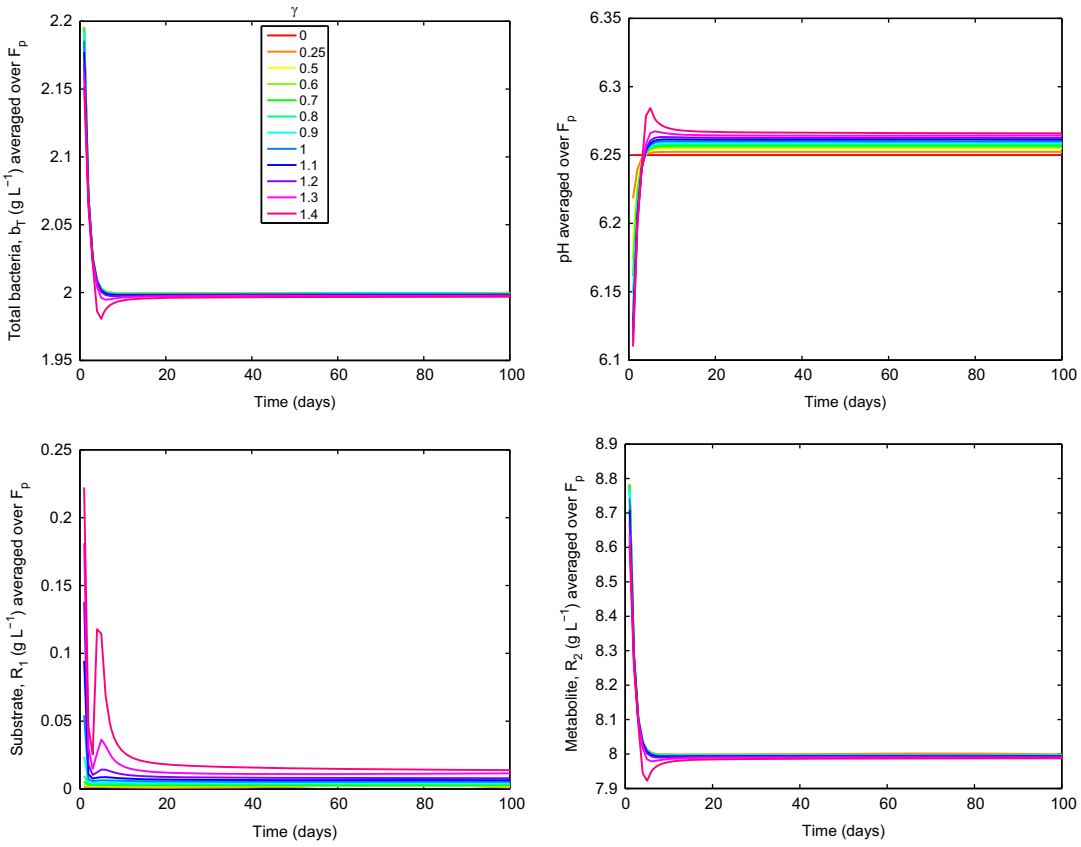

Fig. 2. Total bacteria, substrate and metabolite concentrations and pH averaged over each daily cycle ($Fp=1$) for a 100-day simulation over the range of feedback values with $F_m=1$ and $\Delta H=0.1$. Note that the steady state values of bacteria and metabolite compare well with those given by Eqs. (6) and (7).

fermentor experiments to simulate the human gut, e.g. R_1^{ref} and W from Walker et al. (2005) and u from Cummings (1997). Note that we assume that all the strains are adapted to the reference conditions so that their preferred pH, pH_{opt}, is the same as the reference pH (Eqs. (8) and (9)). Hence, growth limitation depends on the perturbation in pH away from pH_{opt} and their tolerance range, but not on the value itself.

To enable comparisons between model runs we ensure that the bulk properties of the system (total biomass concentration, pH, and substrate and metabolite concentrations) reach periodic steady state. Fig. 2 shows these properties averaged over successive time periods of length F_p. It is clear that the size of the feedback factor, γ (Eq. (9)), affects the time the system takes to reach steady state; when γ is high the system is not performing as well – evidenced by slightly lower substrate consumption. This is because when γ is high the change in pH for a given change in metabolite concentration is larger, thus the system is taken further away from equilibrium on each cycle creating a lag as the bacterial community reorganises itself before reaching optimal growth.

We run the model with trait values H_i^m between 0.1 and 1 with an interval ΔH, such that ΔH controls the number of strains we choose to comprise the total bacterial biomass. This will clearly make a difference to the amount of diversity we have since each strain has a different trait value. However, as the number of strains increases we expect that there will come a point where increasing the number of trait values does not significantly affect the results. As each strain has its own ODE, finding a balance between using the minimum number of strains and getting accurate results is very useful for minimising CPU time. Thus we have run simulations with $\Delta H=0.005$, 0.01, 0.025, 0.05 and 0.1.

Holding the parameters governing incoming substrate fluctuations at fixed values ($F_m=1$, $F_p=1$ where F_m is dimensionless and F_p has the same units as t) we conduct a set of simulations to establish the relationship between the feedback factor and diversity. In a further set of simulations we explore how changes in the fluctuations in incoming resource affect diversity for a fixed feedback factor. We run this second set of simulations for 20 days since after this time the total biomass and metabolite concentrations are very close to their steady state values (Fig. 2).

3. Results

3.1. The effect of environmental feedback on diversity

To investigate the effect of environmental feedback on trait diversity we begin by setting $F_m=1$ so that the fluctuation in incoming resource is the size of the average resource (i.e. R_1^{in} oscillates between 0 and $2R_1^{ref}$); and we set $F_p=1$ day to represent food entering during the day but not at night. We then vary the feedback factor γ in Eq. (9) to investigate how the strength of the environmental feedback affects the phenotypic diversity, $\overline{V}$ (Eq. (12)).

Interestingly, Fig. 2 shows that once the system has self-organised after the initial period, the bulk dynamics of the system do not change significantly with the size of the feedback factor despite the change in size of the effect of the perturbation in environmental pH. Given that the diversity clearly changes with feedback factor (Fig. 3a) this implies that whether the system consists of a small number of dominant strains or a large number of strains with similar concentrations, the system is performing at its optimum with the largest possible amount of biomass present given the incoming resource concentration. However, when $\gamma>1.4$ (not shown) a small change in R_2 leads to such a large

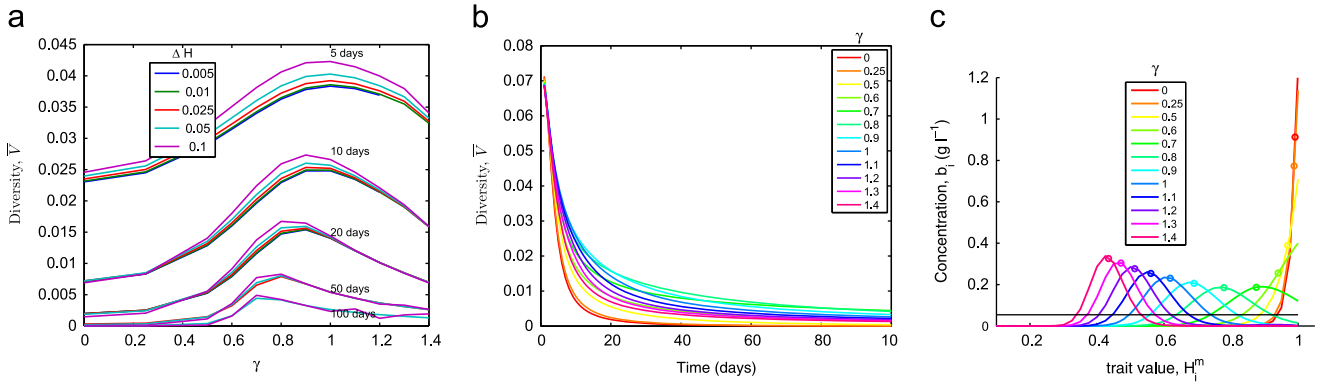

Fig. 3. (a) Effect of feedback on diversity, $\overline{V}$, at given points in time for a range of trait intervals; (b) the effect of feedback on the time taken for diversity to decrease for $\Delta H = 0.05$; (c) the trait distributions after 50 days for $\Delta H = 0.025$, for a different range of γ values. The dashed black line shows the initial trait distribution at $t=0$.

change in pH that the system can not reorganise fast enough through strain succession, and the system crashes. This is because once bacterial growth becomes severely pH-limited, metabolite production decreases which then causes the pH to increase further and bacterial growth is yet further inhibited (positive feedback). It is also useful to note here that if there was no phenotypic diversity and the bacterial functional group was represented by a single strain i.e. with only one pH limitation function (e.g. only one of the curves in Fig. 1a), then the system would not perform at its optimum as it would not have the ability to adapt to the changes in the environment through strain succession.

Fig. 3a shows how diversity varies with γ at given times in the simulation for a range of ΔH and indicates that the number of trait values (within the range from 10 ($\Delta H = 0.1$) to 181 ($\Delta H = 0.005$)) does not alter the qualitative behaviour of the model (which is advantageous in terms of CPU time). Fig. 3b shows that the diversity decreases with time but the rate of this decrease is determined by the size of the feedback factor. When there is little or no feedback ($\gamma \leq 0.25$) then the diversity decreases rapidly, such that by 50 days there is only one strain, ($\overline{V} = 0$, and competitive exclusion has occurred. However when $\gamma = 0.7$ there is more than one strain surviving after as many as 100 days and the decrease in diversity is very slow. Fig. 3c shows the trait distributions after 50 days for different values of γ (for $\Delta H = 0.025$). When there is no feedback, there is no change in pH and so the most specialist strain dominates to the exclusion of all others (red line). As γ increases from zero the system's optimum trait value moves away from the specialist end of the distribution ($H^m = 1$) and towards the generalist end ($H^m = 0.1$) and the number of strains with comparable biomass (and hence the phenotypic diversity) is increased (Fig. 3c). However, as γ increases beyond 0.7 the diversity begins to decrease (Fig. 3c) as generalist strains become more successful at the expense of the specialists. In the absence of system collapse, we believe that as γ increases the trait distribution would gradually move towards the generalist end in the same way it moves towards the specialist end as $\gamma \to 0$.

For an optimal value of the feedback factor, $\gamma = 0.7$, we perform an invasibility test to demonstrate coexistence in our system under equilibrium conditions. A low concentration of a generalist strain grows when introduced into a community consisting of a specialist strain at its periodic attractor (Fig. 5a). When instead the specialist strain is introduced at low concentration to a community consisting of only the generalist strain at its periodic attractor (Fig. 5b), it too grows and hence the system facilitates coexistence between generalists and specialists.

Within our system there is an optimum value of γ for phenotypic diversity where the environmental pH responds to changes in metabolite concentration but is not over sensitive to it. Around this value of γ both generalist and specialist strains co-exist after 50 days. Away from this value the system is either unstable or consists of just specialist or generalist strains and therefore is considered to be less diverse as measured by the phenotypic variance. Due to our choice of γ_{ref}, a value of $\gamma = 0.7$ indicates that the optimum situation for diversity in our system is when the environmental pH changes by 0.7 pH unit in response to a halving of the metabolite concentration from an equilibrium value. For ethical and practical reasons the human intestine is difficult to study and hence it is difficult to relate this optimal intermediate level of pH feedback to the real system. However it is known that the epithelial layer of the intestine plays an important role in absorbing metabolites and hence may influence the observed level of γ.

3.2. The impact of resource supply R_1^{in}

Fig. 3 indicates that by the time periodic steady state of bulk properties is attained phenotypic diversity is largest for a feedback factor of $\gamma \approx 0.7$. However, the system is fundamentally driven by the incoming resource, R_1^{in}. If this was constant in time then bacterial concentrations, metabolite concentrations and pH would reach constant steady state. The system would be dominated by a specialist strain and there would be no long term diversity regardless of the effects of feedback. Thus we now assess the sensitivity of trait diversity to changes in the magnitude and period of the oscillation (parameters F_m and F_p) of R_1^{in} (Eq. (4)) for $\gamma = 0.7$. To find a balance between CPU time and accuracy we choose $\Delta H = 0.025$ (37 strains) and a simulation period of 20 days. Fig. 4 shows the results from running the model at F_m between 0.1 and 1, and for fluctuation time periods of $F_p = 0.5, 0.75, \ldots,$ 2.5 days.

Fig. 4a shows there is a region around the curve $F_p \approx 1.25/F_m$ (dash-dot line on plot) where diversity is optimised. In other words, diversity is maintained when the fluctuation period is long and the magnitude in the fluctuation is small, or if the fluctuation period is short and the fluctuations are large. The mean trait value (Fig. 4b) decreases (i.e. moves from specialist to generalist) at an increasing rate with F_p and F_m until the system crashes ($\overline{b_T} = 0$ in Fig. 4c) as described above.

In our sinusoidal type of fluctuations the same amount of food is eaten in the long run regardless of period or magnitude. However our results suggest that ingestion patterns and the rate at which food leaves the stomach may have an impact on microbial diversity. Diversity can be promoted whether the period is long or short relative to a period of one day. If it is long then the magnitude of the fluctuations need to be low. Otherwise there will be long intervals where food for the bacteria is scarce and strains will be washed out.

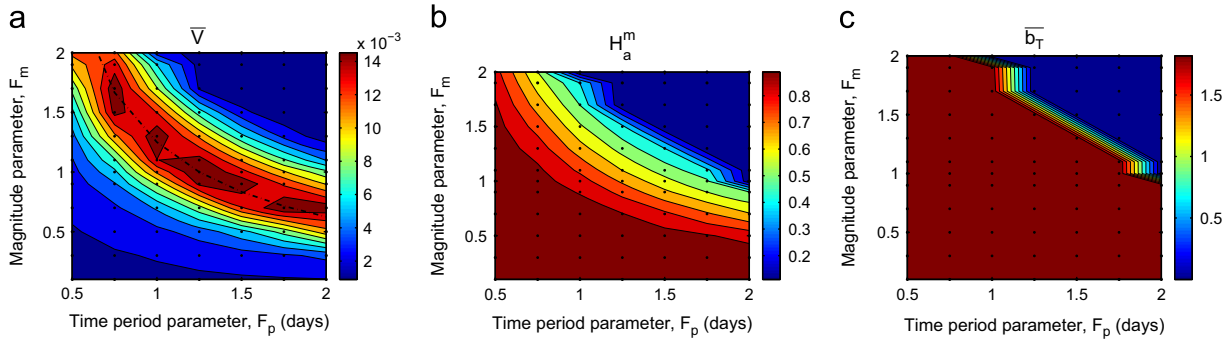

Fig. 4. The effect of the magnitude and time period of the fluctuation in incoming resource (parameters F_p and F_m in Eq. (4)) on (a) the diversity, $\overline{V}$ (Eq. (12)) where the dash-dot line shows the relation $F_m = 1.25/F_p$; (b) the mean trait value, H_a^m (Eq. (10)) and (c) the total biomass, at the end of the 20-day simulation for a fixed feedback factor $\gamma = 0.7$ and $\Delta H = 0.025$ (37 strains). Black dots indicate the points in the parameter space at which the simulations were run.

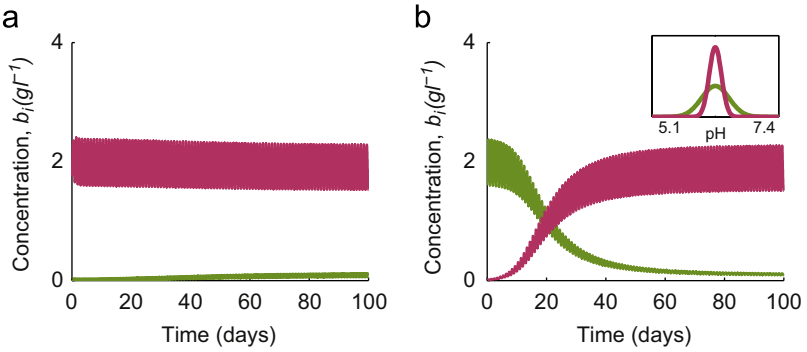

Fig. 5. An invasibility test of equilibrium coexistence. In (a) a community composed of a single specialist strain (grey curve) at its periodic attractor is invaded by a single generalist strain (black curve) at low concentration. In (b) a community composed of only the generalist strain at its periodic attractor is invaded by the specialist strain at low concentration. The inset graph displays the specialist (grey curve) and generalist (black curve) strategies as a function of pH. The specialist and generalist strategies are selected from those displayed in Fig. 1a. Taken together (a) and (b) demonstrate that coexistence of generalist and specialist strategies can occur in our system since the invader increases in concentration in both cases. In (a) and (b) $\gamma = 0.7$, $F_m = 1$ and $F_p = 1.25$ so that substrate fluctuations and feedback factor match the dash-dot line from Fig. 4a.

If the period is short then the magnitude needs to be high. Otherwise the conditions will not change enough for a range of phenotypic values to be favoured.

4. Conclusion

By modelling the dynamics of a large number of bacterial strains within a single functional group we have demonstrated that a pH feedback on bacterial growth (from the release of acidic by-products), in the presence of a time-varying incoming resource, is a potential mechanism for promoting phenotypic diversity in the large intestine. A key phenotypic trait which distinguishes strains is their acid tolerance and here we have theoretically explored the competition dynamics of a spectrum of strains from generalists (which can tolerate a wide range of pH) to specialists (which have a narrow pH range but higher growth rates than the generalists) in environments which fluctuate to differing degrees. On a generic level we have shown that in a resource competition situation where there is a trade off between growth range and growth rate, it is possible to promote diversity through an environmental feedback which keeps the system from reaching a constant fixed state.

The system is driven by a single incoming substrate, if this was constant in time then bacteria and metabolite concentrations and hence pH would reach constant (not periodic) steady state. This would rapidly lead to competitive exclusion as the strain with superior competitive ability would dominate to the exclusion of all others. On the other hand, if the fluctuations in incoming substrate are too large or too slow the bacteria die through lack of substrate. We have shown that there exists a range within which diversity is maximised, when the system is far from equilibrium, where the magnitude of the resource fluctuations is inversely related to the time period of the oscillation. Thus, these results may suggest one of probably many ways in which dietary patterns can influence bacterial diversity. Moreover, the potential relation between the intestinal epithelium, the tissue layer lining the gut wall, and pH feedback may suggest a manner in which damage to the epithelial layer can negatively impact bacterial diversity (for example epithelial damage and microbial communities significantly perturbed in composition relative to normal microbiotas are associated with inflammatory bowel disease (Frank et al., 2007).

Stable community theory has shown that two competitors can coexist on one fluctuating resource (Armstrong and McGehee, 1980; Chesson, 1994) with the equilibrium state corresponding to a periodic attractor. Relative nonlinearity in their resource uptake operates as a stabilising mechanism (Chesson, 2000). Empirical research in intestinal bacteria, however, has highlighted differences in growth limitation due to pH between strains within functional groups (Walker et al., 2005; Duncan et al., 2009). Focusing therefore on environmental feedback resulting in fluctuating pH and relative nonlinearity in bacterial response to pH, we have demonstrated coexistence between generalist and specialist strategies at equilibrium (Fig. 5a,b). In the absence of fluctuations only the strain that can persist at the lowest resource level will survive and therefore this mechanism of stable coexistence is fluctuation dependent. Since resource uptake terms are the same across the strains of our functional group resource fluctuations

alone would not support coexistence and therefore this mechanism is dependent on fluctuations in pH (and hence pH feedback).

We have modelled only one idealised bacterial functional group. However, the intestinal system consists of many functional groups with cross feeding between groups. It is therefore feasible that the overall system (with multiple functional groups) spends much of its time away from equilibrium. In our system fluctuating pH and its feedback to bacterial growth results in fluctuations in the competitive ranking of strains. This equalising mechanism (Chesson, 2000) slows down competitive displacement and the result is increased maintenance of otherwise unstable diversity (Fig. 3b). Thus the fluctuating resource and environmental feedback in this model promotes diversity under both equilibrium (i.e. a periodic attractor) and far from equilibrium conditions.

There are other candidates for mechanisms that facilitate co-existence of bacterial strains in the large intestine such as resource partitioning, patterns of resource use and bacteriophage selection (Shapiro et al., 2010). Here we have suggested a new mechanism by which diversity in the colon can be promoted. As a consequence of this diversity the metabolic pathway remains fully utilised (Fig. 2) despite substantial daily fluctuations in growth limiting pH. This is a demonstration of the insurance hypothesis (McNaughton, 1977; Yachi and Loreau, 1999) which states that diversity helps to stabilise communities. Recent developments in models of microbial fermentation in the colon which do not account for diversity may therefore under-represent community stability. Incorporating a feedback between microbial metabolism and growth through environmental pH may be key to capturing pH dynamics as well as functional diversity. This work highlights the importance of intra-functional diversity, and recent advances in trait-based approximations, e.g. Norberg et al. (2001), allowing functional groups to be represented by just a few distributional moments, mean that this can now be incorporated in full models for little computational expense.

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