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Quantitative Effects of Medium Hardness and Nutrient Availability on the Swarming Motility of *Serratia liquefaciens*

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We report the first controlled measurements of expansion rates for swarming colonies of *Serratia liquefaciens* under different growth conditions, combined with qualitative observations of the organization of the colony into regions of differentiated cell types. Significantly, the results reveal that swarming colonies of *S. liquefaciens* can have an increasing expansion rate with time. We compare and contrast the expansion rate results with predictions from a recent mathematical model which coupled key hydrodynamical and biological mechanisms. Furthermore, we investigate whether the swarming colonies grow according to a power law or exponentially (for large times), as suggested by recent theoretical results.

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1. INTRODUCTION

In natural and generally hostile environments, bacteria are often found to live in communities attached to surfaces (biofilms). The supply of nutrients is an

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important factor in determining the success of a given colony and so the underlying mechanisms that allow bacterial communities to translocate and expand across surfaces, and thus have access to a larger supply of nutrients, are of fundamental importance. Moreover, bacterial self-organization is generally recognized as playing a major role in the expansion of the colony and, hence, its survival [e.g., see Shapiro (1995)].

Several well studied bacteria are known to exhibit swarming motility on surfaces of varying hardness and nutrient availability, such as members of the genera *Bacillus*, *Chromobacterium*, *Clostridium*, *Escherichia*, *Proteus*, *Salmonella* and *Vibrio* [e.g., Eberl *et al*. (1996b, 1999), and references therein]. The process of swarming in thin fluid films incorporates both the rapid colonization of surfaces as well as the differentiation of bacterial cells into elongated, multi-nucleated, aseptate, hyperflagellated, energetically moving swarmer cells Eberl *et al*. (1999). The swarmer cells are 5–50 μm compared with 1.5–3.0 μm for the standard swimming cells Eberl *et al*. (1996b). These swarm cells have the ability to move on top of the agar surface, in contrast to the breeder cells, and get their name by analogy to the phenomenon of swarming bees. Not only is it thought that the swarmer cells have a role in the rapid expansion of the colony, but they may also be important for other reasons, such as increased resistance to predation (Ammendola *et al*., 1998). Swarming colony expansion has been observed to be either continuous and sustained (such as for colonies of *S. liquefaciens*; see below) or can occur in periodic bursts [such as for *Proteus mirabilis*; Rauprich *et al*. (1996), Esipov and Shapiro (1998) and Czirok *et al*. (2001)].

In this paper, we shall present quantitative results on the size of colonies of *S. liquefaciens* as a function of time, nutrient concentration and medium hardness, and shall directly compare and contrast these results with simulations from the Bees *et al*. (2000) model of bacterial swarming. Furthermore, we shall investigate whether scaling laws could better capture the expansion rate of the colony, as suggested by recent theory (Bees, 2002). First, we shall briefly describe some other forms of bacterial translocation in order to place swarming motility in context [also, see Henrichsen (1972), for a description of the six major bacterial surface translocation methods, including swarming].

Since the observation of highly complex (fractal) bacterial patterns growing slowly on the surface of agar plates (Matsuyama *et al*., 1989; Fujikawa and Matsushita, 1989), there has been renewed theoretical and biological interest in the mechanisms and processes for bacterial colony growth. In particular, much effort has been devoted to understanding the qualitative mechanisms which lead bacteria to create different colony morphologies. Colonies either inhabit the water-filled channels within the agar matrix in very soft media or may sit upon the surface of hard media (especially on the surface of dried agar plates). Colonies that occupy the space within the agar matrix are known to produce regular patterns of dots and strips, the dynamics of which are mostly linked to chemotactic and diffusive mechanisms (Budrene and Berg, 1995; Woodward *et al*., 1995). On very hard

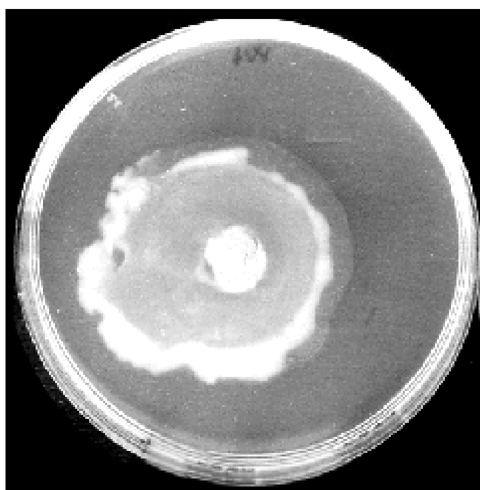

Figure 1. A swarming colony of *S. liquefaciens* approx. 600 min after inoculation. The agar concentration is 0.6% (w/v) and the casamino acid concentration is 0.2% (v/v). The shading is due to the light source reflecting off of the surface of the mostly transparent culture.

media, the colonies can form fractal structures, some of which are reminiscent of diffusion-limited aggregation (DLA) processes. For such systems, most models are phenomenological and are focused on generating the patterns particular to different species of bacteria (Ben-Jacob *et al*., 1995; Li *et al*., 1995). Many models are discrete and based on cellular automata or 'communicating walkers' (Ben-Jacob *et al*., 1994; Ben-Jacob, 1997). Although it may be argued that these models are oversimplified, their ability to reflect the geometrical and temporal features of the bacterial patterns has provided good insight into the interaction of the biological and physical processes responsible for the development of complex morphologies. Recently, continuous models of bacterial expansion, in this context, have been explored by several authors. In particular, non-linear diffusive processes have been employed in models to help explain observations of bacterial motion within a thin film of fluid. In this case, the non-linear diffusion phenomenologically models the effect of a self-generated lubrication fluid (Kitsunezaki, 1997; Golding *et al*., 1998; Matsushita *et al*., 1998; Kozlovsky *et al*., 1999; Lacasta *et al*., 1999).

For intermediate medium hardnesses, swarming motility can occur and is characterized by a very rapid colonization of surfaces. An important feature of the swarming colony (see Fig. 1) is its ability to extract fluid from the underlying medium and to use this to help the colony spread on the dried surface of the agar plate, for which the production of an extracellular biosurfactant becomes very useful (Lindum *et al*., 1998; Rasmussen *et al*., 2002).

S. liquefaciens is an opportunistic pathogen which colonizes a wide variety of surfaces in soils, plants and humans. *S. liquefaciens* is, in general, motile in liquid media by means of peritrichous flagella and the standard swimming form is almost

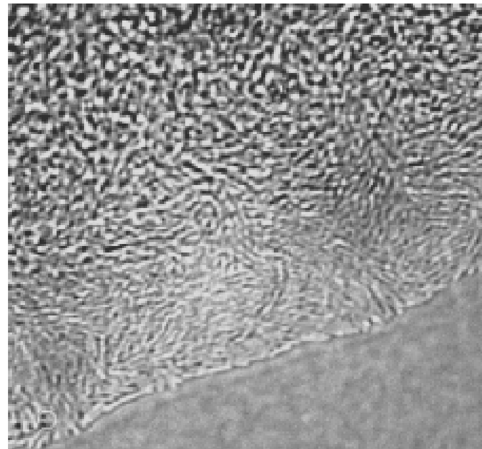

Figure 2. Close-up of the edge of a swarming colony. A monolayer of long, fast moving swarmer cells is visible at the edge of the colony.

non-motile on hard surfaces. In order for *S. liquefaciens* to colonize hard surfaces it differentiates into so-called swarmer cells that appear at the edge of the colony (Fig. 2). The cells have a much higher motility potential on solid surfaces than ordinary, growing and swimming bacteria, hereafter denoted breeder cells. The differentiation into the swarmer cell state is coupled to the expression of certain virulence factors (Givskov *et al.*, 1995). As coordination is a necessity for the colony to further grow, rapidly expand and thereby acquire new sources of nutrients it is an example of a self-organized process. Moreover, the swarmer cells themselves show a high degree of co-operativity that appears to be necessary in order for the motion of the swarmer cells to be useful (Eberl *et al.*, 1996b; Rasmussen *et al.*, 2000; see below).

To obtain a more comprehensive understanding of the factors by which *S. liquefaciens* is stimulated to differentiate into the swarm cell state as well as the overall behaviour of the colony itself, Bees *et al.* (2000) constructed a mathematical model of the growth mode of the expanding colony, implementing elements of thin-film flow as well as relevant biological information. In order for the model to be as comprehensive as possible, several experiments were performed to obtain crucial parameter values as well as to study the basic characteristics and behaviour of the bacterial colony. This paper reports in detail on some of these experiments. Furthermore, the measurements of colony expansion are contrasted with the predicted solutions from the model. A recent theoretical refinement of the modelling approach allows for distinct regions within the swarming colony (Bees, 2002) which is more in line with observations of colony structure (Eberl *et al.*, 1999). Systematically matched asymptotics of the hydrodynamic equations allow for a significantly simplified system of equations, the full details of which will be reported elsewhere (Bees, 2002). However, the form of the theoretical predictions together with the experimental results reported herein suggest that we should inves-

tigate whether the bacterial colonies do indeed expand exponentially for large time (see below) or whether there is a more suitable power law.

Section 2 describes in greater detail features of the biological model. In Section 3 we describe the experimental methods and in Section 4 we report the results of the experiments. The results are compared with results from the Bees *et al*. (2000) model in Section 5. We go on to discuss simple rate laws for the system and compare these with the experimental results.

2. Biological System

At least two control systems are involved in the initiation of cell differentiation and in the formation of a swarming culture in *S. liquefaciens* (Givskov *et al*., 1997, 1998). The major stimulus for the differentiation process, i.e., the transformation from short unicellular breeder cells into elongated, multi-nucleate, hyperflagellated swarm cells, is the sensing of surface contact. This is channelled through and controlled by the master operon encoded by the *flhDC* operon (Eberl *et al*., 1996a), which controls both the expression of flagella genes and is also involved in the regulation of cell division. Thus when *S. liquefaciens* senses a surface the stimulus is channelled through the *flhDC* operon and the bacteria become increasingly flagellated and multi-nucleate, due to the suppression of cell division. The mechanisms by which *S. liquefaciens* senses a surface as well as how the signal is transmitted to the genes of the flagellar regulon are unknown (Tolker-Nielsen *et al*., 2000). In contrast to *P. mirabilis*, surface-induced swarm cell differentiation is neither accompanied by a substantial increase in *flhDC* transcription nor *flhDC* mRNA content. It is speculated, but not proven that swarm cell differentiation occurs as a result of an increased half-life of the FlhDC protein complex (Tolker-Nielsen *et al*., 2000). As to the mechanism, *Vibrio parahemolyticus* has been demonstrated to measure the viscosity of its environment with its polar flagellum, which functions as a tactile sensor measuring external forces (McCarter *et al*., 1988). On very soft media the effective viscosity is not high enough to qualify as a surface and the differentiation process is not initiated. Furthermore, since the media for such low agar concentrations consist of many water-filled channels the bacteria may swim through these, thereby expanding the colony and removing the growth limiting factor of nutrient supply. On soft to hard media the bacteria do sense the surface, and the differentiation process is initiated at the edge of the colony (Givskov *et al*., 1997).

The high level of motility (operation of the many flagella) displayed by the swarm cells is considered to be metabolically demanding and, therefore, requires a high nutrient level in the surrounding medium. Insufficient nutrient leads to consolidation of the swarmer cells back into breeder cells (Givskov *et al*., 1997). Thus it may be speculated that if the concentration of supplied casamino acids becomes limited there are simply not enough building blocks and energy to synthesize and operate

the hundreds of flagella produced during differentiation (Eberl *et al*., 1999). This suggests the presence of a lower bound on the required nutrient availability for cell differentiation and, furthermore, explains why the differentiation process is always initiated and restricted to the perimeter of the colony (Eberl *et al*., 1996b).

The other major requirement for the rapid expansion of a swarming colony is the presence of a conditioning film that changes the wettability by reducing the surface tension of the culture medium. The major stimulus for the production of this conditioning film is the cell density of the colony. *S. liquefaciens* MG1 employs cell-to-cell signalling via a quorum sensing mechanism. The creation of the surfactant is regulated by the presence of two extracellular signal molecules *N*-butanoyl-L-homoserine lactone (BHL) and *N*-hexanoyl-L-homoserine lactone (HHL) [which are present in a molar ratio of approximately 10 : 1; Eberl *et al.* (1996b)]. The conditioning consists of the secretion of the extracellular lipopeptide serrawettin W2, controlled by the quorum sensing target gene *swrA* (Lindum *et al*., 1998), which causes the reduction of the surface tension. The surfactant thus allows the colony to expand under the collapse of the associated thin fluid droplet and the wetting of the dried agar surface. The production of the surfactant was initially thought to be controlled both by breeder as well as swarmer cells. However, our recent, unpublished data (Rasmussen *et al*., 2002) suggests that the breeders may play the dominant role in secreting the serrawettin. The above hypothesis is supported by the fact that a mixture of non-differentiating *flhDC* mutants (capable of producing surfactant but non-motile) and differentiating *swrA* mutants (unable to produce the surfactant) of *S. liquefaciens* can give rise to a swarming colony (Eberl *et al*., 1999). In this mixed expanding colony, the only strain that can produce the surfactant is the non-differentiated *flhDC* strain which is locked in the breeder state. These individuals have recently been found to express *srwA* mainly in the swarmer band (Rasmussen *et al*., 2002). The reason for this is unclear, but is consistent with observations of non-differentiated cells being transported within the swarmer regions (Eberl *et al*., 1999). The mechanism for extraction of fluid from the underlying medium is unknown, but is thought to be a combination of chemical and biomechanical factors (such as the energetic and coordinated motion of the swarmer cells). Such a system may be similar to that of *P. mirabilis* for which wetting agent extraction is facilitated by an extracellular acidic capsular polysaccharide (Gygi *et al*., 1995; Rauprich *et al*., 1996). In contrast to *S. liquefaciens* however, colonies of *P. mirabilis* do not produce detectable quantities of surfactant [see Rauprich *et al*. (1996)].

Within a colony on very hard media, the bacteria at the perimeter differentiate into swarm cells due to the sensing of a high effective viscosity associated with a surface. Nevertheless, the colony does not necessarily form a swarming culture if all forms of the bacteria are unable to extract fluid from the underlying medium. For a swarming culture on media of intermediate hardness, the bacteria in the region behind the swarm region may experience a reduction in medium hardness due to the excessive production of wetting agent, thereby suppressing the

process of differentiation into swarm cells. The cells in this region can also experience a depleted nutrient supply and thus swarm cells in this region consolidate into breeder cells (Givskov *et al*., 1997). A flow diagram of the known biological interactions (as discussed above) is given later in Fig. 6.

3. EXPERIMENTS—MATERIALS AND METHODS

A wild-type strain of *S. liquefaciens* MG1 (Givskov *et al*., 1988) was used for all experiments.

The bacteria were grown in circular Petri dishes (diameter 10 cm) on minimal AB medium (Clark and Maaløe, 1995) with added 0.1% glucose. The medium was further supplemented with a mixture of amino acids (casamino acids), since the differentiation into swarmer cells in *S. liquefaciens* is not promoted by any single amino acid (Eberl *et al*., 1996a,b). After preparation, the dishes were left to dry for exactly one hour in order for any excess surface fluid to evaporate. (Qualitative experiments were performed to determine the effect of the dryness of the plates. It was found that if the plates were allowed to dry for one to three hours the motility of the colony itself wasn't affected, although the time for the colony to initiate swarming appeared slightly delayed for the dryer plates—data not presented.) The hardness of the growth medium was controlled by varying the agar concentration (Difco agar), with a range from 0.1% (soft) to 1.5% (hard).

To study the effects of nutrient supply upon the differentiation process the concentration of supplemented casamino acids was varied. Under normal growth conditions a 0.2% mixture of casamino acids was added to the growth medium, but measurements of expansion rates with concentrations between 0.05% and 0.4% in combination with different agar concentrations were also performed.

The plates were inoculated with a small amount of *S. liquefaciens* MG1 at the centre of the dish (marked on the top cover), with minimal disruption to the agar surface, and thereafter placed in an incubator where they were left to grow at a constant controlled temperature of 30 °C. At regular time intervals the plates were taken out of the incubator and the radius of colony was measured along fixed, but arbitrarily chosen, equiangular directions (i.e., every 120°). These measurements were continued for up to 11 h. A typical colony is displayed in Fig. 1. One may observe that the colony is roughly axisymmetric. Three measurements of radius were taken in order to capture information both on the mean radius and deviation from the mean.

In parallel experiments, cultures were grown under identical growth conditions (i.e., agar and casamino acid concentrations) as above. One culture was used to measure the expansion of the colony whereas the others were removed only at appropriate times to make *in situ* observations with a microscope, in order to monitor the organization of the colony. The above procedure was adopted since pro-

Table 1. Example of experimental measurements l_i, $i = 1, 2, 3$, with mean $\langle l \rangle$ and standard deviation, that form the basis for the expansion values depicted in Fig. 7. Parameter values are 0.5% agar concentration and 0.2% casamino acid concentration.

Time (min)	l_1 (mm)	l_2 (mm)	l_3 (mm)	$\langle l \rangle$ (mm)
0	2.0	2.5	2.0	2.17 ± 0.29
130	2.0	3.0	2.0	2.33 ± 0.58
200	5.0	4.0	4.0	4.33 ± 0.58
250	5.5	5.0	5.0	5.17 ± 0.29
295	6.5	6.5	7.0	6.67 ± 0.29
335	8.0	8.0	7.0	7.67 ± 0.58
370	9.0	8.5	8.5	8.67 ± 0.29
415	11.0	10.0	10.5	10.50 ± 0.50
450	11.5	11.0	12.0	11.50 ± 0.50
495	14.0	13.0	14.0	13.67 ± 0.58
535	17.5	15.0	15.5	16.00 ± 1.32
580	20.0	19.0	19.0	19.33 ± 0.58

Table 2. Experimental measurements l_i, $i = 1, 2, 3$, with mean $\langle l \rangle$ and standard deviation, as in Table 1. Parameter values are 0.5% agar concentration and 0.4% casamino acid concentration.

Time (min)	l_1 (mm)	l_2 (mm)	l_3 (mm)	$\langle l \rangle$ (mm)
0	1.5	1.5	1.5	1.5 ± 0.0
90	1.5	1.5	1.5	1.5 ± 0.0
135	2.0	2.0	2.0	2.0 ± 0.0
220	4.0	5.0	3.0	4.0 ± 1.0
265	5.0	5.0	4.0	4.67 ± 0.58
310	5.0	6.0	4.0	5.0 ± 1.0
385	8.5	8.5	7.0	8.0 ± 0.87
445	10.0	10.5	10.0	10.17 ± 0.29
505	13.0	14.0	14.0	13.67 ± 0.58

longed observation with a microscope at room temperature can inadvertently affect the swarming motility of the cultures.

4. Results

The measurements of the expansion rates for swarming colonies with different agar concentrations are presented in Tables 1 and 2, and Figs 3 and 4. First note that the colonies exhibited a characteristic time lag of approximately 120 min before noticeable growth was observed. Secondly, the expansion rates depended upon the agar concentration, with a maximum expansion rate observed at 0.6% (average radial expansion rate of approx. 2 mm h^{-1}). As described in the previous section the measurements of the colony radius were made in three directions and

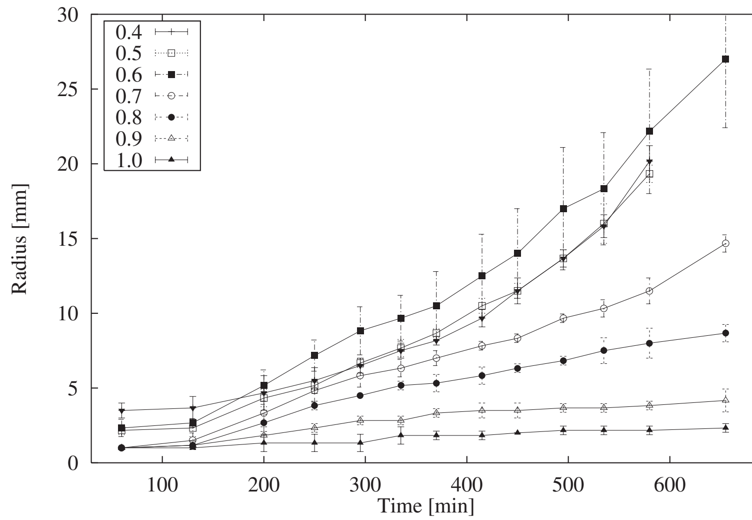

Figure 3. Measured colony radii for swarming bacterial colonies of *S. liquefaciens* with different agar concentrations (see key) and 0.2% casamino acids. The fastest expansion was measured for 0.6% Difco agar. Note the time of approx. 120 min before the colonies start to expand. The error bars indicate the statistically determined standard deviation of the radius measurements.

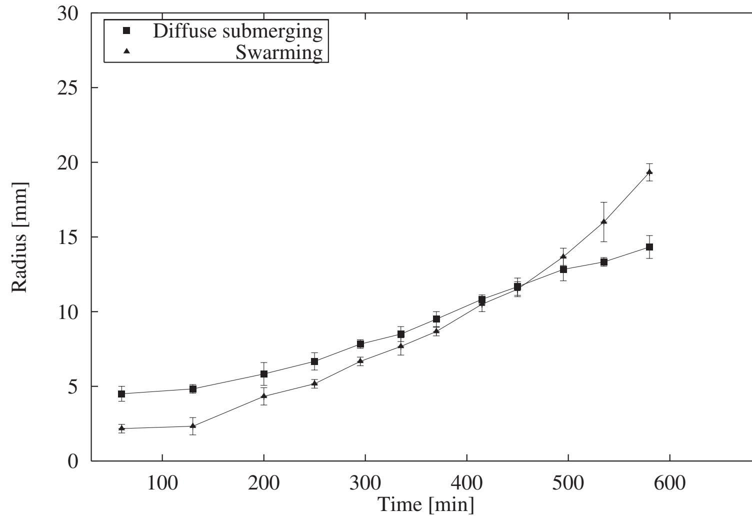

Figure 4. Measured colony radii for a colony exhibiting both swarming (▲) and diffuse submerging (■) motility with 0.5% Difco agar and 0.2% casamino acids. Initially the expansion rate is greatest for colonies employing diffuse submerging within the medium itself. After approximately 500 min the swarming colony on the surface of the medium overtakes the swimming bacteria. The error bars indicate the statistically determined standard deviation of the radius measurements.

the plotted measurements are averages over these three directions. However, a characteristic of some of the swarming colonies was that the growth around the perimeter of the colony was not entirely homogeneous. Rather, the colony some-

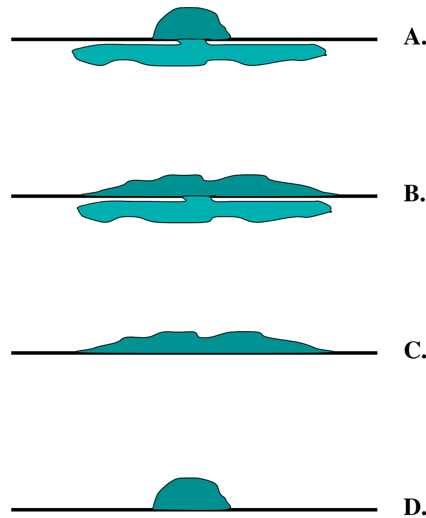

Figure 5. Different morphologies of the colony under different growth conditions quantified by the agar concentration (ac). (a) Diffuse submerging with well-packed non-swarmer colony on surface; very soft surface, ac $< 0.4\%$. (b) Diffuse submerging with swarmer colony on surface; soft surface, $0.4\% <$ ac$<0.6\%$. (c) Swarmer colony on surface only; intermediate surface hardness, $0.6\% <$ ac $< 1.2\%$. (d) Well-packed non-swarmer colony on surface; hard surface, $1.2\% <$ ac.

times grew with slightly different rates at different locations along the front. This may indicate that there is some sort of front instability or, perhaps more likely, that the medium or swarmer distribution are inhomogeneous in the band around the perimeter of the colony. For instance, a locally high concentration of swarmer cells may lead to a reduction in the available nutrient and also the extraction of more fluid, possibly resulting in a locally greater expansion rate, but limited by an advanced consolidation rate back into breeder cells. This scenario clearly requires further investigation. Mostly, however, the colonies have a roughly circular appearance and the standard deviation of radius measurements at a given time was of the order of millimetres (see Tables 1 and 2, and Fig. 3).

In addition to these quantitative measurements certain qualitative features of the bacterial colony and its organization were observed through regular inspections of identical plates under a high-resolution microscope.

On soft media, i.e., low agar concentrations below 0.4%, a small well-packed colony formed on the surface of the medium at the inoculation point, while other bacteria spread within the medium itself creating classic chemotactically induced rings [diffuse submerging; Matsuyama and Matsushita (1996)]. This type of motility is a consequence of the low agar concentration, as the structure of soft medium is characterized by many water-filled channels. The breeder cells are motile in liquid media and can swim through these water-filled channels, thereby expanding the colony [see Fig. 5(a)].

For slightly higher agar concentrations (0.4–0.6%) the bacteria initially followed the same developmental scenario as for the softer media. Observations with a microscope revealed that, contrary to the above case, the cells at the perimeter of the surface colony had undergone a differentiation process whereby the short unicellular flagellated breeder cells were transformed into elongated, multi-nucleate, hyper-flagellated swarm cells [in agreement with Eberl *et al*. (1999)]. After approximately 120 min the colony on the top of the surface suddenly and rapidly began to expand, and soon overtook the bacteria swimming within the media (see Figs 4 and 5(b)). Note that it is not just the rates of expansion of the colonies that differ but also the second derivatives of the radii with time. In particular, the expansion rate of the swarming colony increases.

For intermediate agar concentrations (0.6–1.2%) no diffuse submerging occurred and only rapid swarming motility was observed. A characteristic of this process was an initial delay with no apparent spreading followed by a rapid outward movement of the swarm cells at the perimeter of the colony, also accompanied by standard bacterial growth within the colony [Fig. 5(c)]. This type of behaviour led to an extremely fast colonization of all available space and radial expansion rates of up to 8 mm h^{-1} were measured. Furthermore, the expanding colonies showed three distinct regions of organization. The outermost 1–2 mm along the perimeter of the colony was dominated by vigorously moving swarm cells moving in rafts of 3–5 cells and a lesser number of breeder cells which seemed to be caught in the flow created by the swirling motion of the swarm cells [see Eberl *et al*. (1999) and Rasmussen *et al*. (2002)]. No isolated motile swarm cells were observed. Of note along the outer edge of this region was an extremely thin layer of fluid (typically 0.5 mm wide and less than 1 μm deep), devoid of any bacteria, immediately ahead of the expanding bacterial front. Encompassed by the band of swarmer cells, an inner region consisting primarily of breeder cells was observed. The activity in this region was not as high as in the outer region and the cells appear to be swimming independently of one another (in contrast to the organized rafts in which the swarm cells move) in the characteristic manner of periods of short runs interrupted by tumbles. The activity decreased with the distance to the centre of the colony. Finally, at the centre of the colony bacteria were observed with no apparent activity.

For high agar concentrations above 1.2%, i.e., very hard surfaces, the colony remained in the form of a small well-packed colony and no swimming or swarming motility was observed [Fig. 5(d)]. However, inspection with a microscope revealed that at the outer edge of the colony, differentiation into elongated, multi-nucleate swarm cells had occurred in a limited fashion and that these cells, nevertheless, were almost completely non-motile. Apart from the agar concentration, the onset of differentiation was also observed to depend on the concentration of nutrients in the form of casamino acids [as in Eberl *et al*. (1996a,b)]. In particular, for casamino acid concentrations below 0.1% no swarm cell differentiation was observed. However, the expansion rates of the colonies were not hugely dependent on the level of nutrient available as long as the casamino acid concentration was sufficiently

greater than 0.1%. Tables 1 and 2 tabulate the radial colony expansion measurements for casamino acid concentrations of 0.2 and 0.4%. It is clear that the results only differ by a small amount. This difference may be best quantified by comparing the form of the expansion curves (see below).

5. Comparison Between Theory and Experiments

Bees *et al*. (2000) constructed a mathematical model to describe the phenomenon of swarming, based on coupling biological processes with key hydrodynamic mechanisms. In particular, the model is centred on the assumption that the colony expands due to the wetting of the dried agar surface by fluid, which the bacteria extract from the underlying media. The fluid is a complete wetting fluid due to the presence of a biosurfactant. In the thin-film approximation of the Navier–Stokes equations, the governing equations for the fluid height, $h(x, y, t)$, is derived from mass-balance considerations and proper consideration of the boundary conditions, and reads

$$h_t = -\nabla \cdot (U(x, y, t)h) + h\Gamma(x, y, t). \tag{1}$$

$\Gamma(x, y, t)$ describes the extraction of fluid by the bacteria, depending on the concentration of swarmer and breeder cells, and is activated by the quorum sensing mechanism previously described. Furthermore, $U(x, y, t)$ is the vertically averaged horizontal fluid velocity

$$U(x) = \frac{\gamma}{3\mu(x)} h^2 \nabla \nabla^2 h - \nabla \cdot \Pi(h) - \frac{\rho g}{3\mu(x)} h^2 \nabla h, \tag{2}$$

where γ is the surface tension, $\mu(x, y, t)$ is the dynamic viscosity (dependent on the bacterial concentrations), ρ is the fluid density, g is the acceleration due to gravity and Π is a disjoining pressure (see below). The first term in the right-hand side (RHS) of equation (2) describes the effect of surface tension (the dominant term for the macroscopic case) and the third term on the RHS represents the effect of gravity (which can be ignored for thin enough films, such as the films described here).

The biological interactions (i.e., growth, nutrient depletion, cell-to-cell signalling, differentiation, consolidation, viscosity sensing, etc.; see Fig. 6) are included through reaction–diffusion equations, describing the rate of change of concentration within a small fluid column. Hence,

$$(hR)_t = hI_R - \nabla \cdot (hUR - hD_R \nabla R), \tag{3}$$

where $R(x, y, t)$ is the reactant concentration (breeder cells, swarmer cells, signal molecules), I_R is the reaction term for the reactant, R, and D_R is the associated diffusivity. Furthermore, a simple reaction–diffusion equation describes the nutrient concentration within the agar medium.

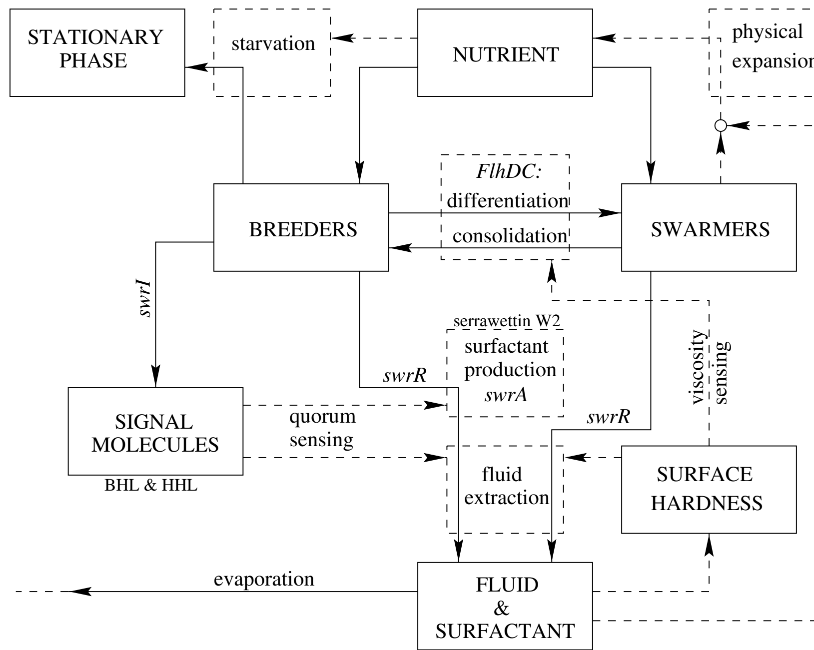

Figure 6. A flow diagram which illustrates the biological interactions involved in the formation of a swarming culture. Solid lines indicate transfer of biomass and dotted lines indicate modes of control. Note the positive feedback loop (↻) between the swarmers and the nutrient supply, this is the feedback loop which drives the expansion of the colony.

The disjoining pressure, Π, employed in Bees *et al.* (2000) was based on the van der Waals interaction free energy with a cut-off below the molecular scale, although the form of the term (and whether such a term should be included) is a controversial issue. There are two main reasons for including such terms. Firstly, they may be physically inspired and, secondly, they relieve a major mathematical difficulty associated with weak solutions and no-slip boundary conditions. This difficulty is closely linked to the stress singularity that results in the Navier–Stokes equations when the contact line is forced to move over a no-slip surface (King, 2001; Shikhmurzaev, 1997). There are several popular ways in which this problem can be circumvented; the above equations can be regularized in several ways [for example see King (2001), and references therein]. Some examples are the explicit inclusion of slip near the contact line, the use of non-Newtonian fluids (such as shear-thinning fluids), evaporation and the inclusion of long-range van der Waals interactions. There is insufficient space here to describe the behaviour and vagaries of such systems, and we instead refer the reader to the references stated above. Moreover, there are many physical attributes of the agar medium which are unknown and thus require investigation before the correct form of the regularization can be formulated. Intriguingly however, correctly matched asymptotic solutions of the macroscale and microscale dynamics (Shikhmurzaev, 1997; King, 2001) reveal universal laws, to first order, between macroscopic (apparent) contact

angles and the speed of the contact line for complete wetting fluids, independent of the form of regularization (except possibly for a constant of proportionality which may depend logarithmically on the regularization). These universal scaling laws may prove very useful in simplifying the hydrodynamical elements of the swarm colony model. With these issues in mind, we first qualitatively compare the experimental results with the Bees *et al*. (2000) model of bacterial swarming and move on to consider appropriate scaling laws for the bacterial colony, subject also to a biologically prescribed fluid source term.

Numerical simulations of these equations with experimentally determined parameters have shown good qualitative agreement with previously observed dynamics of the swarming colony (Bees *et al*., 2000), with one notable exception. The simulations captured elements of the self-organized structure of the colony through the processes of differentiation and consolidation in response to viscosity, nutrient and quorum signals as well as the time scale associated with this reorganization. Furthermore, significant changes in the theoretical expansion rates were observed at the early stages of the swarming process when the hardness of the medium was varied (via the fluid source term) which are in agreement with the experiments. Crucially however, the simulations did not generically reveal accelerating (and maybe even exponential) growth as was generally observed in the experiments (Figs 3 and 4).

In order to assess the appropriate expansion law for colonies of swarming bacteria we first investigate whether the radius expands according to the following expression:

$$r(t) = C\,t^{n}, \tag{4}$$

where $r(t)$ is the average radius of the colony measured at time t, C is a constant and n is the expansion exponent. If the expansion is governed by diffusive effects, one would expect that $n = 0.5$, whereas a constant radial expansion, where $n = 1.0$, would be evident for simple travelling wave solutions. The wetting of a solid surface by a conserved quantity of fluid typically exhibits a long-time expansion rate of $n = 1/10$ for two-dimensional systems and $n = 1/7$ for one-dimensional systems [application of Tanner's laws, de Gennes (1985), Brenner and Bertozzi (1993), Shikhmurzaev (1997) and King (2001)], while if gravitational forces dominate, the exponent is $1/8$ [also for porous media, Aronson (1986)]. The manner in which to analyse the experimental data with respect to such an expression is to produce a log–log plot of the mean radius of the colony vs time (see Fig. 7), whence the gradient of a straight line fit (using least squares) reveals the expansion rate. The generally linear behaviour of these plots for intermediate times after the initiation of swarming indicates that the radial expansion rate could obey a scaling law of the above form. This is particularly true for media with higher concentrations of agar, such as for the experiment with 1.0% agar concentration, which appear to obey the scaling law also for large times. However, one may clearly observe that data for the rapidly swarming colonies (such as on 0.5% agar)

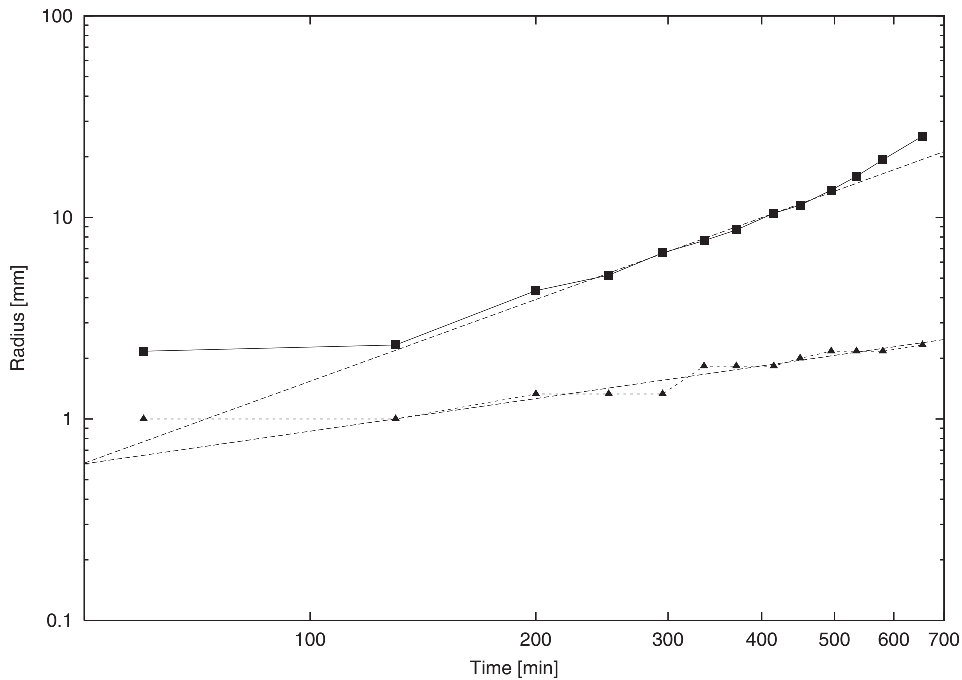

Figure 7. Plot of two fits of the expansion measurements for swarming colonies on intermediate [0.5% agar concentration (■)] and hard medium [1.0% agar concentration (▲)]. The log–log plot shows linear behaviour for intermediate times. The dashed lines are determined by a least squares fit (excluding the first 120 min; the data points after 500 min were also excluded for the 0.5% agar data set). Table 3 lists the complete set of calculated expansion exponents. The casamino acid concentration is 0.2%.

Table 3. Computed radial expansion exponents for colonies grown on media of differing hardness and nutrient availability. The two last measurements (ds) are for the case of diffuse submerging (all data points excluding the first one were used) while the rest are for swarming colonies. Swarming colonies on 0.4–0.6% agar media generally gave poor fits and so a limited range of intermediate data was used from 120 to 500 min.

Agar concentration (%)	Expansion exponent	
	0.2% casamino	0.4% casamino
0.4	1.21 ± 0.11	1.39 ± 0.24
0.5	1.35 ± 0.04	1.31 ± 0.19
0.6	1.29 ± 0.07	1.27 ± 0.07
0.7	1.21 ± 0.06	0.96 ± 0.10
0.8	0.95 ± 0.05	0.65 ± 0.09
0.9	0.64 ± 0.06	0.46 ± 0.04
1.0	0.54 ± 0.04	—
0.4 (ds)	0.40 ± 0.08	0.46 ± 0.06
0.5 (ds)	0.91 ± 0.04	—

are not well-fitted for large times. In particular, the gradient of the expansion curve on the log–log plot increases with time (Fig. 7; see above). In Table 3 we list the

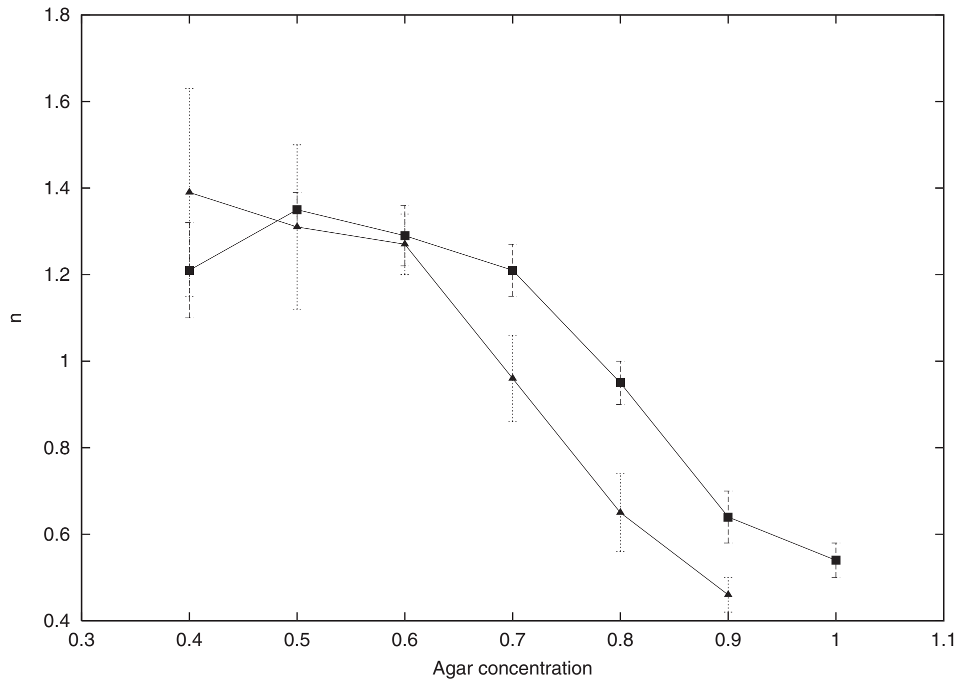

Figure 8. Plot of the expansion exponents n for swarming colonies under different conditions of nutritional supply (0.2% casamino acids, ■; 0.4% casamino acids, ▲) as determined from experiments. Note the large expansion exponents for low agar concentrations and the subsequent decrease as the medium gets harder.

experimentally determined expansion exponents (see also Fig. 8), for the intermediate times. Note how the intermediate expansion exponents have maximum values for low agar concentrations, for which $1 < n < 1.5$. For media harder than 0.5% the expansion exponent is monotonically decreasing. We also note the significantly lower expansion exponents for the diffuse submerging cases. For soft media, the bacteria are thought to swim through the water-filled channels of the medium. This process is likely to be governed by swimming diffusion due to the run and tumble motion of the swimming bacteria and their exponential growth near to the leading edge. This description is in good agreement with two of the three measurements of the expansion exponent when the colony is diffuse submerging (for the softest agar). The higher value of $n = 0.91$ for a swimming colony within medium of agar concentration 0.5 and 0.2% casamino acid may perhaps be explained by the restricted swimming space available to the bacteria due to the structure of the agar matrix, or may be a result of non-linear interactions between bacteria. Further investigation is necessary.

For swarming bacterial colonies, non-linear diffusion induces time-dependent expansion rates due to the biological interactions (through Γ). Employing the model for a swarming colony, we would expect that in the limit of no fluid extrac-

tion, i.e., for very hard media or no biological activity, the expansion exponents should reduce to those obtained for a droplet spreading on a surface. However, the bacterial colony is likely to be densely packed on these occasions and so the colony would not behave as a fluid and the model would break down. Let us return to the governing equations with the aim of assessing the expansion rates of swarming colonies in their simplest form. In order to dramatically reduce the complexity of equation (1), we shall assume that all of the bacteria extract fluid at a constant rate, $\Gamma(x, y, t) = \Gamma_0$, and the viscosity, μ, is also a constant. Furthermore, let us assume that surface tension dominates the macroscopic dynamics and postpone issues of regularization until a later stage. Equations (1) and (2) thus become

$$h_t = -\nabla \cdot (h^3 \nabla \nabla^2 h) + h\Gamma_0, \tag{5}$$

where we have scaled h to remove the constant, $\gamma/3\mu$. Next, consider the following change of variables:

$$h(x, y, t) = e^{\Gamma_0 t}\tilde{h}(x, y, t), \qquad T(t) = \frac{1}{3\Gamma_0}[e^{\Gamma_0 t} - 1], \tag{6}$$

to obtain

$$\tilde{h}_t = -\nabla \cdot (\tilde{h}^3 \nabla \nabla^2 \tilde{h}). \tag{7}$$

Thus we can use the analysis discussed above (with an appropriate regularization of the governing equations) to state that, in two dimensions and for large times, the radius of the colony, r, obeys

$$r \propto T^{1/10}. \tag{8}$$

Therefore,

$$r \propto \left(\frac{1}{3\Gamma_0}\right)^{\frac{1}{10}} [e^{3\Gamma_0 t} - 1]^{1/10}. \tag{9}$$

If $\Gamma_0 t$ is large, then

$$r \propto (\frac{1}{3\Gamma_0})^{\frac{1}{10}} e^{\frac{3}{10}\Gamma_0 t}, \tag{10}$$

indicating the potential for exponential growth of the colony radius for large times.

The generalization of this approach to cultures with more than one region with time-dependent and biologically determined source and viscosity terms will be presented elsewhere (Bees, 2002).

Hence, for large $\Gamma_0 t$ a plot of $\ln r$ vs t is more appropriate. The gradient could thus determine $3\Gamma_0/10$. In Fig. 9 we re-graph the data for an agar concentration of 0.5% and casamino acid concentration of 0.2% and fit a linear function to the last few data points (see Table 4). The graphs become linear toward these data points with greater values of Γ_0 for the very softest agar (as we'd expect, as the bacteria find it easiest to extract fluid when the medium is soft). The computed values of Γ_0 are presented in Fig. 10.

These values for Γ_0 are of the same order as the previously estimated values used in numerical simulations [Bees *et al.* (2000), see their Fig. 13] and pro-

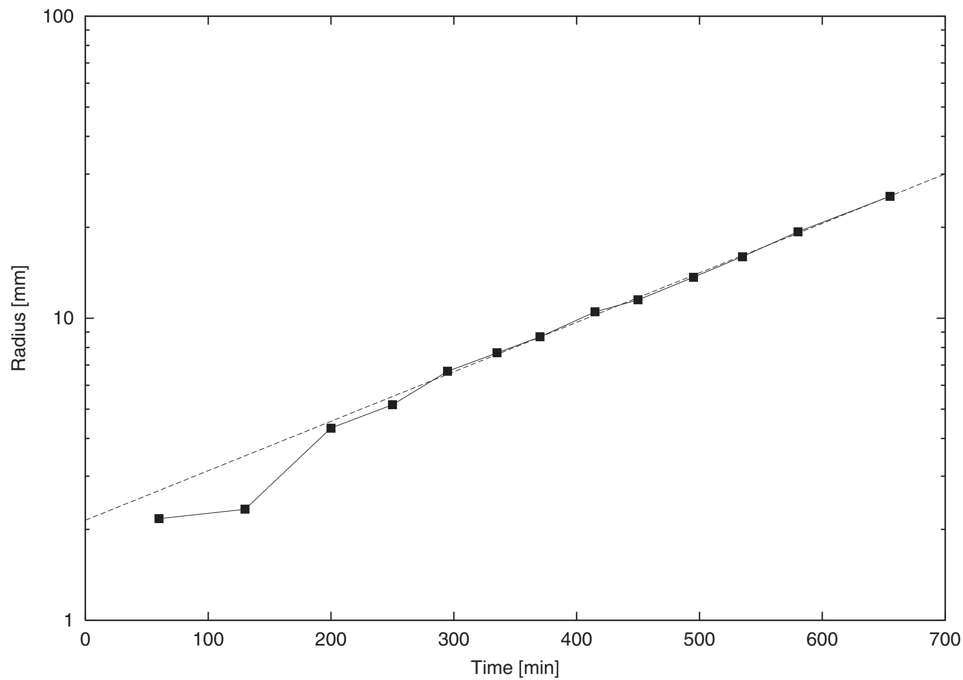

Figure 9. Plot of the expansion rates and linear fit for swarming colonies on intermediate [0.5% agar concentration (■)]. The log–linear plot shows linear behaviour for long times. The dashed line is determined by a least squares fit (last four points). Table 4 lists the complete set of calculated expansion exponents. The casamino acid concentration is 0.2%.

Table 4. Computed values of Γ for colonies grown on media of differing hardness and nutrient availability.

Agar concentration (%)	Γ (min^{-1})	
	0.2% casamino	0.4% casamino
0.4	0.0129 ± 0.0004	0.0124 ± 0.0010
0.5	0.0127 ± 0.0003	0.0125 ± 0.0008
0.6	0.0111 ± 0.0004	0.0117 ± 0.0003
0.7	0.0085 ± 0.0003	0.0121 ± 0.0005
0.8	0.0058 ± 0.0003	0.0075 ± 0.0010
0.9	0.0024 ± 0.0002	0.0048 ± 0.0005
1.0	0.0029 ± 0.0005	0.0035 ± 0.0007

duce similar expansion rates. As a typical example, a value of $\Gamma = 0.08$ min^{-1} was used in the simulations to obtain an expansion of approximately 12.5 mm in 10 h, where we note that the swarmer-to-breeder ratio is roughly 1 : 20 (from their Fig. 11; for which the swarmers alone extract fluid). According to our experiments, an agar concentration of 0.7% and a casamino acid concentration of 0.2% results in an expansion of 12.5 mm in 10 h (see Fig. 3). Table 4 indicates that

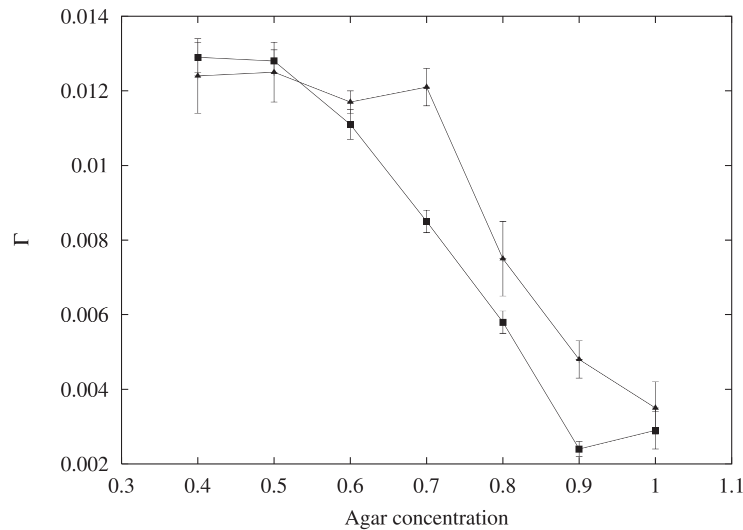

Figure 10. Plot of the values of Γ for swarming colonies under different conditions of nutritional supply (0.2% casamino acids, ■; 0.4% casamino acids, ▲) as determined from experiments.

$\Gamma_0 = 0.0085\ \text{min}^{-1}$ for concentrations of 0.7% agar and 0.2% casamino acid. Allowing for the fact that only 5% of the cells are producing fluid indicates that Γ might be approximated by $20 \times 0.0085 = 0.17$. Thus we have a difference of a factor of two, which represents fairly good agreement. Clearly, better independent measurements of the other parameters in the model are required for a more precise comparison. Furthermore, the comparison may also be aided by a better model of the expansion law dynamics (including more biology, etc.). However, it is also possible that Bees *et al*. (2000) may be misrepresenting the biology (evident by the lack of exponentially expanding colonies in the simulations).

The above theoretically derived exponential expansion behaviour results from the presence of the non-linear diffusion term interacting directly with the biologically prescribed source term. This scenario is not generally associated with the simple diffusion-dominated colony ($r \sim t^{1/2}$; e.g., the diffuse submerging case). The fact that the simulations do not reproduce the exponential increase in radius is thought to be due to the continuous nature of the breeder/swarmer distribution and the resulting dynamic balance between differentiating and consolidating individuals, leading to an inaccurate source term. The results of the simulations indicate that there should be a similar biomass of breeders and swarmers within the swarmer zone at the edge of the colony. This is not observed in experiments under microscopic examination of the colony. Typically, non-overlapping regions of swarmers and breeders are observed, with only a small number of non-differentiated cells being rapidly transported within the swarming zone [Eberl *et al*. (1999) and Rasmussen *et al*. (2002), producing the biosurfactant serrawettin W2]. Perhaps a more

appropriate model would include this fact *ab initio*, and so better reproduce the observed expansion rates.

6. Conclusion

We have performed experiments to analyse the expansion behaviour of swarming colonies of *S. liquefaciens*. The size of the colonies was measured and expansion exponents were calculated, under different medium hardness and nutrient availability conditions. The manner in which the colony develops was compared with a partial differential equation model of the process. This comparison revealed general agreement, but highlighted an inconsistency; the experiments exhibited exponential colony expansion, whereas the model did not. The experiments were also contrasted with a recent theoretical description of expansion rates due to the wetting of a dried agar surface by a bacterial colony with a biologically prescribed source term. This description indicates that exponential growth of the colony radius can occur, in accordance with the experiments. We argue that an improved model with discrete zones of breeder and swarmer cells would be more in keeping with microscopically observed characteristics in experiments.

In essence, we have demonstrated how non-linear diffusion terms in the governing equations can allow the biological interactions to control the expansion behaviour of the colony. We have shown that this description is consistent with the experiments. Furthermore, it also allows us to directly calculate parameters from the data, such as the rate at which the bacteria extract fluid from the underlying media. Quite surprisingly, both theory and experiments indicate that swarming colonies can expand exponentially with time. Such easily measurable characteristics could be used for purposes of bacterial identification.

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