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On the 'Listeria' propulsion mechanism

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Abstract. We give a short overview of three different aspects of the work done in our laboratory on the propulsion mechanism of the bacterium named 'listeria'. They concern (1) the mechanical properties of the comet of the native bacterium, (2) the polymerization/crosslinking process of actin gels in spherical geometry, and (3) a theoretical analysis of the mesoscopic aspects of the propulsion.

Keywords. Bacterial propulsion; polymerization of actin gels.

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1. Introduction

A large amount of work is currently being done in order to understand cell motility [1]. This is a considerable task which involves on the same footing biochemistry, cell biology and soft condensed matter physics. For these reasons biologists have considered with great interest a somewhat simpler problem: the propulsion mechanism of a bacterium named 'listeria' [2, 3], which retains only a fraction of the processes involved in cell motility, namely the polymerization process of an actin gel [4]. This allows us to bypass questions related to cell adhesion, cell signaling, internal transport etc. . . , all of which are important in the general case. Experiments are also facilitated from the fact that they can be carried out in vitro [5–7]: placed in a suitable cell extract the bacterium develops a tail which provides the propulsion (figure 1) [8]. Enough is known of the biochemistry to consider that polymerization occurs at the bacterium surface, without interference from any internal process [9, 10]. Thus this propulsion example is as simple as it can be. One can hope to be able to dig out its main characteristics. In addition, it is not a unique case: other bacteria such as 'shigella' [11], a dangerous one killing several million people a year, or even viruses such as vaccin viruses, use the same propulsion trick [12]. Furthermore, recently it has been shown that a cell under osmotic stress creates many vesicles which move, propelled by actin tails very similar to the ones used by 'listeria' [13]. Hence we are dealing with a generic problem.

The bacterium is about one to two microns long and not more than a micron wide. Its shape is that of a cylinder of revolution capped with two half spheres. It is a Gram positive bacterium which means it has a solid peptidoglycan layer at its outer surface but no external lipid membrane [14]. The tail which can be shown to be an actin gel, can be typically tens

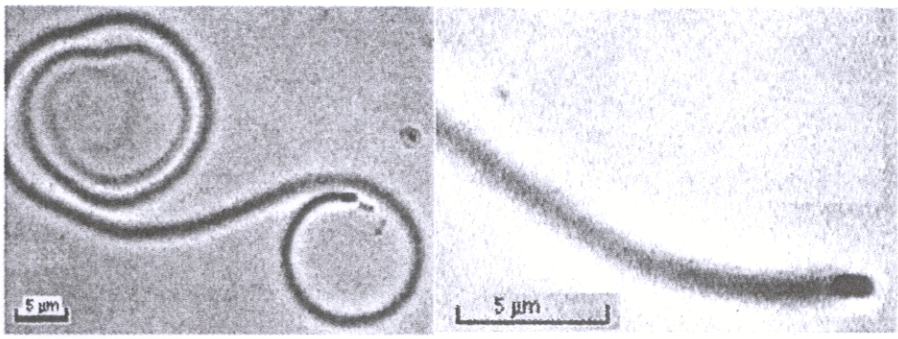

Figure 1. Listeria monocytogenes seen by phase contrast microscopy in human blood platelets cytoplasm extract. The dark ellipsoid is the bacterium itself, and the shaded tail is a crosslinked actin gel. The bacterium moves at about 8 µm. min^{-1}. The tail may measure more than 100 µm in length.

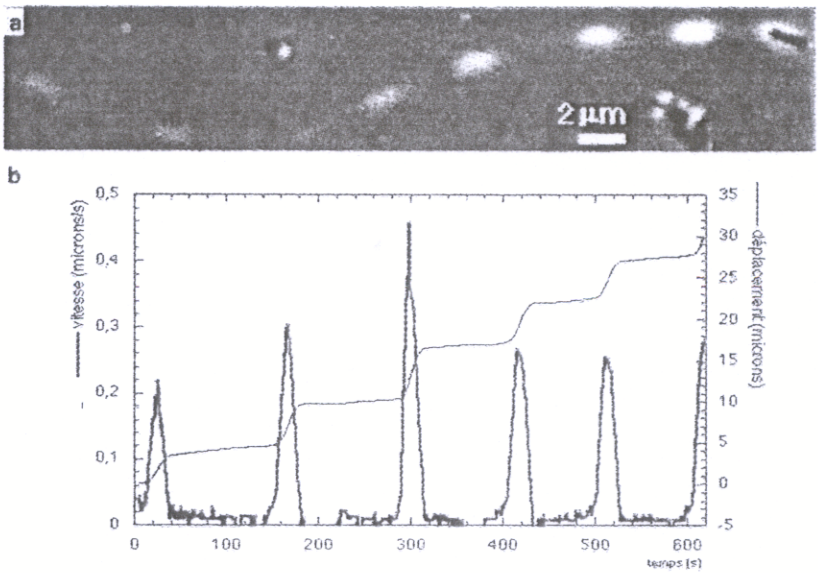

Figure 2. (a) Motion of the $ActA_{\Delta 21-97}$ listeria mutant in the cytoplasm infected cell seen by fluorescence and phase contrast microscopy (Courtesy Lasa *et al* EMBO, 1997). The mutant creates a 'dashed tail' as shown by the fluorescent actin marked with rhodamin. (b) Kinematic study of the motion of the same bacterium. The plot shows the speed and the displacement of the mutant bacterium. Data were obtained from image analysis of the video using NIH image. The average speed of the mutant is about half of the wild type in the same medium, but its peak speed can be up to four times greater.

of microns long and one or two microns wide (i.e. of the same order of magnitude as the bacterium's width). One can see in figure 1 that the contrast of the tail gradually fades out as the distance from the bacterium increases: this shows that actin depolymerises progressively after having been polymerised at the bacterium surface: this process fixes an average length to the comet in steady state. We will not discuss this point any further here. Actin filaments have a persistence length of the order of tens of microns [15, 16] so that we are dealing with a semirigid gel. In vivo, the bacterium uses actin monomers, other proteins

and the energy of the cell it is infecting. It can push its way through the cells membranes, first mechanically then with the help of a phospholipid digesting enzyme [17]. Hence it can infect many cells, duplicating itself every twenty minutes. Infections are dangerous for immune depressed people. The use of cell extracts such as Xenopus eggs extracts [5] or platelets extracts [7] allow to observe a moving bacterium directly under the microscope with DIC or fluorescence. Indeed all the necessary ingredients are present in these types of extracts. Their minimum number is not known, so that one cannot use simpler solutions. What is known is that a protein named ActA plays a primordial role in the polymerization catalysis [18, 19]. Its absence or the deletion of its N-terminal part stops the polymerization altogether [20]. A well chosen mutation [21] profoundly changes the motion: the wild type bacterium motion is steady whereas that corresponding to the mutant is saltatory (figure 2). Can physics give us a clue about what is going on? How can a surface polymerization/reticulation process transmit momentum to the body over which it is taking place? Can we give general guidelines on how to formulate the problem? We address these questions in three steps. First we show experimentally that the physical connection between the comet and the bacterium plus the existence of a well defined gel modulus, imply the build up of stresses which are ultimately responsible for the propulsion of the bacterium. Second by studying the effect of topology on the polymerization dynamics we give experimental evidence for and evaluation of the stresses. Eventually we show how a modelisation can take reasonable account of the observed phenotypes.

2. Comet mechanical properties and connection to the bacterium

The first question which comes to one's mind concerns the connection of the comet with the bacterium. If enzymes promote the polymerization of actin filaments, are these filaments bound to the enzymes permanently or on the contrary only at some very rare occasions? The first possibility is quite unlikely since the bonds involved in the process are non-covalent. The second possibility really means a time short compared to a typical waiting time, between the addition of a new monomer. This is a priori possible, but less generic than a situation in which a mechanical connection would be maintained on average during a number n of monomer additions, and for which it would take a number m of time cycles for a connection to be made. Two experiments allow us to set lower bounds on the listeria/comet friction coefficient.

In a first series of experiments, we used an optical trap to move the bacterium and try to detach it from its comet (figure 3a). The calibration of the trap on bacteria devoid of comet teaches us that the force exerted on the bacterium is of the order of ten piconewton. We can maintain this force for a few seconds. Knowing that we would detect a displacement of the order of a micron, we can deduce a friction coefficient ξ_b larger than 10^{-5} Pa.m.s. To give a comparison, the hydrodynamic friction coefficient of a micron size bead in water is of the order of 10^{-8} Pa.m.s. and in an extract 10^{-7} Pa.m.s, that is hundred times smaller.

In a second series of experiments we used an electric field to exert a force on the listeria (figure 3b). The achievable force is a little smaller (ie: a few piconewton) but the time over which it can be maintained is much longer (5 min) than in the previous experiments. Again we could not detect any motion of the bacterium with respect to

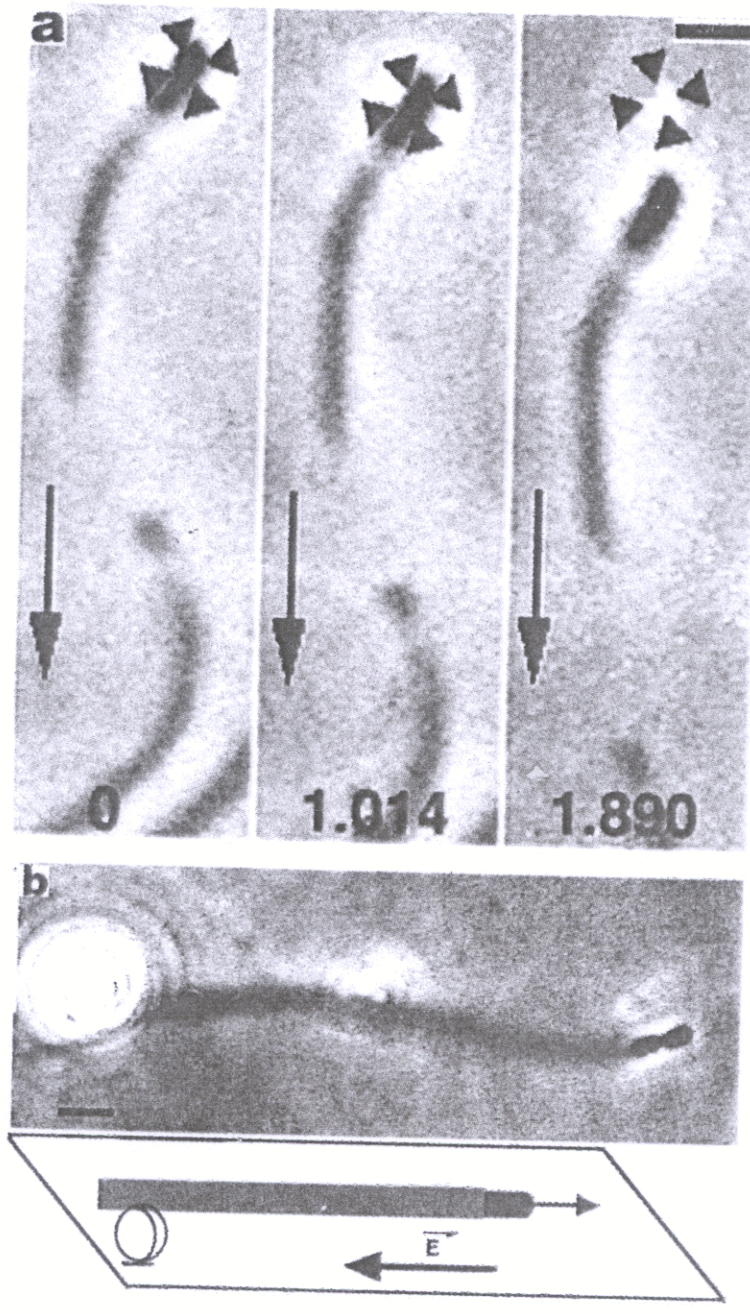

Figure 3. (**a**) In this experiment the tail has been cut by green laser irradiation. The cross shaped marker indicates the position of an optical tweezer grabbing the bacterium. By displacement of the tweezer relative to the microscope stage the bacterium is pulled through the medium in order to induce a viscous force on the tail. The force balance implies that the same force is exerted on the bacterium-tail connection. The bacterium escapes the optical trap before any release of this connection can be detected. Thus the bacterium and the tail are linked by bonds that can resist the force of an optical tweezer (here about 10 pN) over a few seconds. Numbers indicate time in seconds. (**b**) The bacterium being negatively charged, an electric field is used to exert a force calibrated to be in the order of 1 picoNewton. The tail is held on the substrate by means of a bead fused to the tail and the substrate. This experiment allows tractions over much longer time than with the optical tweezer: here about 100 s. It is still not sufficient to detach the bacterium from its tail. Bar = 5 μm.

the comet which sets a lower limit for the friction coefficient ξ_b of 10^{-3} Pa.m.s, that is ten thousand time larger than the hydrodynamic friction of the bacterium on the surrounding fluid!

Implicit in the preceeding discussion is the fact that the comet transmits forces as an elastic body, that is it has a gel structure. If this is true one can measure the elastic bending modulus of the comet. A first estimate may be obtained by looking at the response of the bacterium to optical trapping attempts. Setting the trap on the bacterium and trying to stall it always fails: first the bacterium stops, then the comet buckles and the listeria starts to move away. Once the bacterium has fully moved out of the trap the comet comes back to its original shape showing that the deformation was indeed elastic. Near the buckling threshold the force exerted by the comet on the bacterium and hence by the bacterium on the trap is of the order of the bending modulus divided by the length of the bent region squared. This length being typically ten microns and recalling that the trap force is ten piconewton at its maximum,we find a modulus K of 10^{-21} Newton.meter^2. In terms of polymer physics, we can associate a persistence length of a fraction of a meter to the comet rigidity.

One can get better measurements by combining microsurgery and optical trapping. Using 200 mW visible laser light it is possible to cut well defined pieces of the comet. Then three appropriately treated latex beads are attached to the comet segment: two at the extremities and one at the center. With a sequentially addressed laser trap it is possible to push the middle of the segment and pull the extremities (or vice versa), and study the bending of the comet (figure 4). For a large number of measurements one gets K values ranging over almost two orders of magnitude, i.e, persistence length varying from centimeter to meter, and a typical spread (i.e, half width at half maximum in the distribution) of over one order of magnitude. One explanation for this is the slow depolymerization we already mentioned. This effect can be studied separately, and cannot take account of all the spread. It could either be intrinsic to the bacterium population, or result from the experimental inhomogeneities.

We can first try to infer from these measurements whether the filaments are crosslinked or not. The very shape of the comet already tells us that the filaments want to stay together. This proves that they tend to maintain some average distance between them, but does not tell us wether they can glide past each other or not. In a free gliding picture the comet bending modulus would just be the filament bending modulus K_f times the number of filaments. One would thus expect typically a few tens of thousands of filaments in the comet. Analysis of electron microscopy data, suggests a number smaller by a factor of a few tens. Thus as expected by biologists the filaments must be cross-linked: the comet is a real gel. Estimating how cross-linked the filaments are, requires a knowledge of the gel shear modulus. Extracting an elastic modulus requires further assumptions. For order of magnitude purposes we assume that the gel is homogeneous and incompressible. Then it is easy to invert the formulae given in textbooks for the bending rigidity of beams as a function of the material shear modulus $C = (4/\pi)K/r^4$. We find values centered around one thousand Pascal. If we now estimate the gel shear modulus on dimensional grounds to be:

$$C \simeq \frac{K_f}{l^4} \tag{1}$$

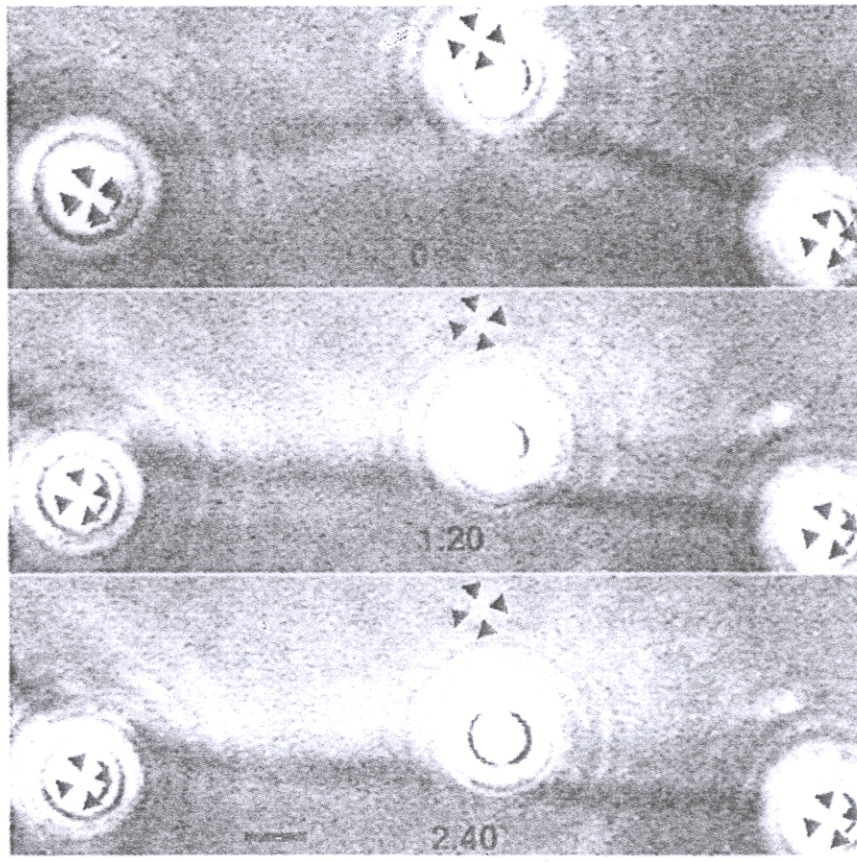

Figure 4. Experiment to measure the bending modulus of the listeria tail. Three beads have been fused to the tail to be used as handles. Each of them is held in an independent optical trap and the bar is deformed by elementary steps until the elastic force from the deformed tail overcomes the known maximum optical force. Here, a filter hides the optical traps. The first image corresponds to the release of the central bead from its trap. The other images show the relaxation of the tail to its mechanical equilibrium.

in which K_f is the bending modulus of a single filament, and l the mesh size of the gel. We get

$$l \simeq \left(\frac{K_f}{K}\right)^{(1/4)} r. \tag{2}$$

that is a mesh size in the thousand Angstrom range. This is very reasonable since it is also the average distance between filaments at the surface of the bacterium. This last figure can be inferred by dividing the bacterium radius by the square root of the number of filaments. To sum up, these experiments tell us that:

- the comet is a genuine gel able to transmit stresses
- there is mechanical integrity between the comet and the bacterium.

The problem is now to show that the gel does exert a stress on the bacterium surface and then to understand how this stress is transformed into a movement.

3. Polymerization in spherical geometry

In order to evaluate the possibility of the gel exerting a stress on the surface over which it polymerizes, we study the polymerization process on a spherical bead. Indeed, suppose that at a given time a gel of thickness $e(t)$ has already grown. After a time dt a new shell of thickness d$e(t)$ tries to develop between the already existing layer and the bead. For that purpose it has to push the existing layer which becomes streched and as a result exerts a compressing stress on the bead (figure 5). This in turn should modify the polymerization process. Standard chemistry tells us that the polymerization rate, at the bead surface reads:

$$\left(\frac{\mathrm{d}n}{\mathrm{d}t}\right) = \varpi_1^i c^i - \varpi_2^i. \tag{3}$$

In which ϖ_1^i is the polymerization probability per unit time and per unit monomer concentration, c^i the monomer concentration in the vicinity of the bead surface, that is at the inner gel surface, and ϖ_2^i the depolymerization probability per unit time. Both ϖ_1^i and ϖ_2^i depend on the force the filament is exerting on the enzyme, that is eventually on the stress σ_1 the gel is exerting on the bead. According to rate theory,

$$\varpi_1^i(\sigma) = \varpi_1^i(0)\exp\left(\frac{-\sigma l^2 a_1}{k_B T}\right), \tag{4}$$

$$\varpi_2^i(\sigma) = \varpi_2^i(0)\exp\left(\frac{\sigma l^2 a_2}{k_B T}\right), \tag{5}$$

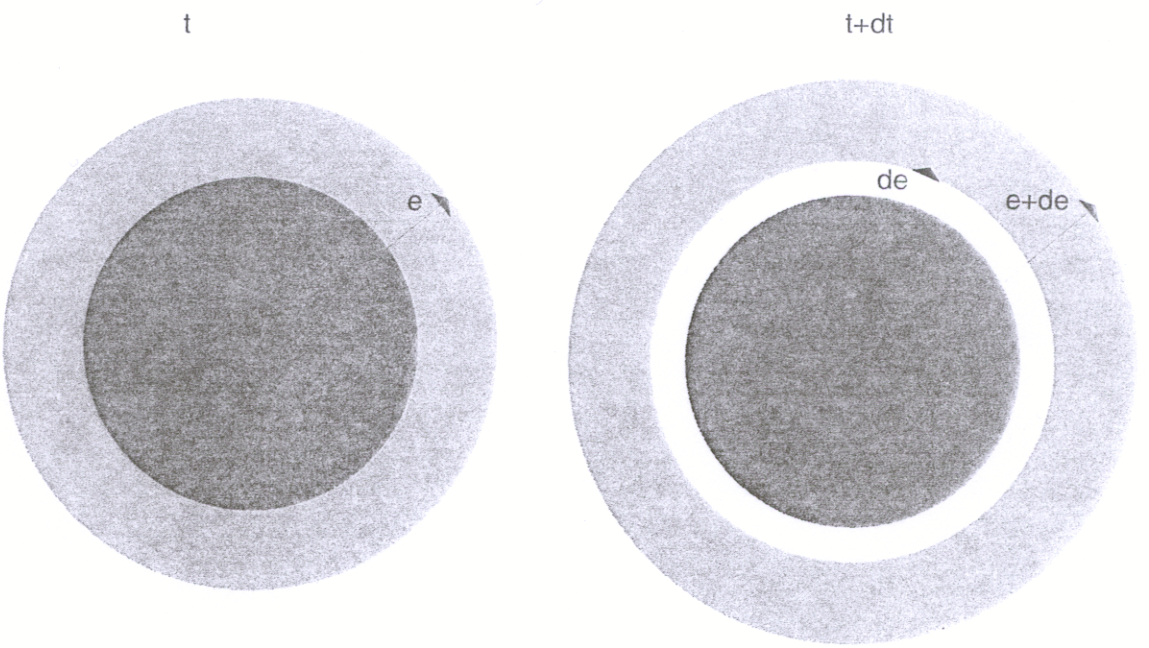

Figure 5. At time t, the bead (dark grey) is covered by an actin gel of thickness e (clear grey). As polymerization proceeds on the bead surface, the addition of a new layer of actin (in white) of thickness de must expand the older layer. This process streches the old layer, which in turn exerts a compressing normal stress on the surface.

in which l is the average distance between enzymes, and a_1, a_2 are lengths of monomeric size ($a_1 + a_2 \simeq a$, the monomer size).

Note that ϖ_1^i decreases with stress whereas ϖ_2^i increases. Thus a stress build up in a spherical topology should result in a decrease and eventually a halt of the polymerization process, and the gel should stop growing at a well characterized thickness e^{TM} such that $dn/dt(\sigma) = 0$. The knowledge of the thickness e^{TM} and that of the elastic modulus of the gel allow us to estimate the stress. In fact the problem is a little more complex since, as we have already seen, the gel depolymerises at its outer edge. The simultaneous polymerization at one end and depolymerization at the other end of actin filaments results from the polarity of the filaments, the presence of the enzyme at the inner surface combined with the out of equilibrium nature of the process. The steady state thickness is now determined by the equality of the stress reduced polymerization rate at the inner surface and the depolymerization rate at the outer surface.

In principle the inner polymerization rate is also reduced by the fact that the local monomer concentration is smaller than that of the extract, since monomers have to diffuse from the solution to the bead surface. This effect alone would be sufficient to give a finite thickness. Let us show that it is not the limiting factor with micron size beads. Suppose first that we are dealing with a flat surface. No stress can build up in this case and steady state is obtained when the stress free polymerization rate at the inner surface, the stress free depolymerization rate at the outer surface and the diffusion flux are simultaneously balanced:

$$\varpi_1^i(0)c^i - \varpi_2^i(0) = -(\varpi_1^e(0)c^e - \varpi_2^e(0)) = l^2 D\left(\frac{c^e + c^i}{e}\right), \tag{6}$$

in which the superscript e stands for quantities at the external surface and D is the monomer diffusion coefficient. Clearly $\varpi_1^i c^i \geq\geq \varpi_2^i$, $\varpi_2^e \geq\geq \varpi_1^e c^e$, which allows to express the limiting thickness as

$$e = \left(\frac{Dl^2 c^e}{\varpi_2^e}\right). \tag{7}$$

We know $D \simeq 10^{-7}\,\mathrm{cm^2\,s^{-1}}$ [1]. The monomer extract concentration is about 1 μM. From the wild type listeria tail length and velocity in the same extract we can infer $\varpi_2^e \simeq 3.10^{-4}\,\mathrm{s^{-1}}$. Anticipating $l \simeq 10\,\mathrm{nm}$, we deduce $e \simeq 1\,\mathrm{m}$ which means that there is no effect due to diffusion in our experimental conditions, since the typical thicknesses are of the order of thousand angstrom to a fraction of micron. Thus the limiting factor does come from the stress build up.

Let us estimate in a very simple way the kind of thicknesses we expect. The stress build up at the inner surface is due to the streching of the gel at the outer surface. Simple geometry tells us that his streching is essentially the thickness of the gel divided by the bead radius. It sets the gel layer into a global tension:

$$\gamma = C\left(\frac{e}{r}\right)e, \tag{8}$$

where C is the gel shear modulus and r the bead radius. This tension translates to a normal stress very similar to Laplace's pressure:

$$\sigma = \frac{\gamma}{r} = C\left(\frac{e}{r}\right)^2. \tag{9}$$

Clearly, the steady state is reached when the argument in the exponentials of equations (4) and (5) are of order one, that is:

$$\sigma l^2 a \simeq k_B T$$

from which we can extract the limiting thickness:

$$e = \left(\frac{k_B T}{aC}\right)^{1/2} \left(\frac{r}{l}\right). \tag{10}$$

Taking again $C \simeq k_B T \lambda / l^4$ one finds: $e \simeq lr/(a\lambda)^{1/2}$. With a dense enzyme coverage of the bead, knowing that the actin persistence length is about ten microns one expects a gel thickness about ten times smaller than the bead radius.

A better estimate would replace $k_B T$ by the chemical potential difference between polymeric and monomeric actin (per monomer) in formula 10, and take explicit account of the depolymerization process at the external edge of the gel, but this changes the e value in a non essential way.

The experiments do confirm these expectations. Genetic manipulation has been used to prepare an enzyme mutant which can bind covalently with polystyrene. The surface density of the enzyme on the bead has been measured by biochemical means to correspond to a nearly close packed structure [22]. Proper control experiments have been done to show that the observed polymerization was indeed catalysed by the enzyme

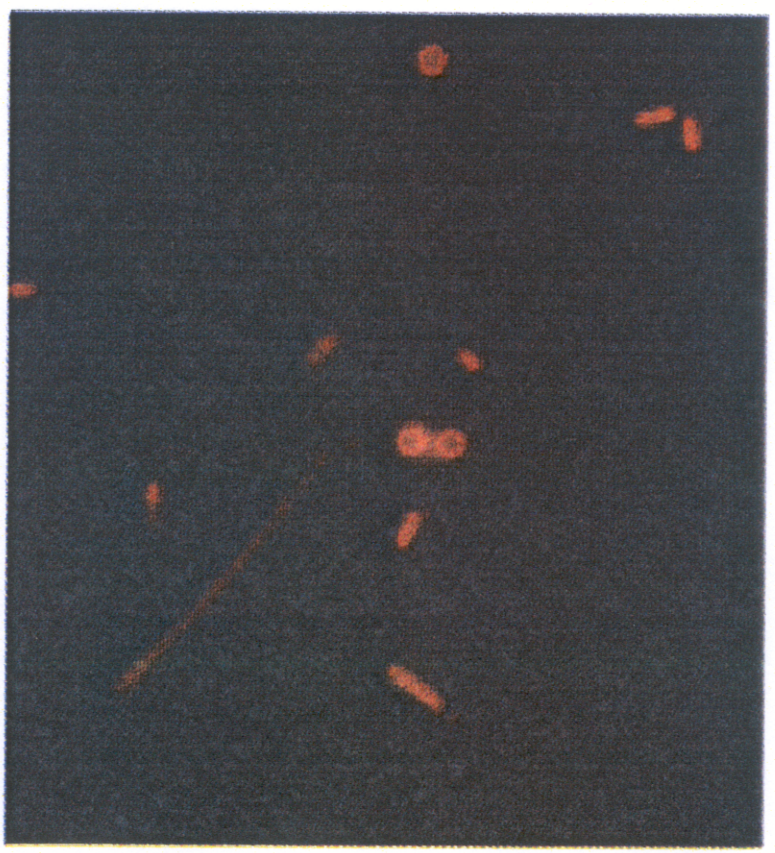

Figure 6. Mixture of beads of 2 μm in diamater (round objects) coated with the ActA protein (GST–ActA–His construct) and bacteria listeria monocytogenes (elongated objects) a few hours after incubation in cytoplasm extract of HeLa cells. Fluorescence microscopy.

Table 1. r_i is the average radius of the beads; e is the thickness of the actin gel around beads of various diameters; the distance between ActA was deduced from the amount of proteins coupled to the beads (determined by subtracting the quantity of protein in the supernatant after incubation to the initial amount of protein in the solution).

r_i (μm)	e (nm)	Quotient (e/r_i)	Estimate of the distance between ActA
0.48 ± 0.02	94 ± 10	2×10^{-1}	77 Å
0.95 ± 0.04	146 ± 10	1.5×10^{-1}	56 Å
4.72 ± 0.48	503 ± 20	1×10^{-1}	42 Å
10.1 ± 0.5	790 ± 20	0.8×10^{-1}	42 Å

ActA. An example of fluorescent visualization of the gel layer around beads is given in figure 6. Measurements of the gel thickness as a function of the bead radius were done with electron microscopy and are summarized in table 1. A ratio e/r close to 0.1 is obtained as expected. This shows that the stress exerted by the gel on the surface is about one atmosphere, that is a force of 10 picoNewton per filament (i.e: every nanometers square). This experiment thus provides a direct proof of the existence of a sizeable normal stress due to the gel in spherical topology. In the geometry of revolution of the listeria, the problem is clearly the same. The enzyme density may be estimated to be 100 times smaller, which means that the gel exerts a stress of 10^{-2} atmosphere on the bacterium surface, which corresponds to a global force of a few nanoNewtons: this explains why an optical trap is unable to stop a bacterium.

4. Elastic model for Listeria propulsion

We are now in a position to give a simple theoretical account of the propulsion mechanism. For the sake of simplicity, we propose an analysis which is more adapted to the bacterium *Shigella flexneri* than to the bacterium listeria (which in general has a solid tail): confocal microscopy observations show that Shigella tails are hollow (P Cossart, personnal communication). We simply quote the result obtained in the more general case.

4.1 *Energy flux*

The situation is schematized in figure 7. The gel is polymerized on the side surface of the bacterium which has a symmetry of revolution. From our experiment in the spherical geometry, we know that the gel exerts a normal stress on the surface: the corresponding net force adds up to zero, except in the region of the bacterium cap where it is in the direction of motion (ie to the left in figure 7).

Equivalently, in the rest frame of the bacterium, the gel is pulled in the opposite direction, that is to the right of figure 7. This means that any motion requires a slip of the gel on the bacterium surface. In a steady state the elastic energy released per unit time, when the gel moves from the bacterium sides to the tail, must balance the dissipation which comes essentially from the gel/bacterium friction.

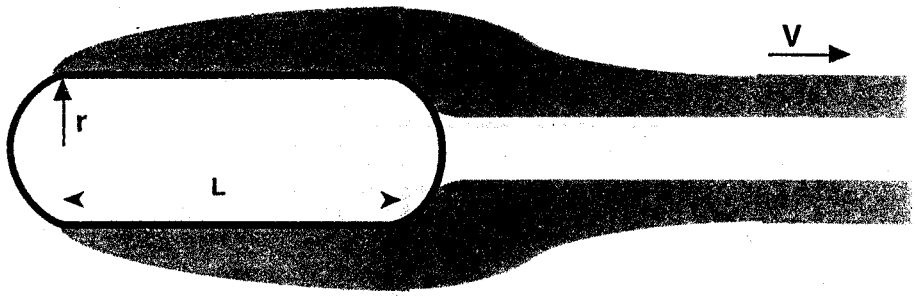

Figure 7. Schematic diagram of the gel profile around the bacterium.

The elastic energy release in a time $\tau = L/V$ is of the order of the stored elastic energy W^e around the bacterium.

In scaling form

$$W^e \simeq C\left(\frac{\delta}{r}\right)^2 rL\delta = C\frac{\delta^3 L}{r} \tag{11}$$

in which (δ/r) measures the strain of the gel, $rL\delta$ its volume and C its shear modulus.

The energy dissipated during the time $\tau = L/V$ is

$$W_D \simeq Vf(V)rL\tau = f(V)rL^2 \tag{12}$$

in which $f(V)$ is the frictional force due to the slip of the gel against the bacterium surface and rL the area over which friction occurs.

In steady state and if we neglect the polymerization dependence on stress which we discussed in the preceding section

$$\frac{L}{V} = \frac{\delta}{V_p}, \tag{13}$$

where V_p is the polymerization rate (the neglect of the stress dependence of V_p requires $\delta \ll e$; the opposite limit in which $\delta \simeq e$ is also easy to discuss).

So the velocity V is given by the relation

$$V^3 f(V) = C\left(\frac{L}{r}\right)^2 V_p^3. \tag{14}$$

Equation (14) shows that the knowledge of the friction force $f(V)$ is just as important as that of the polymerization rate. Currently, only a lower limit of $f(V)$ is known experimentally, as explained in § 3. One can however model the friction of the gel on the bacterium as is done in the following subsection.

4.2 *Gel/bacterium friction*

The average friction force may be estimated by studying the average "life" of a filament. Typically, a small filament starts to grow out of the enzyme which is mechanically coupled to the 'solid' bacterial surface (figure 8). As long as the filament is not cross-linked to the gel, it is not strained and it exerts no force on the bacterium. After some time

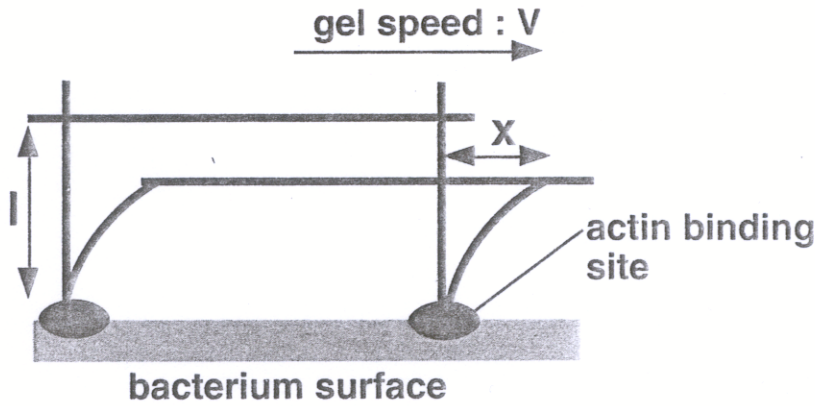

Figure 8. Schematic representation of the links between the gel and the bacterium surface. The gel moves at speed v so the filament tips move by a distance $x = vt$ and bend.

τ_c the filament gets cross-linked to the gel and because of the gel motions, gets deformed: in turn it exerts a force on the bacterium. This force starts from zero and grows continuously until either the filament breaks, or it detaches from the enzyme. It is more likely that it detaches from the enzyme and we will concentrate on that process, altough the other case would lead to a very similar discussion. The typical force f_t exerted on the bacterium by the filament depends on its elastic properties and the bound time τ_b.

If we assume linear elasticity

$$f_t \simeq C_s[V\tau_b], \tag{15}$$

where C_s is the filament elastic modulus, and $(V\tau_b)$ the typical displacement of a cross-link.

The average force per unit area $f(V)$, is simply the product of the force per filament f_t, times the number of filaments per unit area l^{-2}, times the probability for the filaments to be connected:

$$f(V) \simeq f_t l^{-2} \frac{\tau_b}{\tau_b + \tau_c} \tag{16}$$

or, taking into account the 'contact' surface friction ξ_s due to the slippage of the disconnected gel on the bacterium surface:

$$f(V) = \xi V \simeq \left[C_s \frac{\tau_b^2}{(\tau_b + \tau_c) l^2} + \xi_s \right] V. \tag{17}$$

For vanishing velocities τ_b and τ_c are determined by the natural life time τ_b^0 of the filament/enzyme life time on the one hand and the natural time τ_c^0 required for connection on the other. Both are asymptotically independent of velocity. In this limit, (17) defines a simple linear law of friction with a constant friction coefficient:

$$\xi^0 = C_s \frac{(\tau_b^0)^2}{(\tau_b^0 + \tau_c^0) l^2} + \xi_s. \tag{18}$$

For 'large' velocities, the work done by the filament on the enzyme brings down the effective potential barrier maintaining the filament bound to the enzyme to zero in a time

short compared to τ_b^0. In this regime:

$$C_s V \tau_b a \simeq W_b^0, \tag{19}$$

where W_b^0 is the potential barrier in the absence of force. The relation (19) holds (with a logarithmic correction) when $V \gg W_b^0/\tau_b^0 a C_s$. Now, the bound time τ_b is inversely proportional to the velocity, and so is the force per unit area (as long as $\tau_c \simeq \tau_c^0$, and $\xi_s V$ negligible)

$$f(V) \simeq \frac{(W_b^0)^2}{l^2 a^2 C_s V \tau_c}. \tag{20}$$

At some point the normal surface friction takes over and the frictional force increases again with velocity. The existence of the three regimes implies the existence of a maximum and a minimum in the $f(V)$ curve. The full $f(V)$ curve may be obtained from a simple extension of the arguments developed here. A typical plot is shown in figure 9: this is a familiar type of dependence ubiquitous in the solid on solid friction. It is well known to give rise to the so-called 'stick-slip' motion when suitable conditions are met.

4.3 *Steady state and beyond*

Equation (14) expresses the condition for steady state. This condition is cast in a slightly different form in figure 9: the friction per unit area $f(V)$ must be balanced by the

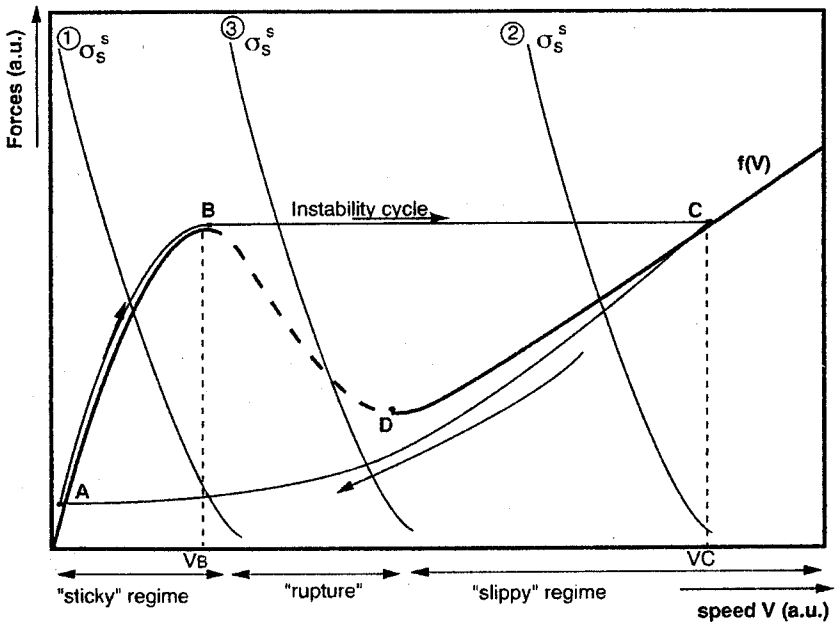

Figure 9. Plot of the friction force $f(V)$, (thick line) and the tangential stress σ_s^s (curve 1, 2 and 3 (thin lines)) as functions of the velocity V. Intersections of $f(V)$ with 1, 2 and 3 define solution to eq. (14); for curves 1 and 2 they correspond to a steady state. For curve 3, the solution is not stable, and the system enters the cycle, oscillating between the points A, B, C and D.

shear stress $\sigma_s = C(\delta^3/r^2L)$ that is $\sigma_s^s = C(L/r)^2(V_p/V)^3$ in steady state (curves labeled 1, 2, 3).

The loading conditions defined by curves 1 and 2 lead to a stable steady state: indeed, if for some reason, the gel thickness is larger (resp. smaller) than the steady state value, the velocity V gets larger (resp. smaller) than the steady state velocity, and the gel thickness decreases (resp. increases) until steady state is recovered.

Curve 3 requires a separate analysis: although its intersection with the $f(V)$ curve corresponds to a locally stable situation, it is in fact not globally stable according to the following argument. Suppose the bacterium starts growing a gel. At the begining the layer thickness δ is small, and so are $\sigma_s(\delta)$ and the corresponding sliding velocity $V(\delta)$ (point A). Since $\sigma_s(\delta)$ is smaller than σ_s^s, the gel thickness increases as already argued. However, when it reaches a value $\delta_{\max} \lesssim V_p(L/V_B)$ such that $\sigma_s(\delta_{\max}) = f_{\max}$ the maximum value of the $f(V)$ curve (point B), the links between the filaments and the enzymes break in a time τ_b whereas the gel thickness has no time to change (ie $\tau_b \ll L/V_c$), and the effective friction decreases in a very significant way. The result is an abrupt velocity increase to the value V_C of figure 9 (point C). It takes a time $t_d \simeq L/V_C$ for the gel thickness to decrease to a smaller value $\delta_s \simeq V_p t_d = V_p(L/V_C)$ which corresponds to a much smaller σ_s value. As a result the bacterium is back at the starting point A and a new cycle begins. This corresponds to the observed saltatory behavior.

5. Discussion

At steady state in the domains where the friction coefficient is nearly constant using eqs. (14) and (17) one can rewrite the bacterium velocity in the following simple way:

$$V \simeq V_p \left(\frac{V_i}{V_p}\right)^{1/4} \left(\frac{L}{r}\right)^{1/2}, \tag{21}$$

where $V_i = C/\xi$ is an 'intrinsic' velocity comparing the gel strength and the surface friction ξ_s. Since on the one hand the geometrical factor $(L/r)^{1/2}$ is always of order one, and on the other hand variations of the factor (V_i/V_p) are strongly damped by the power $1/4$, the bacterium velocity at steady state depends essentially on the polymerization rate. Knowing $C \simeq 10^3 - 10^4$ Pa (see § 3), we only need to estimate ξ in order to get a reasonable value of V_i/V_p. Our experimental data provide a lower limit: $\xi = \xi_b/2\pi rL \simeq 10^8\,\text{Pa.m}^{-1}\text{.s}$: $V_i \simeq 10^{-5} - 10^{-4}\,\text{m.s}^{-1}$ ie $V_i \simeq 10$ to $100\,\mu\text{m.s}^{-1}$. Such values are consistant with: $V \simeq 10V_p \simeq 0.1\,\mu\text{m.s}^{-1}$ (where we have ignored the $(L/r)^{1/2}$ factor of order one and ξ_b is the total friction defined in § 2). These orders of magnitude mean that 'hollow tail' bacteria can indeed move significantly faster than the polymerization rate.

If the bacterium is moving steadily under conditions such that the friction is dominated by the 'surface' friction ξ_s, the steady state velocity would not be very different from the preceeding estimate: indeed, a lower value for ξ_s, ignoring the entanglements would be

$$\xi_s \gtrsim C_s \tau_f / l^2, \tag{22}$$

where τ_f is the time scale for transverse fluctuations of wavelength l of a single filament: $\tau_f \simeq (\eta l^4/K_f)$. Estimating $C_s \simeq Cl$, we get $\xi_s \gtrsim Cl^3(\eta/K_f)$. In the local environment of

the surface the viscosity is probably higher than in the bulk of the extract (η in the range of 10^{-2} Pa.s.) and one can reasonably estimate $\xi_s \gtrsim 10^7$ Pa.m.s.

The intrinsic velocity could easily be ten times larger than in the preceeding case but the ratio $V_i/V_p \simeq 15$ is not so different from the one found in the previous estimation.

Vesicles in similar conditions should move faster. The friction in that case comes essentially from the motion of the enzymes with respect to the membrane and should scale as (ignoring logarithms): $\xi_{\text{ves}} \simeq \eta_m w/l^2$ in which η_m is the membrane viscosity and w the membrane thickness. η_m is typically hundred times the viscosity of water, and the factor (w/l) is about $1/20$ if we keep the same enzyme density as in the rest of the article. We hence expect $\xi_{\text{ves}} \simeq 10^5$ Pa.m.s, and $V \simeq 100 V_p$! Experiments on vesicles cannot be directly compared to this expectation since the enzyme density and polymerization rates are not known; it is however rewarding to see that the tails are hollow [13].

If in all cases V is significantly larger than V_p, one would expect the tails to be always practically hollow, or that the inner part of the tail should always be unable to maintain the mechanical integrity of a gel. This is not quite true: if we fill up the inner part of the tail in figure 7, clearly since the outer part tends to move faster than the natural polymerization rate, the inner part of the gel will be under tension. *A priori* two scenarios can be imagined:

- beyond some critical tension filaments growing at the back of bacterium do not have time to connect to the gel and the core of the gel is essentially hollow or disconnected.
- below that tension the outer gel is slowed down and simultaneously the inner polymerization rate is speeded up, acording to eqs (3), (4) and (5); as a result a uniform gel velocity with respect to the bacterium is obtained and the tail can be full.

A 'two gel model' developed along those lines can reproduce all the phenotypes known to date [23].

At last let us discuss the saltatory behavior of the $\text{ActA}_{\Delta 21-97}$ mutant: the peak velocity observable in figure 2 is of the order $0.2 - 0.3\ \mu\text{m.s}^{-1}$, that is of the order of the steady velocity of the wild type, whereas the average velocity is about half of it and the lowest velocities are twenty times smaller. These observations are consistent with the cycle of figure 9. The fact that the peak velocity of the mutant is of the order of the steady velocity of the wild type implies that the polymerization rate V_p is smaller in the mutant than in the wild type. If the mutation had an influence on V_p only, one could then deduce that the wild type steady state is in the neighborhood of point C of figure 9. However, it is quite possible that the $F(V)$ curve itself is significantly perturbed by the mutation and a steady state in the high friction regime cannot be ruled out for the wild type.

Eventually, one might wonder why we did not investigate the possibility of oscillations of chemical origin. In such a case, one would expect the high speed sequence to correspond to a high polymerization rate, and conversely the low speed sequence to a low polymerization rate; the gel thickness variations should be small since the relation $\delta \simeq (Lr)^{1/2}(V_p/V_i)^{1/4}$ is obeyed. This is definitely not what is observed. The build-up of the gel coating around the bacterium occurs during the low velocity period and clearly the velocity develops once a 'slip' threshold is reached: this is an example of 'living stick-slip'.

Last, it is important to point out that a key to the understanding of the occurance of phenotypes will be the patient construction of dynamical diagrams of state: in our

example here, the saltatory phenotype does not necessarily occur as a result of a genotypic change, but can arise as a consequence of extract modifications, temperature variation etc.... The type of mesoscopic model presented here will have to be developed in many other situations.

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