Is quorum sensing a side effect of diffusion sensing?

Rosemary J. Redfield

Many bacteria appear to communicate by releasing and sensing autoinducer molecules, which are believed to function primarily as sensors of population density. However, this quorum-sensing hypothesis rests on very weak foundations, as neither the need for group action nor the selective conditions required for its evolution have been demonstrated. Here, I argue for a more direct function of autoinducer secretion and response – the ability to determine whether secreted molecules rapidly move away from the cell. This diffusion sensing allows cells to regulate secretion of degradative enzymes and other effectors to minimize losses owing to extracellular diffusion and mixing.

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Interest in the bacterial phenomenon known as quorum sensing is exploding – many minireviews and at least nine major reviews have been published in the past two years [1–9], and the annual number of publications is growing exponentially (Fig. 1). The shared mechanism of all quorum-sensing systems is regulation by autoinducers released into the cell's environment (Fig. 2a). Typically, cells produce a small extracellular autoinducer molecule – usually a peptide, a boron derivative of ribose or an acyl homoserine lactone (Fig. 2b–d) – and simultaneously sense the concentration of the autoinducer at the cell surface. If the concentration exceeds a threshold, gene expression is induced, usually leading to the production of other extracellular products. Some well-studied examples are listed in Table 1.

The term quorum sensing is derived from the interpretation that these regulatory circuits exist to sense population density [10]. Because the concentration of autoinducer in liquid cultures exceeds the induction threshold only when the culture exceeds a critical cell density, these systems are thought to have evolved to allow bacteria to detect cell density and thus optimize the expression of functions that are most beneficial when simultaneously carried out by large populations of cells. Regulation by quorum sensing was originally thought to be restricted to specialized functions in a few species (e.g. light production in *Vibrio fischeri* and competence development in *Streptococcus pneumoniae* [11,12]). However, the number of known regulatory systems and the diversity of phenomena regulated are growing dramatically, and it now appears that most bacteria possess at least one quorum-sensing system [2,3,5].

Problems with the quorum-sensing model

The appeal of the idea that bacteria act cooperatively has caused the postulated benefits of quorum sensing to be accepted uncritically as the explanation for the role of autoinducers in gene regulation. However, although autoinducer-controlled processes typically do act outside of the cell, there is little direct evidence that their benefits depend on group action. Instead, discussions of function usually begin by assuming that the benefits must be group-limited, and then evolutionary 'just-so stories' are postulated to explain how this benefit arises. For example, secretion of degradative enzymes such as cellulase and pectin lyase by the plant pathogen *Erwinia carotovora* is regulated by autoinducer accumulation [13,14]. Host plants respond defensively to such exoenzymes, so it has been proposed that regulation by quorum-sensing allows *E. carotovora* cells to delay exoenzyme secretion until the population is large enough to overwhelm host defences [2]. However, no attempt has been made to test this hypothesis by showing either that exoenzyme production by an isolated cell triggers the host responses, or that growth of this cell into a population large enough to overcome these responses does not require the nutrients exoenzymes provide. Many other superficially plausible but untested hypotheses about the benefits of quorum sensing have also been proposed. The stories are usually developed in isolation, often invoking benefits that in other stories are treated as costs to be avoided. In one extreme example, quorum regulation of natural competence was proposed as an adaptation to decrease the proportion of con-specific DNA and, a page later in the same review, as an adaptation to increase it [2].

'The appeal of the idea that bacteria act cooperatively has caused the postulated benefits of quorum sensing to be accepted uncritically...'

The evolution of quorum sensing also poses major problems. Genes for quorum sensing will only evolve if cells that invest individual resources for a shared benefit reproduce better than cells using their resources selfishly. The difficulty of maintaining genes for cooperative strategies in genetically mixed populations makes this a notoriously weak and controversial mode of selection, especially where interspecies quorum sensing is proposed to benefit the members of a mixed-species biofilm [9]. Because direct selection on individual benefits is much more effective than indirect selection on group benefits, evolutionary biologists usually reject explanations relying on the latter unless individual benefits have been ruled out [15].

Where cells live exclusively in single-clone populations, natural selection can indeed favour clones that cooperate over clones whose members act selfishly.

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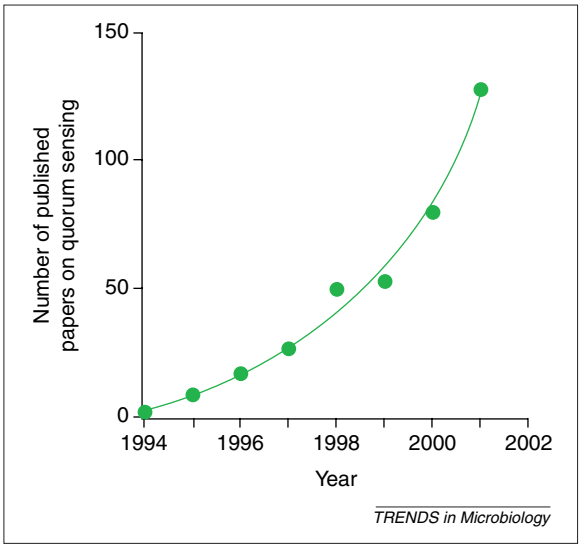

Fig. 1. The rate of publication of papers on quorum-sensing. The data were obtained using a Web of Science search for papers with 'quorum sensing' in any field.

However, in mixed populations engaged in quorum sensing, selection will favour any selfish cells that can passively obtain the benefits without the expense of producing or responding to the signal. Consider a population of cells obtaining their amino acids by secreting a quorum-regulated protease. Cells that do not participate in sensing or protease secretion will be at an advantage, as they obtain the amino acids for free. These 'cheats' could be mutants that arose within the population or members of other strains or species. The benefits of cheating have been demonstrated by Velicer and co-workers, who found that non-cooperating mutants of *Myxococcus xanthus* can outcompete their relatives during cooperative formation of fruiting bodies [16]. Because bacterial populations are rarely clonal outside of the laboratory (see references in [3]), cells investing in quorum sensing to obtain shared benefits should be under constant competition from non-cooperators. However non-cooperators have not been reported, suggesting that regulation by autoinducers might confer a substantial benefit on individual cells.

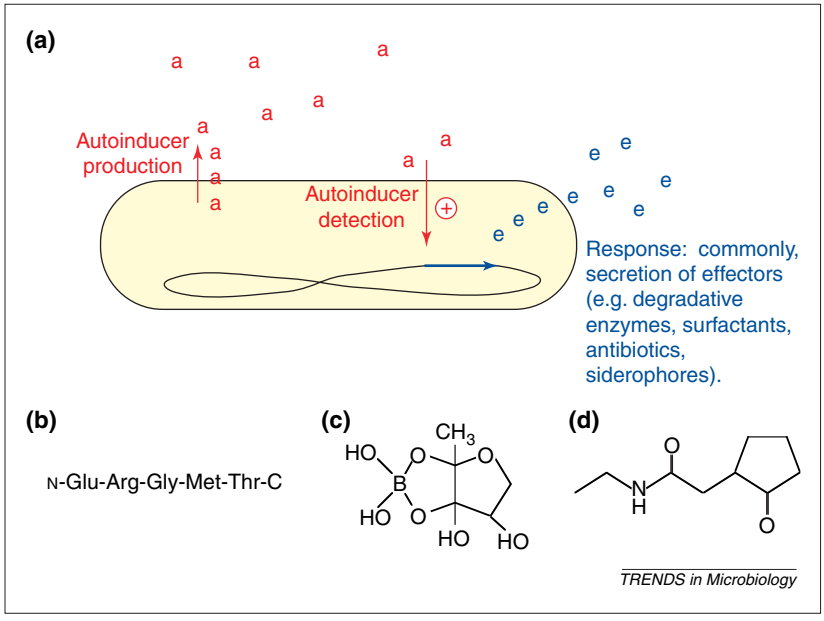

Fig. 2. (a) Regulation by autoinduction. Many bacteria produce autoinducers (red 'a's) which diffuse or are secreted into the cell's environment. If the concentration of autoinducer detected by the cell exceeds a critical threshold, genes are induced and effectors secreted (blue 'e's). (b) The competence and sporulating stimulating factor (CSF) autoinducer of *Bacillus subtilis* [17]. (c) The AI-2 autoinducer produced by many bacteria [7]. (d) The *N*-butyryl-homoserine lactone of *Pseudomonas aeruginosa* [7].

Why diffusion matters

The evolution and postulated benefits of quorum sensing are both problematic, but why else would a cell produce and detect an autoinducer? One important candidate function is the ability to detect the extent of diffusion and mixing in the cell's microenvironment. Because bacteria cannot use phagocytosis, they rely on the secretion of degradative enzymes to break down macromolecules into subunits that can then be taken up (Fig. 3). Bacteria also increase nutrient availability by secreting siderophores, antibiotics, surfactants and other secondary metabolites. All of these can provide benefits to a single cell, but will be effective only when diffusion and mixing in the cell's microenvironment are limited, so the secreted molecules remain close enough to the cell for the benefits to be realized. Figure 4 illustrates how an autoinducer serves as a molecular sensor, with its local concentration reflecting the molecular processes occurring in the cell's immediate environment. Diffusion and flow in natural environments can be extremely unpredictable (consider soil before and after a rainfall, or nasal mucosa before and after encounter with an allergen), and cells that produce and detect autoinducers can assess these changes directly and regulate gene expression accordingly.

Many of the properties of quorum-sensing systems support this hypothesis. As predicted, autoinducers commonly regulate the production of substances that are secreted into the extracellular environment. Some of these functions are indicated in Table 1. Others include secreted proteases of many types, cellulases, pectinases, collagenases, chitinases, antibiotics, siderophores, surfactants, lipases, cytolysins and the majority of virulence factors. Molecules that have evolved to function as diffusion sensors should also be cheap to produce and not naturally present in the environment, properties which are typical of the known autoinducers.

Testing hypotheses about autoinducer regulation

Quorum sensing holds its position as the current paradigm for autoinducer regulation by default, and both quorum sensing and diffusion sensing should be treated skeptically until each has been rigorously tested. Simply observing density-dependent regulation is not informative, as any autoinducer-regulated process will inevitably be regulated by population densities if mixing is high but very local (i.e. able to disperse the autoinducer among the population but unable to disperse the population itself). Such conditions are typical of laboratory cultures, where populations are actively mixed but constrained within culture vessels. Thus, demonstrations of quorum sensing in the laboratory tell us nothing about the roles of autoinducers in the natural environment. We instead need to ask

Table 1. Processes regulated by quorum sensing

Organism	Autoinducer(s)	Processes regulated	Refs
Agrobacterium tumefaciens	Acyl-homoserine lactone	Ti plasmid transfer	[39]
Erwinia carotovora, Erwinia vietnamiensis	Oxohexanoyl-homoserine lactone	Carbapenem antibiotic[a], polygalacturonase[a], pectate lyase[a], cellulase[a], protease[a], Harpin[a]	[40–42]
Burkholderia cepacia	Octanoyl- and hexanoyl-homoserine lactones	Protease[a], siderophore[a], surfactant[a],biofilm formation[a], swarming motility	[43–45]
Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas aureofaciens	Oxo-dodecanoyl-homoserine lactone, *N*-butyryl-homoserine lactone	Antibiotics[a], biofilm formation[a], pigment[a], lectin[a], rhamnolipid surfactant[a], elastase[a]	[33,46–50]
Vibrio fischeri, Vibrio harveyii, Vibrio anguillarum, Vibrio cholerae	Furanosyl borate diester, various *N*-acyl homoserine lactones	Bioluminescence, cholera toxin[a], metalloprotease[a], enterobactin[a], hemolysin[a], serine, pigment[a], biofilm formation[a], motility	[29,51,52]
Bacillus subtilis	Oligopeptides	Surfactant[a], bactilysin antibiotic[a], competence, sporulation	[53,54]
Staphylococcus aureus, Staphylococcus epidermidis	Thiolactone peptides	Toxic shock toxin[a], α-toxin[a], enterotoxin B[a], protease[a], many other virulence factors[a]	[30,55]
Enterococcus faecalis	Unknown	Cytolysin[a], gelatinase[a], serine protease[a]	[56,57]
Streptomyces sp.	Butyrolactones (factor A)	Streptomycin[a], superoxide dismutase[a], mycelium formation	[58]

[a]Products known to be secreted.

whether the regulation acts under natural conditions where quorum sensing is possible (high mixing in a bounded environment) and whether sufficient autoinducer is produced under these conditions. Where quorum sensing is shown to be physically possible, we still need to determine whether the hypothesized evolutionary benefits exist. Is the measured benefit to each cell greater when cells cooperate than when they act individually? Alternatively, do isolated cells experience diffusion-limited microenvironments where self-induction would be expected? Does the consequent expression of inducer-regulated genes increase growth and/or survival? Because natural selection acts more strongly on individual benefits than on those shared between members of a population or community, explanations relying on group benefits should not be accepted unless potential benefits to individuals (or demonstrably pure clones) have been rigorously sought and unambiguously shown to be inadequate.

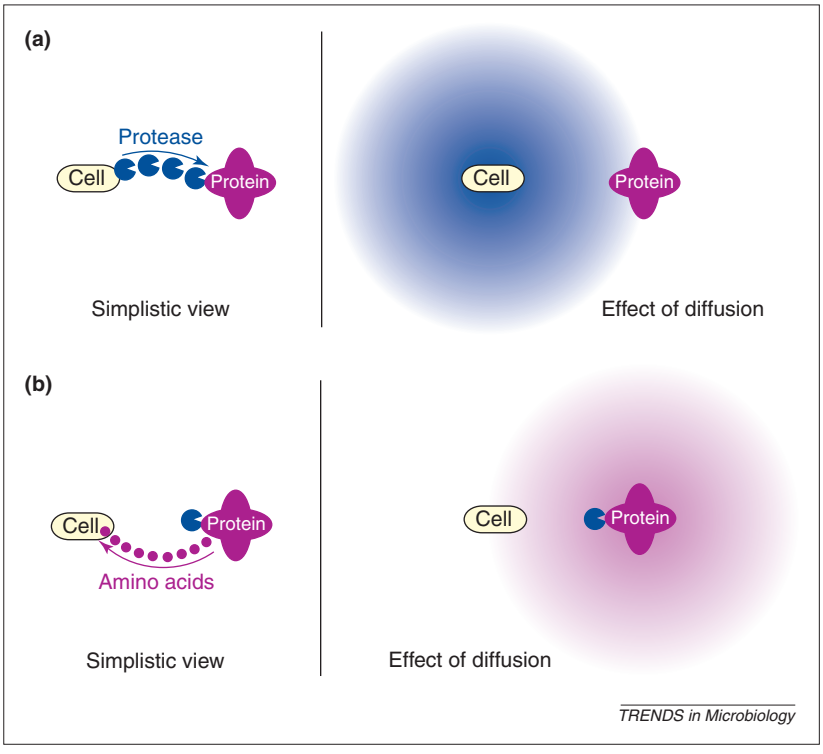

Fig. 3. How cells use secreted proteases (and other effectors) to obtain nutrients. Most cells secrete exoenzymes and other effector molecules as a mechanism to convert nutrients into a form suitable for uptake. The impact of diffusion on these processes is usually ignored (left panels) and is illustrated here (right panels) for a secreted protease. Diffusion limits both the amount of protease that reaches a target protein [(a); blue particles and cloud] and the amount of amino acids that reaches the cell [(b); pink particles and cloud].

One experimental prediction of the diffusion-sensing hypothesis is that isolated cells should be able to produce enough autoinducer for self-induction under plausible natural conditions. (The quorum-sensing hypothesis does not predict this but does not preclude it either.) Most of the available data are consistent with this prediction. For example, in laboratory cultures of the soil bacterium *Bacillus subtilis*, autoinducers reach inducing concentrations at culture densities $<10^8$ cells ml^{-1} [17], implying that each cell can fill a volume of 10^4 μm^3 with an activating concentration of inducer. This is well within the volumes bacteria are constrained to in drying soil (Fig. 4b) [18]. However, direct experimental testing of this prediction is warranted, especially because the amounts of autoinducer produced depend strongly on culture conditions (M. Surette, pers. commun.).

New perspectives on regulation by autoinducers

Diffusion does not play an obvious role in several autoinducer-regulated processes. For some of these, further investigation might demonstrate true quorum-sensing systems. Others might be found to be more dependent on diffusion than previously suspected. Consider natural competence. In both *Bacillus* and *Streptococcus* the ability to take up DNA is regulated by autoinducers [12,19], and scenarios have been suggested whereby this reflects the benefits of high population density. However, the *B. subtilis* autoinducers also regulate secretory functions (including a surfactant and a peptide antibiotic) [20,21] and the coinduction of competence could reflect their ability to increase DNA availability

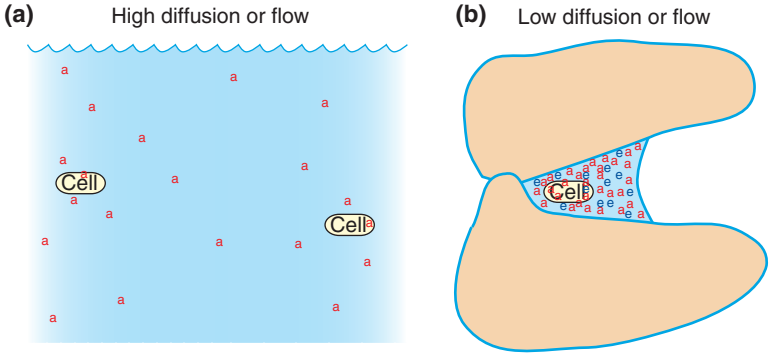

Fig. 4. A secreted autoinducer will function as a sensor of diffusion. Autoinducer molecules (red 'a's) produced under high-diffusion conditions are rapidly lost from the cell's microenvironment (a); this prevents wasteful production of exoenzymes and other secreted effectors. However, if the diffusion in the microenvironment is constrained by physical boundaries such as soil particles and air–water interfaces (b), autoinducer will accumulate to inducing concentrations and exoenzymes (blue 'e's) will be produced.

rather than a need for group action. Similarly, Li and co-workers have shown that the competence-stimulating autoinducer of *Streptococcus mutans* also stimulates biofilm formation and development of acid resistance [22,23], suggesting that DNA uptake is part of a suite of as yet poorly understood environmental adaptations in *Streptococcus*.

The regulation of motility by autoinducers could also reflect the benefits of sensing the physical structure of the environment rather than the presence of other bacteria. The presence of a solid barrier will cause the autoinducer to accumulate, and might induce shifting to a mode of motility better suited to movement along surfaces rather than to movement free in solution. Although motility is most often induced by accumulation of the autoinducer, in principle, repression of motility by autoinduction could allow bacteria to increase investment in flagella or other motility structures only when sufficient water became available to make movement an option. (The water films on soil particles and other environmental surfaces can easily become so thin that bacterial movement is precluded [18].) Similar ideas can be applied to autoinducer regulation of biofilm formation and sporulation.

This new perspective might also clarify other aspects of regulation by secreted peptides. Conjugative plasmids in *Enterococcus faecalis* identify potential recipient cells by their secretion of peptides. Although these peptides are often described as sex pheromones, that is, recipient adaptations to attract sources of potentially useful genes [24], they have many of the hallmarks of autoinducers. Flannagan and Clewell have now shown that the peptides are derived from the signal sequences of unrelated lipoproteins [25]; whether they function as autoinducers has not yet been investigated. One possibility is that, rather than being invited in by eager recipients, the plasmids are targeting new hosts by the peptides they release, in the same way that mosquitoes exploit the CO_2 we emit.

Bacterial bioluminescence remains problematic. Most discussions focus on bioluminescent species symbiotic in the light organs of marine animals, and emphasize the indirect benefits that quorum-regulated light production confers on the host. However many non-symbiotic bacterial species also produce light, and some of these are known to use autoinduction to regulate their bioluminescence [26]. Furthermore, light production has been shown to benefit bacterial cells directly by recycling reducing equivalents and by providing photoreactivating wavelengths for DNA repair [27,28]. Further investigation could show that light organ environments do have the physical properties required for true quorum sensing, and that autoinducers produced there do indeed function as quorum-sensing systems. However, this would probably be seen as a secondary adaptation of an as-yet-unknown function of autoinduction in free-living bioluminescent bacteria.

The various autoinducer systems characterized so far exhibit both surprising unity and surprising diversity. Both characteristics have been explained as adaptations for communication but could result instead from selection on environmental sensing. Many organisms use two or more different autoinducers – these usually differ in physical properties and so are likely to convey differing information about the environment. They are also usually differentially regulated, and so could themselves regulate the different responses appropriate to, for example, starvation, drying or sporulation. Autoinducers shared by many unrelated bacteria, such as the AI-2 specified by *luxS* [29], have been interpreted as adaptations for inter-species communication [2,7]. Under the diffusion-sensing hypothesis, sharing would instead occur under conditions where individual benefits are rarely confounded by signals from competitors. The opposite situation is seen in the many cases where autoinducers differ dramatically even between isolates of the same species [19,30–32]. In pathogens such diversification of autoinducers could be caused by frequency-dependent selection, perhaps driven by antagonistic host-recognition systems rather than by any need for 'private-channel' communication [33].

There is currently substantial interest in developing drugs that target quorum sensing, and autoinducer transgenes that can be used to manipulate plant–pathogen and plant–symbiont interactions [34–36]. A clearer understanding of the function of autoinducer production might suggest roles for these. For example, interventions against virulence genes controlled by autoinducers might be more effective than previously expected if the response to autoinducer is not restricted to conditions of high bacterial density.

Conclusions

Hypotheses about function are rarely given the level of scientific scrutiny applied to hypotheses about mechanisms. Instead, those explanations that mesh well with our preconceptions are often uncritically accepted and those that do not are scorned. This is unfortunate because a misunderstood function can seriously delay interpretation of mechanism. For example, molecular biologists spent decades trying to match the behaviour of bacterial 'recombination' proteins with their assumed function of promoting genetic exchange, before finally realizing that their major roles are in DNA replication and repair [37].

Failure to test functional hypotheses is partly owing to the mistaken belief that they are not really testable, and partly to misplaced confidence in our ability to evaluate them at an intuitive level. This overconfidence is particularly perilous for molecular biologists and microbiologists because events at the scale of single cells and molecules are beyond the range of human experience, and we frequently rely on extrapolation from laboratory conditions instead of investigating conditions in the natural environment. We seem to be most prone to errors with those processes that most strongly distinguish us from bacteria – our sexuality and our sociality. Sex plays a central role in our lives, and we have rarely questioned whether it is also important in bacteria [38]. Similarly, perhaps because we are social animals, we find the idea that bacteria have evolved communication and cooperation very appealing. By failing to question the assumption that autoinducers exist to allow quorum sensing, we might have allowed foundations of sand to underlie what is otherwise excellent research into new mechanisms of gene regulation.

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Is the molecular basis of metronidazole resistance in microaerophilic organisms understood?

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Metronidazole is an antibiotic that has been effective against many microaerophilic microorganisms with importance in medicine and animal husbandry. The development of increasing resistance against current treatments by many of these organisms has created an urgent need to establish the molecular bases of resistance, knowledge which will help to develop novel diagnostic methods and identify new therapeutic targets. Significant progress has been made in understanding resistance to this antibiotic in the human pathogens *Helicobacter pylori* and, to a lesser extent, *Campylobacter* spp. However, insufficient knowledge of the physiology and genetics of these and other related bacteria has led to investigations based on hypotheses that themselves must be established more thoroughly. This review presents the status of our current knowledge of metronidazole resistance and outlines reasons to explain some of the conflicting evidence and controversy in the interpretation of results in this area.

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Infectious diseases claimed more than 20 million lives in the year 2001, with most of those deaths occurring in developing countries. These same regions of the world serve as incubators for emerging strains of bacteria, fungi and parasitic protozoa that are resistant to current antimicrobial therapies. In addition, the overuse of hitherto potent antibiotics in agriculture and medicine in both developed and developing countries also contributes significantly to the pool of resistant microorganisms.

Understanding the molecular basis of resistance to antibiotics would be an advance in our knowledge of pathogens of intrinsic scientific value, with many potential applications. Practical outcomes include the design of more effective antibiotic compounds, and of faster and more accurate methods to diagnose the susceptibility of infections to antimicrobials; proper targeting and shorter time lags in determining the resistance status of an infection can be critical factors in its elimination.

Metronidazole (Mtr) is an important component of therapeutic regimes currently used against many bacterial and parasitic pathogens. It is a 5-nitroimidazole (Fig. 1) with a nitro group of –415 mV redox potential. Mtr is administered as an inactive prodrug and is converted to a cytotoxic form by anaerobic or microaerobic organisms; the drug is ineffective in aerobic microorganisms or mammalian cells, and this is the basis of its selective toxicity. In the classical definition of susceptibility, a bacterial strain is considered susceptible to Mtr if it does not grow *in vitro* at or above a specific concentration of the antibiotic, commonly set at 8 μg ml^{-1}.

The mode of action of Mtr is well characterized in anaerobic microorganisms, where the 5-nitro group of the imidazole ring is reduced via a one-electron transfer to the nitro-radical anion intermediate capable of damaging DNA [1]. Activation of Mtr occurs

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