Antonie van Leeuwenhoek **67:** 289–295, 1995.

Secretion of an antibacterial factor during resuscitation of dormant cells in *Micrococcus luteus* cultures held in an extended stationary phase

Galina V. Mukamolova[1], Arseny S. Kaprelyants[1] & Douglas B. Kell[2,*]
[1] *Bakh Institute of Biochemistry, Russian Academy of Sciences, Leninskii prospekt 33, Moscow 117071, Russia;*
[2] *Institute of Biological Sciences, University of Wales, Aberystwyth, Dyfed SY23 3DA, UK (* requests for offprints)*

Received 26 May 1994; accepted 5 September 1994

Key words: antibacterial factor, dormancy, *Micrococcus luteus*, resuscitation, stationary phase

Abstract

A high proportion of *Micrococcus luteus* cells in cultures starved for 3–6 months in spent medium following growth to stationary phase in batch culture lost the ability to grow and form colonies on agar plates, but could be resuscitated from dormancy by incubation in liquid medium containing supernatant taken from the late log phase of viable cultures of the same organism (Kaprelyants et al. 1994). In the present work, we found that during the first 50–70 h of such resuscitation the dormant cells actually divide for 10–17 generations in lactate minimal medium containing yeast extract whilst remaining nonculturable on agar plates. Further incubation results in a decrease in the total cell number in liquid medium. The addition of viable (culturable) *Micrococcus luteus* cells in concentrations of up to 10^4 ml^{-1} to test tubes containing either resuscitating cells or supernatant from these cultures revealed the excretion of a factor or factors which inhibited the proliferation of otherwise viable cells. The maximum production of this factor took place after some 96 h of incubation of starved cells in resuscitation medium. Supernatant from late logarithmic phase batch cultures of *M. luteus* abolished the antibacterial effect of starved cultures incubated in resuscitation medium. It is concluded that the stimulating effect of viable cells, and of supernatant taken from batch cultures, on the resuscitation of dormant cells might be connected in part with overcoming the activity of an antibacterial factor causing self-poisoning of dormant cells during their resuscitation.

Introduction

The nonsporulating Gram-positive coccus *Micrococcus luteus* starved for 3–7 months in spent growth medium following growth to stationary phase in batch culture exhibited very low viability (i.e. culturability: less than 0.001% as estimated by plating on agar plates) whilst the total count remained close to its initial value at the onset of starvation (Kaprelyants & Kell 1993; Kaprelyants et al. 1993). Using flow cytometry with appropriate probes and conditions (Kell et al. 1991; Kaprelyants & Kell 1992) we found that at least 50% of *M. luteus* cells in 3-month-old populations could be resuscitated to normal, colony-forming bacteria (Kaprelyants & Kell 1993). This indicated that a significant number of cells in the starved populations were not dead but dormant, where we used the term 'dormancy' to describe a reversible state of low metabolic activity in which a cell can persist for extended periods without division (Kaprelyants et al. 1993). It has occasionally been suggested that two other groups of nonsporulating bacteria, viz. the so-called viable-but-non-culturable (VBNC) forms (Roszak & Colwell 1987; Barer et al. 1993; Oliver 1993) and the ultramicrobacteria (Morita 1990) found in natural water habitats, might also exhibit dormancy (for a review see Kaprelyants et al. 1993). It has also been suggested that dormant cells can be converted to normal, colony-forming bacteria following a special procedure of resuscitation involving cultivation in liquid media (MacDonell & Hood 1982, Allen-Austin et al. 1984). However, and although in our own work Kaprelyants & Kell 1993 we could exclude these arguments on quantitative grounds, these other studies were subjected to the criticism that the resuscitation observed might merely be the regrowth of the small number of cells that

were viable prior to the resuscitation (Allen-Austin et al. 1984; Rose et al. 1990; Morgan et al. 1991). More recently by using the Most Probable Number (MPN) method we resuscitated cells in media which (statistically) contained no 'initially-viable' cells and found a huge (1000 to 100,000-fold) increase in the number of potentially culturable (viable) bacteria in starved populations as compared to the numbers determined by the agar plate method. The important condition for such a resuscitation was the addition of sterile supernatant taken from normal batch culture of *M. luteus*. It was concluded (Kaprelyants et al. 1994) that viable cells can produce a factor which stimulates the resuscitation of dormant cells. By monitoring by flow cytometry the kinetics of reappearance of various cell properties during the resuscitation we confirmed this suggestion and found that a small fraction of viable cells at the onset of resuscitation facilitated the recovery of the majority of the remaining (dormant) cells, again presumably due to the excretion of some factor(s) which promote the transition of cells to a colony-forming state (Votyakova et al. 1994). However the chemical nature and mechanism of action of this factor remain to be elucidated, as well as the processes which take place during the initial stages of resuscitation.

In the present study we found that during the resuscitation of starved cells without the supernatant from viable cell cultures, the dormant cells are able to undergo a limited number of divisions but that they excrete an antibacterial substance(s), presumably inhibiting their further multiplication.

Materials and methods

Organism and media

Micrococcus luteus NCIMB 13267 (previously described as 'Fleming strain 2665') was grown aerobically at 30° C in shake flasks (30 ml medium volume) in a lactate minimal medium containing L-lactate, exactly as described previously (Kaprelyants & Kell 1992).

When the culture had reached stationary phase (some 4.10^9 cells ml^{-1}), agitation was continued at 30° C for up to 3 months. Cultures were then held aerobically at room temperature without agitation for periods of up to a further 3–9 months. To avoid growth of any "initially" viable (culturable) cells resuscitations were performed under MPN-like conditions (i.e. at high dilution) using lactate minimal medium supplemented by 0.05% of yeast extract as described (Kaprelyants et al. 1994). No penicillin pretreatment was performed. Test tubes were incubated aerobically at 30° C with agitation during 120–180 h. In some experiments resuscitation medium was diluted by supernatant (in amounts ranging from 10–100% v/v) taken from late-logarithmic phase batch cultures of *M. luteus* grown on lactate minimal medium.

For preparing sterile supernatant, fresh cultures of *M. luteus* were grown in lactate minimal medium until late logarithmic phase ($OD^{1\,cm}_{650\,nm}$ = 1.8–2.2) was centrifuged and supernatants were passed through a 0.22 μm filter (Gelman or Millipore).

Estimation of antibacterial activity

To test antibacterial activity during resuscitation 4.10^2 to 4.10^7 of early stationary phase *M. luteus* cells were added to test tubes (2 ml) that contained either starved cells that had been incubated in lactate minimal medium containing 0.05% w/v yeast extract for an appropriate time, or filtered (0.22 μm filter) liquid from these cultures. In control experiments the same concentration of viable cells was inoculated into resuscitation medium that was either fresh or had been kept for 70–120 h at 30° C without starved cells or supernatant from these cells. All test tubes were incubated with agitation at 30° C for up to 180 h. In the disc method 30–100 μl of filtered culture liquid taken from starved cells (initial concentration 400 ml^{-1}) that were incubated for 96 h in lactate minimal medium containing 0.05% yeast extract and was added to a 1 cm diameter paper filter disc placed on the surface of an agar plate containing nutrient broth E (Lab M) on which had been spread a lawn of freshly harvested *M. luteus*. Plates were kept at 30° C for 120 h.

Colony-forming units and total cell counts

The number of viable cells (cfu) was judged by plating on agar plates containing Nutrient Broth E (Lab M) and 1.5% w/v purified agar, Lab M No1, as described (Kaprelyants & Kell 1992). Total cell counts were performed on unstained cells using a phase-contrast microscope and a modified Neubauer counting chamber. Under low cell concentration the samples were concentrated by centrifugation before counting.

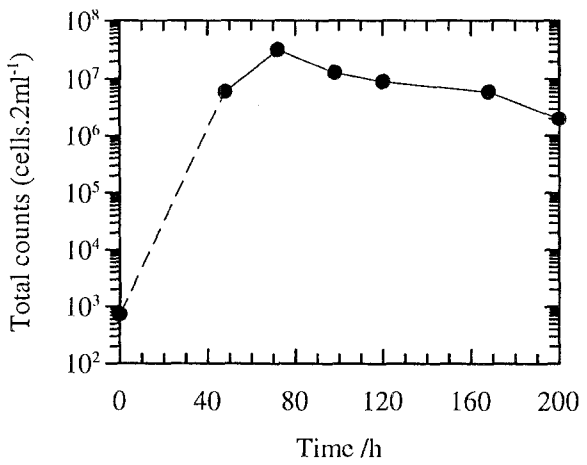

Fig. 1. Changes in the total counts of diluted starved *M. luteus* culture during incubation in growth medium. 9-month-old cells (total count 7.10^9 cells.ml^{-1}), viable counts 5.10^5 ml^{-1} were diluted and inoculated into test tubes containing 2 ml of lactate minimal medium with 0.05% yeast extract to a final cell concentration of 360 ml^{-1}. 0.5–2 ml of culture were concentrated by centrifugation and cells were counted microscopically.

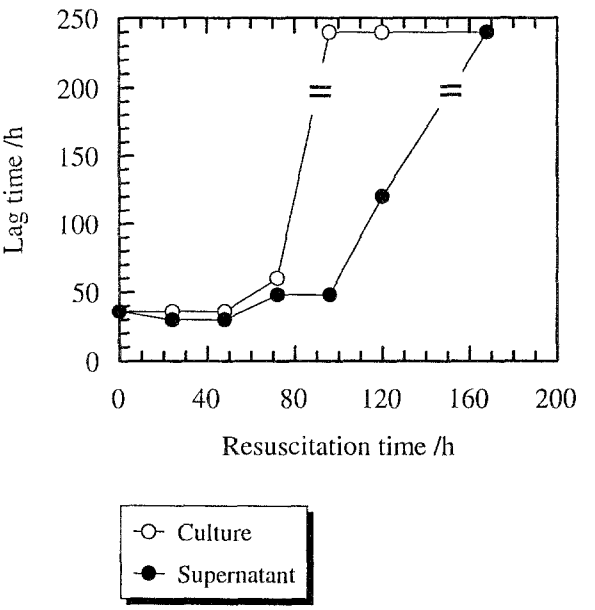

Fig. 2. Lag periods before the visible growth ($OD^{1cm}_{650nm} \geq 0.5$) of fresh *M. luteus* inoculated into test tubes with diluted, starved *M. luteus* cells or with filtered supernatant from these cultures at different time of starved cells incubation. The experimental conditions are similar to those described in the legend to Fig. 1. Viable, late logarithmic phase cells were added at a concentration of 700 ml^{-1} to a culture of starved bacteria (open circles) or to supernatant prepared from these cultures by filtration (closed circles). For points marked as 240 h there was *no* growth for at least this period. There was no growth in test tubes containing starved bacteria without the addition of viable bacteria.

Chemicals

Nutrient Broth E, yeast extract, agar were obtained from Lab *M.* L-lactate (Li salt), was obtained from Sigma. Other chemicals were of analytical grade.

Results

In order to shed further light on the behavior of *M. luteus* cultures during resuscitation we incubated small numbers of starved *M. luteus* cells in 2 ml lactate minimal medium containing 0.05% yeast extract. In all experiments, the test tubes with these diluted samples did not contain any viable (colony-forming) cells at the onset of resuscitation, and the majority of such cells under these conditions have been shown to be dormant (Kaprelyants & Kell 1993; Kaprelyants et al. 1994). Figure 1 shows that under such conditions the total count of cells nevertheless actually increased within 70 h from 700 to 3.10^7 (the latter being equivalent to an OD^{1cm}_{650nm} of 0.015–0.02), which corresponds to 16 generations. (The range in 6 independent replicates of this experiment was 10–17 generations, not shown.) Further incubation led to a decrease in the total count and to cell degradation and lysis (as observed by light microscopy, data not shown). Parallel measurements revealed that no colony-forming cells appeared in samples taken during the 200 hours of incubation and plated onto agar plates which were incubated for up to a month, suggesting that the cells could maintain their nonculturability for at least the stated period of incubation in lactate minimal medium with 0.05% yeast extract. This is in agreement with our earlier report.

To elucidate the possible reasons for the cessation of cell multiplication (in a medium which would have supported 5.10^9 cells.ml^{-1}), we inoculated tubes containing starved bacteria with a low concentration of freshly harvested (late logarithmic phase), viable micrococci at different times after the onset of resuscitation, and studied the effect of this on the lag time before the viable cells exhibited visible turbidity. The results of these experiments (Fig. 2) revealed the inhibition of bacterial growth when cells were added more than 50 h after the beginning of resuscitation. If viable cells were added to tubes with starved bacteria that had been incubated for more than 80 h they did not grow during more than 10 days of incubation. How-

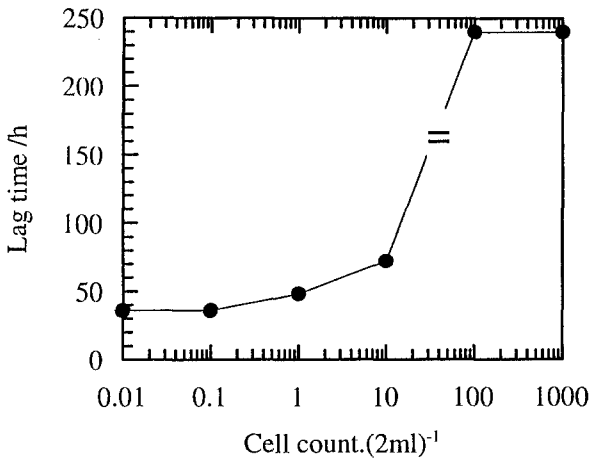

Fig. 3. Effect of the number of starved *M. luteus* cells on the lag period before visible cell growth after addition of fresh late logarithmic phase cells. 700 viable cells.ml^{-1} were added to starved *M. luteus* cells that had been incubated for 96 h. Conditions were otherwise similar to those described in the legends to Fig. 1 and 2. For points marked as 240 h there was *no* growth for at least this period.

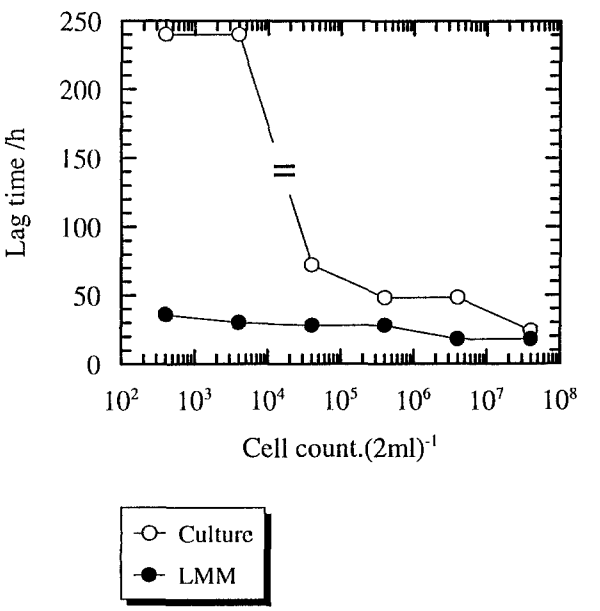

Fig. 4. Effect of the number of viable *M. luteus* cells on the lag period before visible increase in cell growth. Late log cells were added to the lactate minimal medium with 0.05% yeast extract (closed circles) or to the culture of starved *M. luteus* cells incubated during 120 h (open circles). Conditions are similar to those described in the legends to Fig. 1 and 2. For points marked as 240 h there was *no* growth for at least this period.

ever, the simple incubation of resuscitation medium for 70–200 h alone (i.e. without any dormant cells being present) before inoculation with viable cells did not influence the cell growth. Figure 3 provides further evidence that the antibacterial action of incubated starved cultures is indeed connected with the presence of starved cells, since their initial concentration significantly influenced the growth of added viable cells. A similar antibacterial effect was found when viable cells were inoculated into supernatants obtained after filtration of cultures with the appropriate cell concentration and time of resuscitation (Fig. 2). The filter disc method (see Materials and methods) of antibacterial assay also demonstrated the presence of an antibacterial substance in filtered supernatants (as the development of a zone of nongrowing *M. luteus* cells around a disc containing a drop of supernatant, not shown).

The antibacterial factor(s) found in the supernatants of starved bacteria incubated in resuscitation medium preserved their activity after autoclaving or freezing, but disappeared after dialysis. Similar experiments in liquid media revealed that this factor was also active against *E. coli* and *Brevibacterium ammonigenes* (data not shown). The experiments with *E. coli* and *B. ammoniagenes* were done exactly as were those described for *M. luteus* when we tested antibacterial properties using liquid medium (see Methods): late log cells at a concentration of 10^3 cells/2 ml were added to test tubes with lactate minimal medium and 0.05% yeast extract (control) or to a culture of starved *M. luteus* cells that had been incubated for 120 h. After further incubation of tubes for 240 h there was no growth in the test tubes containing starved cultures in contrast to control tubes.

The effectiveness of the antibacterial activity of the dormant, resuscitating culture was found to be dependent on the concentration of viable cells added to the culture after 120 h of preincubation (Fig. 4): the addition of 4.10^7 cells to test tubes (2 ml) containing starved cultures incubated in resuscitation medium resulted in fast growth while 10^4 cells didn't grow under the same conditions (control experiments showed cell growth at all concentrations when added to medium preincubated without starved bacteria (Fig. 4)).

To establish whether the antibacterial activity of starved cultures can be related to our earlier report of the resuscitation-promoting activity of supernatants taken after centrifugation of normal, batch cultures with high viability (Kaprelyants et al. 1994), we studied the influence of such supernatants on the growth of viable cells in test tubes with starved, resuscitat-

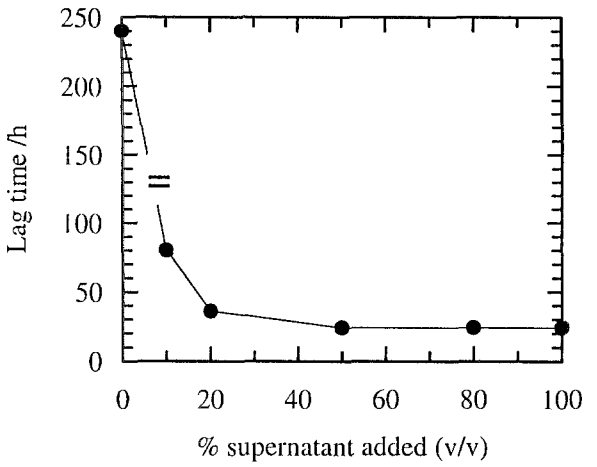

Fig. 5. Effect of the supernatant of late-log phase *M. luteus* culture on the lag period before visible growth was observed after the addition of viable *M. luteus* cells to starved bacteria incubated in resuscitation medium. Conditions are similar to those described in the legends to Figs 1 and 2. 700 viable cells.ml^{-1} were added to starved *M. luteus* cells that had been incubated in lactate minimal medium containing 0.05% yeast extract for 96 h. For points marked as 240 h there was *no* growth for at least this period. Similarly, there was no growth in test tubes containing starved bacteria without the addition to viable bacteria.

ing populations. We found that a mixture of cultures of starved cells that had been incubated for 96 h with filtered supernatant from a culture of late logarithmic-phase micrococci resulted in the restoration of growth of viable cells added to the mixture at a concentration of 700 ml^{-1} (Fig. 5). The same effect was obtained when instead of starved cells that had been incubated in resuscitation medium we used the supernatant of this culture. Simple dilution of starved cell culture with the medium used for resuscitation in the proportion 1 : 1 or 1 : 2.5 did not result in any growth of the test bacteria. These experiments indicated the inactivation of the antibiotic properties of resuscitating starved cultures by the supernatants from viable cultures.

Discussion

The important result of the experiments described here is the finding of the ability of starved, cells that are not observably culturable on solid media to undergo a limited number of divisions under conditions that were previously employed for the resuscitation of dormant cultures of *M. luteus*.

The ability of starved cells to exhibit only a limited number of doublings that we observed is reminiscent of the cognate behavior of some eukaryotic cells in cell cultures (the Hayflick effect; Hayflick & Moorhead 1961). In principle, the observed cessation of growth might be due to 'endogenous reasons' such as mutations consequent upon prolonged starvation, or as a result of the appearance of some antibacterial compound(s) in the culture medium during cell incubation. The results obtained in this study lend strong support to the latter possibility. The data in Fig. 2, and those obtained using the paper disc method, clearly show the excretion of an antibiotic substance 70 h after the onset of resuscitation of starved bacteria. Whether all bacteria or only a fraction of the starved population are responsible for the antibiotic excretion remains to be clarified. At all events, the production of this factor is definitely connected with the resuscitation of dormant cells since incubation of starved cells in minimal medium without lactate and yeast extract for a long time led neither to cell multiplication, nor to resuscitation, nor to any antibacterial effect (data not shown). Weichart et al. (1992) also reported the production of an antimicrobial factor excreted by *V. vulnificus* during the transition of the culture to a nonculturable state caused by lowering the temperature. The supernatant of such cultures decreased the culturability of *V. vulnificus* cells, although it was not stated if cells also produced this factor under conditions in which culturable cell numbers increased (Weichart et al. 1992). *E. coli* starved in the stationary phase also revealed a 'killing effect' with respect to nonstarved cells, although in this case the authors failed to find a 'killer' in the supernatant; it was concluded (Zambrano et al. 1993) that the accumulation of particular mutants during cell starvation could lead to cell death due to cell competition in such a mixed culture. Finally, Shimkets (1990) reviews a number of studies (Wireman & Dworkin 1975, 1977; Varon et al. 1984, 1986; Rosenbluh & Rosenberg 1989; Rosenbluh et al. 1989) in which it has been shows that *Myxococcus xanthus* normally produces an autocide, AMI, during its developmental transition to form a fruiting body (spore).

The antibacterial activity of starved cells could be revealed only when the concentration of viable cells added to starved cultures or to supernatants was below a certain level (Fig. 4). Two interpretations of this are that (i) the antibacterial factor excreted by starved cells is present in limited amounts and is tightly bound upon interaction with the target cells, or (ii) viable cells can inactivate the factor. While we cannot exclude the sig-

nificance of the first mechanism, the data in Fig. 5 support the second. Earlier we found that the number of potentially viable cells in *M. luteus* populations after 3–6 months of starvation could be dramatically increased after dilution of appropriate medium used for cell growth in MPN experiments with supernatants taken from normal late log phase culture (Kaprelyants et al. 1994). Recently, by using flow cytometry we found that the presence of viable cells at the onset of resuscitation of starved culture is otherwise vital for the resuscitation of nonculturable cells. In conditions which excluded the presence of viable cells resuscitation was unsuccessful unless supernatant was added (Kaprelyants et al. 1994; Votyakova et al. 1994). On the basis of the present results it is tempting to suggest that one of the roles of such supernatants could lie in the inactivation of an antibacterial factor produced by starved bacteria. MacDonell & Hood (1982) reported the conversion of nonculturable cells to colony-forming ones by repeated passages ('conditioning') of cells from plate to plate. Plausibly the role of such passages could be the stepwise 'washing' of cells and removal of an inhibitor produced during cell resuscitation on the agar surface. The ratio between the concentration of antibacterial factor and its inactivating factor from the supernatant of viable cultures can determine the final result of *M. luteus* resuscitation as judged by the MPN-like method described earlier (Kaprelyants et al. 1994), and may well be the reason for the significant noise in the stated results. We also found that the variation of the time when 'viable' supernatant is added to test tubes during the incubation of starved cultures in MPN experiments significantly influenced cell resuscitation (not shown), in that administration of the supernatant from viable cultures after 45 h of incubation in resuscitation medium normally led to much higher final MPN counts than when the supernatant was present from the outset.

These experiments clearly, and for the first time, demonstrate the secretion of an antibacterial substance during the resuscitation of dormant bacteria. Such secretions may well be a general phenomenon for starved cells and this should therefore be taken into consideration during resuscitation when employing a dilution method to avoid the presence of any viable cells at the beginning of the resuscitation (Allen-Austin et al. 1984; Rose et al. 1990; Morgan et al. 1991). Complementarily, while even a relatively small number of viable cells can make the results of similar experiments (Allen-Austin et al. 1984; Colwell et al. 1985; Nilsson et al. 1991; Weichart et al. 1992) difficult to interpret, such conditions are arguably just those which are necessary for the resuscitation of nonculturable cells. An important task for the future is the elucidation of the nature, specificity and chemical structure of the antibacterial factor(s) and its inactivator(s), as well as the mechanisms of their action. Finally, we would stress that the resuscitation-promoting and resuscitation-inhibiting factors reveal the importance of cell-cell interactions in the phenomenon of dormancy, a phenomenon long foreshadowed as the 'population effect' showing a cell density-dependent cell survival in starved cultures (Harrison 1960; Postgate & Hunter 1962; Strange & Dark 1962; Strange & Shon 1964; Postgate 1976). It is now becoming evident that signal molecules can influence cell density-dependent biochemical processes in a very wide variety of prokaryotes (e.g. Stephens 1986; Horinouchi & Beppu 1990, 1992; Khokhlov 1991; Jones et al. 1992; Kim et al. 1992; Kuspa et al. 1992; Williams et al. 1992; Kaiser & Losick 1993; Piper et al. 1993; Swift et al. 1993; Zhang et al. 1993; Fuqua et al. 1994; Pearson et al. 1994; Kell et al. 1995).

Acknowledgements

We are indebted to the Biotechnology Directorate of the Science and Engineering Research Council, U.K., and the Royal Society under the terms of the Royal Society/Russian Academy of Sciences Joint Project scheme, for financial support of this work. We thank Professors J. Gareth Morris and John Postgate for many useful discussions.

References

Allen-Austin D, Austin B & Colwell RR (1984) Survival of *Aeromonas salmonicida* in river water. FEMS Microbiol. Lett. 21: 143–146

Barer MR, Gribbon LT, Harwood CR & Nwoguh CE (1993) The viable but non-culturable hypothesis and medical microbiology. Rev. Med. Microbiol. 4: 183–191

Colwell RR, Brayton BR, Grimes DJ, Roszak DB, Huq SA & Palmer LM (1985) Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: implications for release of genetically engineered microorganisms. Bio/Technology 3: 817–820

Fuqua WC, Winans SC & Greenberg EP (1994) Quorum sensing in bacteria – the luxR-luxI family of cell density-responsive transciptional regulators. J. Bacteriol. 176: 269–275

Harrison AP, Jr (1960) The response of *Bacterium lactis aerogenes* when held at growth temperatures in the absence of nutriment: an analysis of survival curves. Proc. R. Soc. B 152: 418–428

Hayflick L & Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res. 25: 585–621.

Horinouchi S & Beppu T (1990) Autoregulatory factors of secondary metabolism and morphogenesis in actinomycetes. CRC Crit. Rev. Biotechnol. 10: 191–204

— (1992) Autoregulatory factors and communication in actinomycetes. Ann. Rev. Microbiol. 46: 377–398

Jones S, Yu B, Bainton NJ, Birdsall M, Bycroft BW, Chhabra SH, Cox AJR, Golby P, Reeves PJ, Stephens S, Winson MK, Salmond GPC, Stewart GSAB & Williams P (1993). The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. EMBO J. 12: 2477–2482

Kaiser D & Losick R (1993) How and why bacteria talk to each other. Cell 73: 873–885

Kaprelyants AS, Gottschal JC & Kell DB (1993) Dormancy in non-sporulating bacteria. FEMS Microbiol. Rev. 104: 271–286

Kaprelyants AS & Kell DB (1992) Rapid assessment of bacterial viability and vitality using rhodamine 123 and flow cytometry. J. Appl. Bacteriol. 72: 410–422

— (1993) Dormancy in stationary-phase cultures of *Micrococcus luteus*: flow cytometric analysis of starvation and resuscitation. Appl. Env. Microbiol. 59: 3187–3196

Kaprelyants AS, Mukamolova GV & Kell DB (1994) Estimation of dormant *Micrococcus luteus* cells by penicillin lysis and by resuscitation in cell-free spent culture medium at high dilution. FEMS Microbiol. Lett. 115: 347–352

Kell DB, Kaprelyants AS, Grafen A (1995) On pheromones, social behaviour and the function of secondary metabolism in bacteria. Trends Ecol. Evolution., in press

Kell DB, Ryder HM, Kaprelyants AS & Westerhoff HV (1991) Quantifying heterogeneity: flow cytometry of bacterial cultures. Antonie van Leeuwenhoek 60: 145–158

Khokhlov AS (1991) Microbial autoregulators. Harwood Academic Press

Kim SK, Kaiser D & Kuspa A (1992) Control of cell density and pattern by intercellular signalling in *Myxococcus* development. Ann. Rev. Microbiol. 46: 117–139

Kuspa A, Plamann L & Kaiser D (1992) A-signalling and the cell density requirement for *Myxococcus xanthus* development. J. Bacteriol. 174: 7360–7369

MacDonell MT & Hood MA (1982) Isolation and characterization of ultramicrobacteria from a gulf coast estuary. Appl. Environ. Microbiol. 43: 566–571

Morgan JA, Cranwell PA & Pickup R (1991) Survival of *Aeromonas salmonicida* in lake water. Appl. Env. Microbiol. 57: 1777–1782

Morita RY (1990) The starvation-survival state of microorganisms in nature and its relationship to bioavailable energy. Experientia 46: 813–817

Nilsson L, Oliver JD & Kjelleberg S (1991) Resuscitation of *Vibrio vulnificus* from the viable but nonculturable state. J. Bacteriol. 173: 5054–5059

Oliver JD (1993) Formation of viable but nonculturable cells. In: Kjelleberg S (Ed) Starvation in Bacteria. (pp 239–272) Plenum, New York

Pearson JP, Gray KM, Passador L, Tucker KD, Eberhard A, Iglewski B & Greenberg EP (1994) Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. Proc. Natl. Acad. Sci. 91: 197–201

Piper KR, Beck von Bodman S & Farrand SK (1993) Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. Nature 362: 448–450

Postgate JR (1976) Death in macrobes and microbes. Symp. Soc. Gen. Microbiol. 26: 1–18

Postgate JR & Hunter JR (1962) The survival of starved bacteria. J. Gen. Microbiol. 29: 233–263

Rose A, Ellis A & Munro A (1990) Evidence against dormancy in the bacterial fish pathogen *Aeromonas salmonicida*. FEMS Microbiol. Lett. 68: 105–108

Rosenbluh A, Nir R, Sahar E & Rosenberg E (1989) Cell density-dependent lysis and sporulation of *Myxococcus xanthus* in agarose microbeads. J. Bacteriol. 171: 4923–4929

Rosenbluh A & Rosenberg E (1989) Autocide AMI rescues development in *dsg* mutants of *Myxococcus xanthus* in agarose microbeads. J. Bacteriol. 171: 1513–1518

Roszak DB & Colwell RR (1987) Survival strategies of bacteria in the natural environment. Microbiol. Rev. 51: 365–379

Shimkets LJ (1990) Social and developmental biology of the myxobacteria. Microbiol. Rev. 54: 473–501

Stephens K (1986) Pheromones among the prokaryotes. CRC Crit. Rev. Microbiol. 13: 309–334

Strange RE & Dark FA (1962) Effect of chilling on *Aerobacter aerogenes* in aqueous suspension. J. Gen. Microbiol. 29: 719–730

Strange RE & Shon M (1964) Effects of thermal stress on viability and ribonucleic acid of *Aerobacter aerogenes* in aqueous suspension. J. Gen. Microbiol. 34: 99–114

Swift S, Winson MK, Chan PF, Bainton NJ, Birdsall M, Reves PJ, Rees CED, Chhabra SR, Hill PJ, Throup JP, Bycroft BW, Salmond GPC, Williams P & Stewart GSAB (1993) A novel strategy for the isolation of *luxI* homologues: evidence for the widespread distribution of a luxR : luxI superfamily in enteric bacteria. Mol. Microbiol. 10: 511–520

Varon M, Cohen S & Rosenberg E (1984) Autocides produced by *Myxococcus xanthus*. J. Bacteriol. 160: 1146–1150

Varon M, Tietz A & Rosenberg E (1986) *Myxococcus xanthus* autocide AMI. J. Bacteriol. 167: 356–361

Votyakova TV, Kaprelyants AS & Kell DB (1994) Influence of viable cells on the resuscitation of dormant cells in *Micrococcus luteus* cultures held in extended stationary phase. The population effect. Appl. Env. Microbiol. 60: 3284–3291

Weichart D, Oliver JD & Kjelleberg S (1992) Low temperature induced non-culturability and killing of *Vibrio vulnificus*. FEMS Microbiol. Lett. 100: 205–210

Williams P, Bainton NJ, Swift S, Chhabra SR, Winson MK, Stewart GSAB, Salmond GPC & Bycroft BW (1992) Small molecule-mediated density-dependent control of gene-expression in prokaryotes – bioluminescence and the biosynthesis of carbapenem antibiotics. FEMS Microbiol. Lett. 100: 161–167

Wireman JW & Dworkin M (1975) Morphogenesis and developmental interactions in myxobacteria. Science 189: 516–522

— (1977) Developmentally induced autolysis during fruiting body formation by *Myxococcus xanthus*. J. Bacteriol. 129: 796–802

Zambrano MM, Siegele DA, Almiron M, Tormo A & Kolter R (1993) Microbial competition. *Escherichia coli* mutants that take over stationary phase cultures. Science 259: 1757–1760

Zhang LH, Murphy PJ, Kerr A & Tate ME (1993) *Agrobacterium* conjugation and gene-regulation by N-acyl-L-homoserine lactones. Nature 362: 446–448