

or absence of other species and this may indicate the occurrence of interactive segregation resulting from interspecific competition. For instance, Welton (pers. comm.) concludes that stone loach and bull-head partition resources in chalk streams by adopting different habitats. Cowx (1989) showed that when dace and roach depart from their "preferred" habitat, or co-exist in the River Exe catchment, the growth rate of one or the other declines. That study was unable to identify whether this was caused by resource partitioning, as a result of interspecific competition, or by habitat selection independent of species interaction. Therefore a further part of this study will be concerned with the elucidation of the mechanisms behind observed segregation and/or habitat shifts, by employing methods such as removal experiments and observations with remote automatic devices.

Ultimately, at the end of three years I hope to more closely define the habitat requirements of some of the above-mentioned fish species throughout their life-history, and for some species to explain how and why habitat-use changes in the presence or absence of other species. Both of these aims should provide information useful to the management of fisheries, leading to recommendations for their maintenance and improvement.

Request for co-operation from landowners and anglers

Because this study will involve the use of many different stretches of river it can only be achieved with the co-operation and good-will of land-owners, angling bodies, and water authorities. Whilst we shall be approaching some of these bodies responsible for suitable waters ourselves, we would very much like to hear from landowners or angling clubs/associations in southern England who would give permission for us to use their water in this study. It would involve a 1- or 2- day visit in a year, during which a variety of measurements would be taken, and an electrofishing survey completed. It will not be necessary to kill any fish. At the same time it will give those interested the opportunity to see at close hand the fish living in their waters; they should initially make contact with Mr A. Ibbotson or Dr M. Ladle at the River Laboratory, Wareham.

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GENETIC ENGINEERING OF MICROORGANISMS: FREE RELEASE INTO THE ENVIRONMENT

J. ALUN W. MORGAN

Introduction

Genetic engineering now makes possible the insertion of DNA from many organisms into other prokaryotic, eukaryotic and viral hosts. This technology has been used to construct a variety of such genetically engineered microorganisms (GEMs). The possibility of accidental or deliberate release of GEMs into the natural environment has recently raised much public concern. The prospect of deliberate release of these microorganisms has prompted an increased need to understand the processes of survival, expression, transfer and rearrangement of recombinant DNA molecules in microbial communities. The methodology which is being developed to investigate these processes will greatly enhance our ability to study microbial population ecology. Once release has occurred, it is generally accepted that recovery of these cells from the natural environment can not be performed. Detecting their presence is the most that one can expect to achieve.

Considerable effort in the construction of GEMs was initially placed on creating both microorganisms and cloning systems (plasmids used in the creation of recombinant DNA) that were considered "safe". These measures were designed to ensure that accidentally released strains would not survive or transfer their "novel" genetic material in the environment. The safeguards included debilitated host bacteria and cloning vectors, incapable of replication or transfer except under defined laboratory conditions. Such biological disabilities were used in conjunction with strict physical containment standards to reduce the likelihood of GEMs entering the environment. Since no hazardous consequences from work with recombinant DNA have been reported such safeguards are considered to have been successful. However, since an accident of this nature could have resulted in very subtle changes, this statement may only reflect our lack of understanding of the environment. Unlike accidental release it is impractical to disable GEMs that are designed for deliberate release. Strains are currently being constructed to survive at least for a limited time in specific target environments. Survival, to allow completion of their intended function, is essential for the application of most of these strains.

The potential benefits from releasing GEMs into the environment are

considered to be great. The microbial detoxification of pollutants may play an important part in reducing environmental contamination, and genetic engineering may improve these abilities. Strains have also been constructed to protect plants from frost damage, to extend the growing season. The application of this technology may be broadened further to include the construction of recombinant vaccines and pesticides. An issue central to the successful application of releasing GEMs is the ability of the strain to perform its desired function within the environment. To allow this, the host would normally have to survive and remain active for a period of time within the environment.

Currently the deliberate release of GEMs is restricted. The few reported cases in the UK and USA have been cautious in design (Gaertner & Kim 1988). This leads to difficulties in assessing the hazards associated with the release of GEMs. An engineered microorganism cannot be released into the natural environment until it is considered to be safe. Safety may have to be determined directly in the environment, since data from the laboratory or enclosed systems may not be suitable in predicting their behaviour after release. The problem may be resolved by performing multistage tests, from laboratory systems through containment facilities to small field plots, before any GEM is considered suitable for large-scale release. Unfortunately a position where there is no risk cannot be achieved and such multistage tests are aimed at limiting the uncertainties in order to minimise the risk. An overall balance between the potential benefits and potential risks of releasing GEMs currently needs to be addressed on a case by case basis. The risk related to the release of GEMs may be considered as the inadvertent creation of a pathogen. Abelson (1977) used a worst case model in which an *Escherichia coli* host carrying polyoma DNA (DNA from viruses that induce tumors) may be found to induce tumors in animals. Although pathogenicity is an important factor in risk assessment, this is likely to be governed by the character or nature of the release strain rather than by the recombinant DNA it carries. More recently concern has been focused on environmental effects, such as changes in primary production, material processing, nutrient cycling, extreme fluctuations in the natural population, altered species diversity, or the loss of critical species.

Marked strains

A simple and generally effective method to identify a particular microorganism is to provide it with a specific genetic marker, or a series of markers that are unlikely to occur at a detectable frequency in the natural environment. The first markers used to study the fate of recombinant plasmids in natural environments relied upon experiments involving

DNA encoding resistance to antibiotics and heavy metals. Any attempts to monitor antibiotic resistance in the aquatic environment are likely to be obscured by a high background of naturally occurring antibiotic-resistant bacteria. The simplest DNA markers are small oligonucleotide sequences synthesised in the laboratory. These markers have been used to tag insect viruses for their identification from similar naturally occurring viruses (Bishop et al. 1988).

Markers where complete genes are used are selected on the assumption that they would offer no advantage to their host. Colorimetric markers such as the β -galactosidase system for fluorescent pseudomonads have been developed. These are limited by the requirement for selection on solid growth media (Drahos et al. 1986) upon which many aquatic bacteria cannot be isolated. As direct detection methods have become more practicable, marker systems not requiring the culture of the microbe have been developed. One such system follows the emission of light from bacterial cells containing genes for bioluminescence, which offers the possibility for very sensitive detection (Shaw & Kado 1986). The presence of naturally occurring luminescent bacteria in aquatic systems, along with the presence of similar genes for bioluminescence that are present within cells that do not emit light (genes that are not phenotypically expressed), limits the potential usefulness of this system in lake water.

If the marker gene offers no apparent advantage then the region of DNA in front of the gene, the promoter, which governs a gene's expression, may play an important role in determining its survival. A promoter which directs high level expression could impose a heavy metabolic burden on its host. On the other hand, low level expression may limit detection of the gene product and reduce sensitivity. A regulated expression system where the promoter can be switched on or off, as required, would overcome these problems. The regulation of a specific viral promoter (λP_R) by its repressor protein (cl_{857}) has been widely exploited to control the high level expression of many genes. At temperatures below 28°C the repressor protein effectively prevents gene expression from the λ promoter. At temperatures above this (up to 42°C), the control is inactivated and high level expression of the gene occurs. Therefore, in lake water where bacterial growth may be below 28°C, control of gene expression should occur, resulting in a low metabolic burden being placed on the cell. In the laboratory the temperature can be increased, resulting in high level gene expression which can be used for the detection of marked cells.

In using such controlled high level expression systems it is important to select a suitable gene, since high levels of the gene product must be tolerated by the host cell. The marker system developed at the Freshwater Biological Association, Windermere, and the Department of Genetics and Microbiology, Liverpool (Winstanley et al. 1989), is based on

the high level expression of a gene isolated from a toluene degradative plasmid, TOL (*xyIE* gene, Fig. 1). This gene encodes an enzyme, catechol 2,3 dioxygenase, that cleaves catechol (a colourless compound) to 2-hydroxymuconic semialdehyde (a bright yellow product). In previous studies, high levels of catechol 2,3 dioxygenase within bacteria have been shown to have no detrimental effect on the cell. The fact that this gene has been sequenced and its product characterized also aided in the development of detection methods.

Survival

GEMs have been constructed for release into a wide range of natural environments. Since a target environment cannot be isolated from neighbouring environments, the release strain also needs to be tested in non-target environments. As a consequence of water disposal systems, drainage of industrial effluent and agricultural run-off, rivers, lakes and their sediments may become the ultimate sink for released microorganisms. Since the release of GEMs into open environments is restricted, laboratory based systems are being used. The model laboratory system (microcosm) is designed to mimic the natural environment, or part of it, and to analyse the behaviour of microorganisms in it. Microcosms have been used to study the interactions between pollutants and bacteria. More recently they have been used to monitor the survival of recombinant bacteria and the transfer of recombinant DNA. In microcosms, it has been suggested that a community establishes itself which is unique relative to the original environment. Therefore, these systems are limited in their ability to mimic natural systems. In addition, it would be impossible to reproduce the diversity associated with such natural environments in these small laboratory based systems. However, the pattern of survival of a recombinant strain of *Pseudomonas fluorescens* that was released into the environment was accurately predicted by microcosm studies (Drahoš et al. 1988). Microcosms may become very useful in evaluating a new strain by using these results in a comparison with those available on GEMs released into laboratory microcosms and into the environment.

The risks related to the release of GEMs depend upon the establishment of recombinant DNA within the environment. Only if the host is able to survive, multiply or transfer its genetic material to other microorganisms in the environment will it have a lasting impact. Lake water contains a characteristic community of microorganisms; non-indigenous species that are introduced commonly decline. In general, microbial communities tend to resist invasion by exogenous microorganisms. Opposing this it has been found that *Escherichia coli*, *Klebsiella* spp., *Vibrio* spp. and *Candida albicans* will survive indefinitely and may

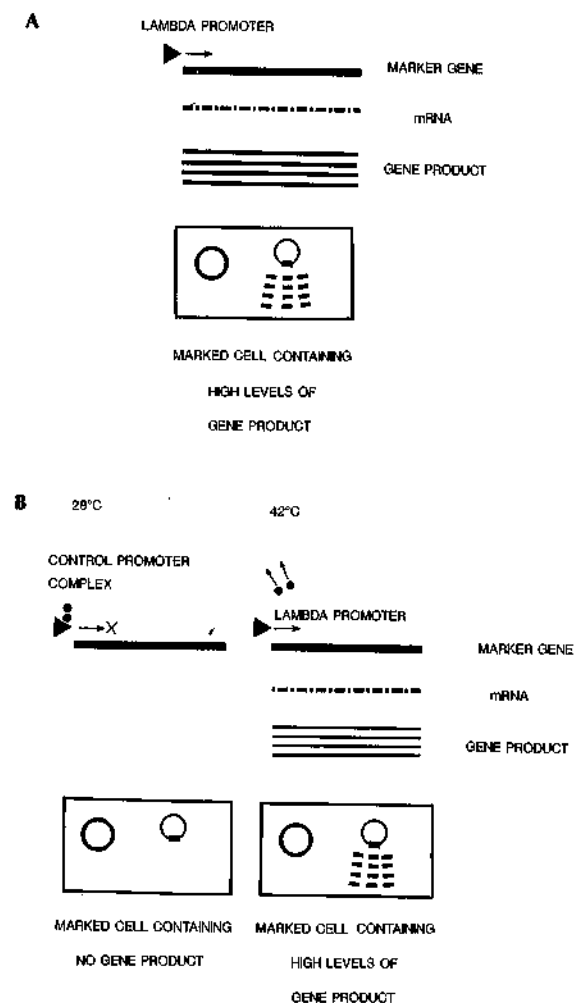


FIG. 1. Plasmid marker systems. A, high level expression system where the gene product is continually produced. B, controlled expression system where at 28°C no gene product is produced while at 42°C high level expression occurs. Chromosomal DNA, large circle; plasmid DNA, small circle; control protein, ●. Inhibition of expression of the gene product by the control proteins is at the level of the synthesis of messenger RNA (mRNA).

even grow in tropical waters (Carrillo et al. 1985; Lopez-Torres et al. 1987; Valdes-Collazo et al. 1987).

Engineering a microorganism to produce a desirable characteristic may serve to debilitate it and thus lessen the likelihood of its survival. In the absence of selection, recombinant DNA may be expected to impose a reduction in fitness probably relating to the burden associated with synthesis of gene products and not to the replication of the DNA. In addition the expression of new gene products may affect normal physiological processes and reduce the fitness of the host. The presence of foreign DNA has in some cases improved the growth rate of bacteria in the absence of any apparent selection (Edlin et al. 1984; Hartl et al. 1983), and a host may even change genetically to accommodate the presence of foreign DNA and improve its fitness (Bouma & Lenski 1988). Survival and plasmid transfer abilities in the natural environment would also depend on any selective advantage conferred. It is likely that the potential advantages of any recombinant DNA have to be considered on a case by case basis.

The survival of *Pseudomonas putida* strains in lake water containing the lambda-promoter marker system was investigated over a 28-day period. A typical set of release results are presented in Fig. 2. After an initial decline in numbers, populations released at high cell densities (above 10^4 colony-forming units, cfu ml⁻¹) survived the 28-day period. Smaller populations fell to undetectable levels. As well as monitoring the survival of hosts containing the controlled marker system (28°C off, 42°C on) a second marker where expression was not controlled (28°C on, 42°C on) was used in a similar survival study. No significant difference in the survival of the two marked hosts was found (M. Hurley, pers. comm.). This suggests that there was no benefit from controlled gene expression, which reduces the metabolic burden placed on the cells in lake water. However, any differences would have been masked by the great variability found in results from replicate experiments.

Detection of GEMs following release

Research into the ecology of microorganisms in the natural environment has focused mainly on their activity at a process level, where the flux of a particular element or compound is extensively examined using chemical methods. As a consequence little is known about the role of particular microorganisms, or about population and community structure even in environments of low complexity. It is well established that the inadequacies of conventional microbiological methods for studying environmental samples have contributed to this problem. Since at present only a small proportion of the bacteria observed in fresh water by direct counting techniques can be isolated, the true diversity of such

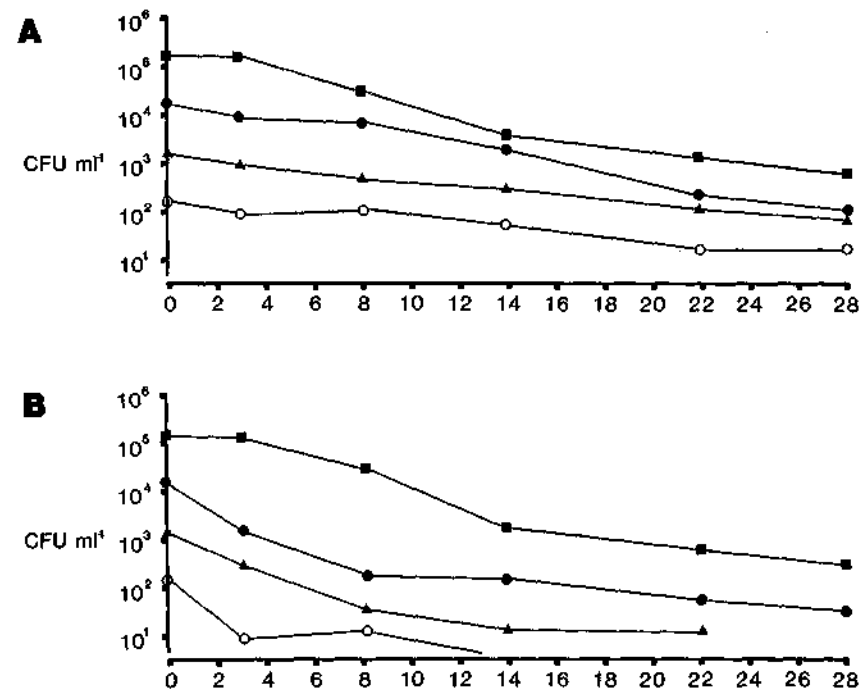


Fig. 2. Survival of *P. putida* in lake water over 28 days (colony-forming units per ml). A, *P. putida* containing the high level expression system; B, *P. putida* containing the controlled expression system.

microbial communities cannot be assessed (Jones 1987). The uncertainty in our ability to culture target microorganisms in environmental samples limits the value of techniques based on cell growth (such as colony hybridization, plasmid analysis, protein profiling and restriction endonuclease fingerprinting). Direct detection offers the advantage of eliminating the need to grow recovered cells.

The acridine orange direct count is commonly used to obtain total bacterial counts in environmental samples. Acridine orange binds DNA and RNA and is limited in its ability to detect viable cells. Kogure et al. (1979) describe a new method to detect possible viable cells. After the addition of nalidixic acid which inhibits DNA replication, and a low nutrient solution, viable cells elongate but do not divide. This method was used to identify non-culturable but viable pathogenic bacteria in environmental samples (Colwell et al. 1985). This method is limited in mixed populations by the presence of cells of varying length.

Fluorescent antibody staining (immunofluorescence microscopy) permits the detection of selected species or strains under the microscope (Bolhool & Schmidt 1980). This method offers the possibility of detecting GEMs only if the gene product is accessible on the cell surface. The fluorescent antibody approach has been used to identify bacteria in soil (Bolhool & Schmidt 1980), water (Ward & Carlucci 1985, and coal refuse (Apel et al. 1976). To distinguish viable cells from dead or particulate material, immunofluorescence has been used in conjunction with vital dyes and the direct viable count. Immunofluorescence flow cytometry offers a number of advantages over immunofluorescence microscopy in automation and sensitivity (Phillips & Martin 1988). However its application to environmental microbiology has so far been limited. Cells have been detected in solid phase immunoassays using antibodies directed towards cell surface components. However, the above methods have not been used in studies with GEMs but were designed to detect specific bacterial populations in environmental samples. We have investigated the potential for directly extracting total protein from environmental samples and attempting to quantify a target, or recombinant protein, by an enzyme-linked immunosorbent assay (ELISA) (Morgan et al. 1989).

Selective enrichment or recovery methods could also prove useful in detecting released strains. Ideally, to avoid problems associated with cell culture any approach would remain independent of cell growth. The method we have used was initially developed for tissue-typing blood cells. A specific monoclonal antibody was produced to react with the bacterial flagella of our release strain (*Pseudomonas putida* mt-2). This antibody was linked to small ($4.5\mu\text{m}$) magnetised polystyrene beads. When the beads were suspended in lake water, target release cells became attached to the beads. The bead cell complexes were then recovered with a magnet and washed (Fig. 3). The accuracy of the recovery process hinges on the specificity of the antibodies and the number of washes used to remove non-target cells. Flagella appear to be sufficiently variable to allow the production of strain specific antibodies. However, flagella appear to be relatively brittle and do not survive vigorous washing. Therefore, the immunocapture strategy could be directed either at the recovery of high numbers of target cells and accepting a low level of contaminating particles, or at obtaining a low number of pure target cells.

Recently the gene probe has been widely adopted as a new detection method. The probe follows the genome specifically and can be directed at a recombinant gene or a specific host sequence. For the detection of cells containing target sequences in the environment, DNA has been directly isolated from water (Somerville et al. 1989), sediments (Holben et al. 1988), and soils (Steffan & Atlas 1988). Most-probable-number determinations have been used to compare the relative abundance of

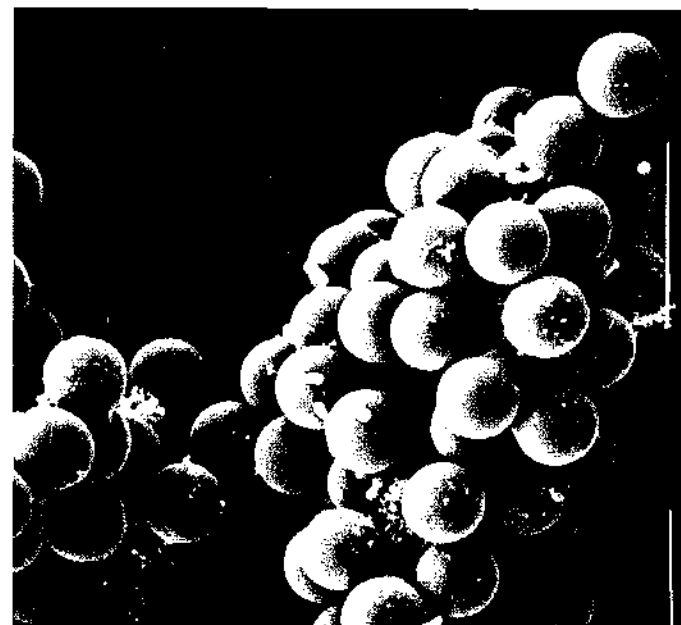


FIG. 3. *P. putida* cells attached to magnetic beads isolated from lake water samples (bar= $1\mu\text{m}$).

target sequences. The sensitivity of the DNA hybridisation method can be improved by amplifying the target sequence by polymerase chain reaction (PCR) (Steffan & Atlas 1988).

New detection techniques are needed to assess population and community structure. Without a basic knowledge of community structure the possible perturbations of natural populations caused by large scale release cannot be determined. Cultural methods suitable for the complete isolation of the aquatic microflora would be difficult, if not impossible to design. The development of new techniques which utilise macromolecular markers for profiling populations such as lipids and ribosomal RNA (Stahl et al. 1985) are promising but are at an early stage of development.

Potential for gene transfer in the natural environment

Many GEMs that are candidates for release into the open environment will probably be engineered to contain additional genes on the chromo-

some or on plasmids that are less likely to be transferred to indigenous populations. Chromosomal gene transfer and recombination in bacteria is by its very nature conservative due to a nominal requirement for homology in both donor and recipient. This restricts chromosomal gene transfer to species with related DNA. However, homology is only needed in the regions flanking the inserted gene and not within the gene itself; this might be an important factor when considering the possibility for chromosomal gene transfer. The RecA protein is central to the process of recombination. In *E. coli* and *Pseudomonas aeruginosa*, the expression of RecA protein has been induced by various environmental stresses (Walker 1984; Rolfe & Holloway 1966). This may increase the rate of recombination in the environment compared to those frequencies observed in the laboratory. The frequency of chromosomal gene transfer when determined by retrospective analysis, even within a population of closely related bacteria, may be relatively rare (Harrison et al. 1989). However, chromosomal gene transfer has been observed in bacteria introduced into both sterile and non-sterile soils (Krasovsky & Stotzky 1987). Plasmids and phages that have a discrete set of genetic markers are being used to provide both a workable genetic system in which transfer can be observed and to provide an upper limit for assessing the potential for gene transfer in the environment.

Conjugation is the transfer of plasmid DNA through cell to cell contact between both donor and recipient cells. It was initially considered that conjugation was the most likely route for gene transfer. Most information about gene transfer in the environment comes from studies on medically important bacteria which address a rather specific and possibly atypical subset of the bacterial population. The past occurrence of genetic events may be inferred by the nature and distribution of specific plasmids. For example, this type of approach was used to monitor the spread of antibiotic resistant plasmids (Barth & Grinter 1974), the evolution of plasmids encoding penicillin resistance in *Neisseria gonorrhoeae* (Saunders et al. 1986) and the distribution of degradative plasmids (TOL Plasmids) in *Pseudomonas putida* (Duggleby et al. 1977).

Much emphasis is currently placed on measuring gene transfer frequencies using model laboratory systems into which engineered strains are released. The information from such studies may be more appropriate as they mimic the deliberate or accidental release of GEMs. The transfer of plasmids from introduced bacteria by conjugation of mobilisation has been demonstrated in sewage treatment facilities (Mancini et al. 1987), lakes (O'Morchoe et al. 1988), rivers (Bale et al. 1987), soils (vanElsas et al. 1987), and on plant surfaces (vanElsas et al. 1988; Knudsen et al. 1988). Plasmid transfer was observed at a reduced frequency in the presence of a natural microflora. The decline of the release strain in such samples may have been the major factor responsible for the

reduced transfer frequency. Transfer to the natural bacterial flora (indigenous population) has also been observed.

Transduction is the transfer of bacterial genes by bacteriophages. Transfer is limited to host populations subjected to infection by the same phage strain. The narrow host-range of most phages reduces the likelihood of gene transfer within a mixed bacterial population. Interspecies transduction was shown to occur in *Vibrio* spp. associated with oysters grown in marine aquaria (Baross et al. 1974). Chromosomal DNA was also transduced in cells released into environmental test chambers incubated in freshwater (Morrison et al. 1978). In addition plasmid DNA was transduced in a similar system (Saye et al. 1987). However, in the presence of a natural microflora the transfer frequency was reduced, possibly due to the decline of the release host. Since phage is released into the environment in a free form, transduction does not require cell to cell contact and may represent an ideal method for dispersing genes in the environment. There is also evidence that the adhesion of bacteriophages to particles can offer protection and prolong their persistence in the environment (Babich & Stotzky 1980).

Transformation is the process in which free DNA is taken into the cell. The presence of extracellular DNA has been demonstrated in fresh water and sea water and a number of bacterial strains have been shown to release or produce extracellular DNA in aquatic environments (Paul & David 1989). In addition, members of the natural population incorporated this free DNA. However, the DNA could have been taken up as gene sequences or as individual bases. In fresh water, DNA turnover is very rapid (Paul et al. 1989) and recombinant DNA sequences from GEMs would therefore not be expected to survive for long periods in the environment. Attachment of DNA to particulate material may improve its survival; however its persistence is still very short (4-24 hours). Protection of DNA in excreted vesicles outside the cell could improve the chance for gene exchange in the environment; such structures have been reported in strains of *Neisseria gonorrhoeae* and *Haemophilus*.

The environment itself poses the first barrier to genetic exchange. Cell to cell contact is required for conjugation and so limits transfer to only those cells with which the donor comes into close physical contact. The formation of microcolonies on the surface of particles may bring hosts into contact with other surface-attached bacteria. In aquatic systems, a large fraction of microbial biomass as well as the majority of metabolic activity has been reported to occur on surfaces of suspended material (Iriberry et al. 1987). Commonly 15% to 30% of the total bacterial count has been found attached to particles, although values as high as 70%-95% have been observed. Conjugal transfer of many plasmids has been shown to be more efficient on solid surfaces. Bradley (1980) has proposed that the sex pili of conjugative plasmids determines whether transfer occurs more effectively in solution or on a solid surface. For

example, the plasmid RP4 encodes a long rigid pilus that is easily broken in liquid culture. Therefore transfer frequencies are much greater on solid surfaces. Other plasmids encode a flexible pili that is suited to liquid transfers. It is not known whether the attachment of cells to the surface of particles implies an increased ability for genetic transfer, recombination, or the expression of exogenous genes. Transfer by transduction does not require cell to cell contact and from this point of view it could be considered an ideal process for gene transfer in the environment. Fine clay particles present in the environment can coat bacterial cells and inhibit conjugation (Singleton 1983) and phage infection (Roper & Marshall 1978 b), as well as protect the host from predation (Roper & Marshall 1978 a). The effects of other environmental factors such as temperature, pH, and the presence of oxygen have been investigated. Overall, these studies have shown that gene transfer can occur under environmentally relevant conditions.

Barriers to the stable establishment of DNA in new hosts may also exist to maintain the genetic integrity of particular species. Restriction enzymes present within cells can cut foreign DNA as it enters the cell. This provides a significant barrier to gene transfer. Although restriction systems are generally considered to be defensive mechanisms for protection against attack by bacteriophages and indiscriminate ingress by plasmids, it has been suggested that these systems may be more important in promoting genetic recombination and have played an important part in the evolution of plasmid genomes (Price & Bickle 1986). Therefore, restriction may actually be creative since recombination events may be stimulated and novel rearrangements of DNA produced (Saunders et al. 1989). Restriction systems do not operate equally against different DNA molecules or indeed against the same DNA substrate entering the cell by different routes. These differences reflect altered topological states of DNA as it enters a potential recipient. The susceptibility of any DNA molecule to restriction phenomena will depend as much on where it has come from as to where it is going (Saunders et al. 1989). Restriction enzymes may also be inactivated by environmental stresses (Rolfe & Holloway 1966; Walker 1984) or the DNA may be modified (methylated) and resist cleavage.

Even if a gene is moving at random through an interconnected, mixed microbial population it is clear that the novel products from such events are rarely selected. Under appropriate conditions, such as environments stressed by the presence of drugs or novel organic compounds (xenobiotics), the products of gene transfer are readily detectable. For example, the detection of plasmids encoding antibiotic-resistance has been directly correlated with the developing use of antibiotics and their release into the environment (Anderson 1975). Therefore, if gene transfer is occurring readily in nature the expression of a particular phenotype may play an important part in its survival. Transcription, translation

and post-translational modification (the processes involved in gene expression) present a series of barriers to the production of a functional gene product. It is interesting to note that some transposons (small pieces of mobile DNA within the bacterial chromosome) may be considered to be natural expression vectors. Insertion of many genes into a range of transposons has resulted in transcription and provided a useful system for gene cloning. However, the genes also have to be inserted in such a way that they can be translated.

Fail safe systems

One approach that has been proposed for overcoming concern over the fact that GEMs cannot be recalled following release, is to create potentially suicidal systems. GEMs could be designed to survive until a specific environmental signal triggers their death. Suicide systems may also be used to prevent the movement of genes from the release host to other organisms, so that recombinant DNA is contained within specific bounds. These systems may be constructed with lethal genes placed under specific gene expression controls. Bej et al. (1988) have fused a lethal gene to the *lac* promoter and cell death is brought about by the introduction of an inducer. This system was designed for the conditional death of the strain. However, microcosm studies revealed that mutations took place at a high frequency and hosts became resistant to the system. This and other systems usually rely on the addition of innocuous chemicals which, combined with the incomplete death of the population and genetic instability, limits the use of a single suicide system. The use of genes and promoters identified and constructed in *Escherichia coli* also reduce the effectiveness of systems finally targeted at other Gram-negative bacteria. Conditions regulating gene expression within each release host would require investigation before an efficient system could be produced. By using suicide systems such as those described, there is also the danger that the lethal gene could have a greater effect on the indigenous population than the "novel" gene of interest. If this were the case then such suicide systems could not be used.

Conclusions

It is hoped that by using a wide range of sensitive techniques an accurate picture of the fate of GEMs in fresh water and other environments can be obtained. Broadening the issue further to answer questions on the impact of releasing such organisms into the environment leads to an even greater set of problems that need to be addressed. At present it is very difficult if not impossible to determine the impact from the release of GEMs on any environment. Since our understanding of the

microbial ecology of the environment is limited, a solution to this problem appears to be a long way off. If, however, the survival and transfer of novel DNA precedes any deleterious effects on the environment, studying such events may provide some very useful information for risk assessment.

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