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Review article.

Photorhabdus and *Xenorhabdus* – Gene Structure and Expression, and Genetic Manipulation

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Abstract

Xenorhabdus and *Photorhabdus* spp. are entomopathogenic bacteria carried symbiotically by nematodes of the genera *Steinernema* and *Heterorhabditis* respectively. This review describes the preliminary work done so far on the molecular genetics of the bacteria. This includes the cloning and partial characterization of less than 20 genes, the use of DNA sequence homology in taxonomic studies of the bacteria, and developments in DNA uptake methods including the preparation of transposon mutant banks. Many of the gene expression studies have focused on genes involved in phase variation and our knowledge of the regulation of this unique phenomenon is summarized.

Keywords: Entomopathogenic bacteria, *Photorhabdus*, *Xenorhabdus*, molecular genetics, gene structure, gene expression, DNA sequence, protein sequence, bioluminescence genes, lipase gene, polynucleotide phosphorylase gene, *mal*B regulatory region, flavin reductase gene, outer membrane proteins, molecular taxonomy, transformation, transduction, conjugation, transposon mutant banks, plasmids, bacteriophage, restriction-modification

1. Introduction

Photorhabdus spp. and *Xenorhabdus* spp. are bacteria carried symbiotically in the gut of nematodes of the genera *Heterorhabditis* and *Steinernema*, re-

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spectively. (A strain of *Photorhabdus* has also been isolated from a human wound: Colepicolo et al., 1989). The nematode/bacterial complex kills insects after the nematode penetrates into the insect larva and releases the bacteria into the hemocoel. The bacteria kill the larva by evading the insect immune response and by producing a variety of non-specific toxins (Dunphy, 1994; Dunphy, 1995; Clarke and Dowds, 1995). They also create an appropriate environment in the insect for the development and reproduction of the nematodes. *Xenorhabdus* and *Photorhabdus* (formerly *Xenorhabdus luminescens*, Boemare et al., 1993) are facultatively anaerobic Gram-negative rods and members of the Enterobacteriaceae family (Holt et al., 1994). Phase variants (different colony forms) occur in all strains of these genera and an unusually large number of phenotypes are affected by the phase shift.

Work has begun recently on the study of the molecular genetics of these entomopathogenic bacteria and has focused mainly on the cloning and sequencing of a limited number of genes, the use of hybridization methods for taxonomy, and the development of DNA uptake methods for the purposes of genetic engineering and generation of transposon mutant banks. The gene cloning, sequencing and expression studies have arisen from investigations into bioluminescence, phase variation, outer membrane proteins and low temperature induced proteins. Transposon mutant banks have been generated to study the mechanisms of virulence, phase variation and other phenomena.

2. Gene Structure and Expression

The following groups of genes have been cloned from *Photorhabdus* strains: the pigment, *lux*, flavin reductase, lipase and polynucleotide phosphorylase operons and part of the maltose uptake regulon. In addition three outer membrane protein (*omp*) genes have been isolated from *Xenorhabdus nematophilus*.

Omp genes

Forst and colleagues have begun to study the outer membrane proteins (Omp) of *X. nematophilus*. In *E. coli*, the OmpF and OmpC proteins form pores which allow diffusion across the outer membrane. The genes encoding them are regulated by several environmental stimuli including changes in osmolarity. This is mediated by a two component regulatory system comprising an EnvZ sensor protein which is activated by environmental changes to phosphorylate the OmpR regulatory protein which in turn regulates expression of the *ompF* and *ompC* genes. Several outer membrane proteins of *X. nematophilus* are

regulated by growth phase, temperature and phase variation (Leisman et al., 1995) and by anaerobiosis and high osmolarity (Forst et al., 1994). Forst et al. (1994) also report the cloning and sequencing of the genes coding for the *X. nematophilus* homologues of *ompF*, *ompR* and *envZ* and found many similarities to those of *E. coli* in terms of sequence and organization. The *opnP* gene homologue of *E. coli's ompF* contained *micF* antisense RNA and OmpR binding sequences similar to those found in *E. coli*, and the *envZ* gene of *X. nematophilus* was fully functional in an *envZ*-minus strain of *E. coli*. However, OpnP was not antigenically related to the *E. coli* Omps.

Genes induced at low temperatures

The subject of cold adaptation in *Photorhabdus* spp. has been reviewed by Clarke and Dowds (1994a). The operon coding for polynucleotide phosphorylase (Pnp) which degrades mRNA, and a ribosomal protein, RpsO, has been cloned from *Photorhabdus* sp. strain K122 (Clarke and Dowds, 1994b). It was found to be linked to an operon which includes the *nusA* gene which codes for a transcription antiterminator. Pnp and probably also NusA are induced by growing *Photorhabdus* at low temperatures (9° compared with 28°C) and both of these proteins as well as others encoded by the *nusA* operon are also induced by cold shock (a transient shift from 37°C to 10°C) in *E. coli*. Regulation of expression of the *rpsO-pnp* operon is complex in *Photorhabdus*. The two genes share two transcription initiation sites but there is also an additional promoter located between the genes which regulates cold inducible transcription of *pnp*. Upstream of this promoter is a consensus sequence for a cold shock enhancer binding site while downstream is an RNase111 cleavage site. In *E. coli* processing of the *pnp* transcript by RNase111 is the first step in a translational autocontrol process.

Some of the outer membrane proteins are also subject to temperature regulation including OpnA and OpnB both of which are induced at low temperatures (Leisman et al., 1995). OpnB accumulated at 19°C but was repressed at temperatures above 30°C while OpnA was optimally produced at 30°C and not present in cells grown at lower or higher temperatures. OpnT production was repressed at lower temperatures.

mal B regulatory region

A piece of DNA from *Photorhabdus* sp. strain K122 was cloned and sequenced and found to have 53.5% identity with the *malB* region of *E. coli* (Clarke and Dowds, unpublished). The cloned region comprises part of the *malE* and *malK* genes and the regulatory region between them. The maltose regulon of *E. coli*

consists of genes involved in maltose uptake (the *mal*B region comprising two operons: *mal*EFG and *mal*K-lamB) and maltose metabolism (*mal*PQ called the *mal*A region). Five proteins are needed for maltose and maltodextrin transport. The LamB protein forms a pore that allows permeation of maltodextrins through the outer membrane. The *mal*E gene codes for the maltose binding protein (MBP) which is located in the periplasm. The products of the *mal*F, *mal*G and *mal*K genes form a complex that interacts with maltose loaded MBP to mediate active transport of maltose across the cytoplasmic membrane (Schwartz, 1987). The *mal* genes are regulated by a transcriptional activator, MalT, encoded by a gene in the *mal*A region. The intergenic region between the *mal*EFG and *mal*K-*lam*B operons contains the control regions that respond to levels of maltose in the media and also to catabolite repression. In *E. coli* this region has four CRP binding sequences and four binding sites for MalT while in *Photorhabdus* it has two CRP and four MalT sites in the same position and orientation as in *E. coli*.

Pigment genes

The phase 1 (primary form) variants of *Xenorhabdus* and *Photorhabdus* produce a pigment, whose colour depends on the strain. The genes coding for the red anthraquinone pigment of *Photorhabdus* sp. strain Hm have been cloned into *E. coli* where they were expressed off their own promoter (Frackman and Nealson, 1990). The clone carried 8.1 kb of *Photorhabdus* DNA which probably did not contain all of the genes required for biosynthesis of the *Photorhabdus* pigment since the recombinant *E. coli* colonies were a deeper red than strain Hm. Further analysis of this clone would be interesting because the pigment, while having no antibiotic activity itself, is related to the polyketide antibiotics of *Streptomyces* spp. In addition, pigment is not produced by phase 2 (secondary form) colonies and so would form a handle with which to analyse the regulation of phase variation.

Bioluminescence genes

The bioluminescence (*lux*) genes of four *Photorhabdus* spp. strains have been isolated in four different laboratories by expression off their own promoter on a plasmid in *Escherichia coli*, the bank being screened for light production (Frackman et al., 1990; Szittner and Meighen, 1990; Xi et al., 1991; Wang and Dowds, 1991; Meighen and Szittner, 1992). Five genes are needed for light production, *lux*C, D and E coding for the enzymes of the fatty acid reductase complex which produces the long chain aldehyde substrate for the luciferase whose two subunits are encoded by the *lux*A and B genes. *Xenorhabdus* spp. are

non-luminescent and it was found that the *lux* genes of *Photorhabdus* did not hybridize to DNA from *Xenorhabdus* spp. implying that this genus does not even have traces of non-functional *lux* genes. This suggests that the bioluminescence genes may have been acquired by *Photorhabdus* by horizontal transfer after the divergence of the two genera. All other known luminous bacteria are of marine origin and fall within the *Vibrio* and *Photobacterium* genera, both in the family Vibrionaceae. Szittner and Meighen (1990) compared the predicted amino acid sequences of the luciferases of *Photorhabdus* and *Vibrio harveyi* and found that the α subunits displayed 85% identity and the β subunits had 60% identity. The organization of the five structural *lux* genes is the same in *Photorhabdus* as in marine luminous bacteria though a *lux*G gene appears to be missing from *Photorhabdus* or is at a different position on the chromosome in this genus (Meighen and Szittner, 1992). If the similarity between the two genera is limited to the *lux* genes, it suggests that *Photorhabdus* may have acquired the bioluminescence genes from marine bacteria by horizontal transfer. Meighen and Szittner (1992) compared the sequence of the *lux* genes of two *Photorhabdus* strains, one isolated from nematodes and the other from a human wound. The sequences were 85–90% identical but diverged in the flanking regions, suggesting that the location of the genes in the chromosome differs between the two strains. Enteric repetitive intergenic consensus (ERIC) sequences are found amongst the *lux* genes – one in *Photorhabdus* strains isolated from nematodes and four in strains isolated from humans (Meighen and Szittner, 1992). The significance of these sequences is not understood, but it is reasonable to suggest that the difference in repetition frequency between the strains is related to the time of divergence of the human and nematode symbionts.

Bacterial bioluminescence occurs when a long chain aliphatic aldehyde and $FMNH_2$ are simultaneously oxidized by molecular oxygen in the presence of luciferase, resulting in the emission of blue-green light. $FMNH_2$ is supplied by the reduction of FMN by NAD(P)H-flavin oxidoreductase (Fre). Zenno and Saigo (1994) cloned *fre* genes from one *Photorhabdus* and three *Vibrio* species. Their products were good suppliers of $FMNH_2$ to the bioluminescence reaction. The *fre* genes were very similar in sequence to the *lux*G gene found in *Vibrio* spp.. However the products of the two genes appear to constitute two separate groups of flavin associated proteins.

Lipase gene

The gene encoding the extracellular lipase of *Photorhabdus* sp. has been cloned and sequenced (Wang and Dowds, 1993) and found to be linked to a gene coding for the pyridoxal phosphate biosynthetic protein, PdxJ. The primary

translation product of the lipase gene contains a leader peptide of 24 amino acids which was removed from the product of the cloned gene in *E. coli* and which is presumably a signal for secretion in *Photorhabdus*. The sequence was found to have little homology (except at a possible active site) to other lipase genes which are, in general, poorly conserved. The active site may be different from that suggested by Wang and Dowds (1993). Stuart Brody (personal communication) suggested that the N-terminal region of the protein (amino acids 7–13 of the mature protein) was a better match to the active sites of a number of other lipases, particularly the acyltransferase of *Aeromonas hydrophila* (Derewenda and Sharp, 1993). Lipase activity is produced by the primary but not the secondary form of *Photorhabdus* sp. strain K122 and the gene was cloned mainly to use as a tool in the analysis of phase variation (see next section).

Regulation of genes coding for phase variant characteristics

Xenorhabdus and *Photorhabdus* produce at least two phase variants, whose phenotypic differences are displayed in stationary phase cultures. The stability of primary phase (phase 1) cultures varies widely from strain to strain, some producing secondary forms (phase 2) at high frequency and some producing multiple form variants (Hurlbert et al., 1989; Gerritsen et al., 1992). Phase 1 differs from phase 2 *Photorhabdus* in the emission of light by the former but not the latter colony forms. The phase variants of *Xenorhabdus* and *Photorhabdus* differ in that the primary but not the secondary is pigmented and produces extracellular lipase, protease and antibiotic activity, it makes fimbriae, a surface capsule and flagella and is preferentially retained by the infective stage nematode and provides better conditions for nematode reproduction (see Givaudan et al., 1995 as one of the more recent contributions to the literature on phase variation). Taxonomic studies including one using DNA hydridization techniques indicate that phase variation is not an artifact due to contamination and that the levels of hybridization between the phases is not significantly different from 100% (Boemare et al., 1993). Neither the function of the phases nor the mechanism of phase variation is understood but molecular genetics has been used as a tool to provide some answers to the latter question. (See Burnell and Dowds, 1996 for speculation about the function of the phases).

Phase variation is a widespread phenomenon in bacterial cultures. The stochastic switching to the alternate phase usually affects one or a small number of gene products and is usually mediated by DNA instability such as DNA inversion (Dybvig, 1993). The phenomenon appears to be different in *Xenorhabdus* and *Photorhabdus* where a large number of phenotypes are

affected and where major changes in DNA structure do not appear to account for the phenotypic switches. Thus the pattern of bands from restriction digests of genomic DNA hybridising to total genomic DNA (Southern Cross experiment: Akhurst and Smigielski, 1994) and to the *lux* (Frackman et al., 1990; Wang and Dowds, 1991) and lipase (Wang and Dowds, 1993) genes is the same for the two phases.

Lipase gene transcription is initiated at the same site in the two phases and both *lux* (Wang and Dowds, 1991) and lipase (Wang and Dowds, 1993) mRNAs accumulate to the same extent in the two phases implying that gene expression in the secondary is repressed at a post-transcriptional level or further downstream. In the case of lipase, it has been shown using antiserum that the lipase protein accumulates and is secreted to the same extent in the two phases. However the lipase protein is inactive in the secondary phase. Protease is also made and secreted in an inactive form in the secondary phase and is probably inactivated in the same way as the lipase since the secondary form of both enzymes can be activated by the same means (SDS treatment) *in vitro* (Wang and Dowds, 1993). The data presented so far suggests that phase specific genes are regulated at a post-translational level by a common control mechanism. The secondary phase might either lack an activator needed for the expression of phase variant characteristics or might produce a repressor of the activities. An experiment of Frackman and Nealson (1990) suggests that the latter is a more plausible model. They transformed the cloned *lux* genes into primary and secondary cells on a multicopy plasmid and found that the amount of light emitted was equal in the two forms and about 10 times higher than in non-recombinant primary phase cultures (Frackman and Nealson, 1990). This suggests that the secondary form produces a repressor of bioluminescence which was diluted out by the excess of *lux* gene products formed from the multicopy plasmid. They noted that light emission occurred slightly later in the growth cycle of the transformed secondary phase culture, suggesting that the proposed repressor had a partial effect, but is possibly unstable.

Evidence about the nature of the proposed repressor comes from work done in Nealson's laboratory (Hosseini, 1994; Hosseini and Nealson, this volume). They found that light emission from primary phase cells begins late in the growth cycle at a cell density of about 4 $O.D._{560}$ units but that it occurs earlier in the growth cycle in the presence of transcription inhibitors such as rifampicin. In addition, rifampicin derepresses bioluminescence in the secondary phase so that it occurs at the same time and to the same extent as in the primary phase treated with rifampicin. Hosseini (1994) noted that an inhibitor of protein synthesis did not have this effect, arguing that the repressor may be an RNA molecule, presumably an antisense RNA that inhibits translation of the *lux* mRNA. Rifampicin did not have the same effect on other

phase variant characteristics, namely pigment, antibiotic production, lipase and protease activity. Also, Wang and Dowds (1993) found that lipase and protease were regulated at a post-translational level, implying that the mechanism of phase variant regulation of extracellular enzyme activity is different from that exerted on bioluminescence.

Several other lines of evidence suggest independent types of regulation of different collections of phenotypes. For example, an intermediate phase isolated in this laboratory displayed protease activity typical of the primary form, lipase and antibiotic activities typical of the secondary and intermediate degrees of pigmentation and bioluminescence (Zeller and Dowds, unpublished). A mutant isolated by Hosseini (1994) over-expressed pigment, light and antibiotics, a different collection of characteristics from the intermediate. Finally, Krasomil-Osterfeld and Ehlers (1994) found that a decrease in osmolarity could induce a primary to secondary phase shift and that increased osmolarity could reverse the shift. [Bacteria are known to respond to changes in osmolarity by altering the composition of proteins in the outer membrane. In this context, it is interesting that two of the stationary phase induced outer membrane proteins of *X. nematophilus*, OpnA and OpnB are not produced in secondary form cells (Leisman et al., 1995).] The phase variant characteristics which responded to changes in osmolarity were cell morphology, inclusion bodies, pigmentation, luminescence and antibiotic production (Krasomil-Osterfeld and Ehlers, 1994). The general trend of this work has been repeated in my laboratory for different strains of *Photorhabdus*. However, low osmolarity failed to reduce lipase and protease activities to the low levels typical of secondary phase cultures and a variety of different pigment colours were produced under different conditions (McDonagh and Dowds, unpublished) implying again, that different phase variant characteristics are separately controlled. However, it seems unlikely that phase variant phenotypes are all controlled independently of each other. It is probable that there is a master switch which differentially affects a number of other regulatory systems that in turn control one or a small number of phase variant characteristics.

3. Taxonomic Studies based on DNA Sequence Homology

The taxonomy of *Xenorhabdus* and *Photorhabdus* has been discussed elsewhere (Boemare et al., 1993; Akhurst and Boemare, 1994), so the summary here is derived only from recent work stemming from the use of hybridization or sequencing techniques. Putz et al. (1990) prepared oligonucleotide probes complementary to particularly variable regions of 16S rRNA that were

capable of distinguishing between the previously defined five species of *Xenorhabdus*. In addition, three strains of bacteria isolated from *Heterorhabditis* sp. and *Steinernema affinis* failed to hybridize to any of the five species-specific probes. Boemare et al. (1993) later used the method of total genomic DNA hybridization to re-evaluate the designation of *Xenorhabdus* species which had been determined by classical methods of bacterial taxonomy. Their work confirmed that *X. bedingii, X. nematophilus, X. bovienii* and *X. poinarii* are valid species whereas the fifth species, *X. luminescens* was transferred to a new genus, *Photorhabdus*. This study showed that the four *X. luminescens* strains exhibited less than 20% identity to any other *Xenorhabdus* strain, whereas the levels of relatedness between the other *Xenorhabdus* strains were usually more than 20% and up to 48%. Overall, *Photorhabdus* is a more homogeneous genus than *Xenorhabdus* and cannot yet be subdivided into species (Akhurst and Boemare, 1994). The same authors note that there is not a strong correlation between DNA relatedness groups of *Photorhabdus* and their *Heterorhabditis* hosts, whereas there is a strong relationship in the case of *Xenorhabdus* and *Steinernema*.

The position of *Xenorhabdus* and *Photorhabdus* genera within the Enterobacteriaceae family has also been studied using total genomic DNA hybridization studies. This shows that *X. nematophilus* was as dissimilar from *X. luminescens* as it was from *E. coli* (Boemare et al., 1993) and that *Xenorhabdus* is only 4% related to *E. coli* (Farmer, 1984). However, *Xenorhabdus* or *Photorhabdus* genes which have counterparts in *E. coli* display much higher levels of sequence identity. For example, the cloned DNA encoding the Pnp and RpsO proteins displayed 75% identity to the homologus region from *E. coli* and the *mal*B regulatory region displayed 53.5% identity. Table 1 shows 50–86% levels of identity for protein sequences predicted from the DNA sequences. There was a somewhat lower level of identity in DNA than in protein sequences because of less conservation in non-coding regions and because of same sense (third base) alterations between the two species. In addition to sequence similarity, the arrangements of the genome and the properties of their encoded proteins greatly resemble each other for these species. For example, the arrangements of the *rpsO-pnp* (Clarke and Dowds, 1994b) and *mal*B (Clarke and Dowds, unpublished) regions of the genome in *Photorhabdus* were the same as in *E. coli*. Similarly the function and antigenicity of many of the outer membrane proteins of *X. nematophilus* complemented or overlapped with those of *E. coli* (Leisman et al., 1995). Thus the similarity between homologous genes in *Xenorhabdus/Photorhabdus* and *E. coli* is much greater than might be expected from the total genome DNA sequence identity of 4% predicted from hybridization studies. This discrepancy suggests that many *Xenorhabdus/Photorhabdus* genes do not have homologues

in *E. coli* and that there is considerable divergence between the species in the non-coding regions. It is also possible that the hybridization technique underestimates sequence similarity. This might arise because of a large number of third base changes destabilizing hybrids and having a greater effect on hybridization than on sequence.

Table 1. Similarity between *E. coli* and *Photorhabdus / Xenorhabdus* protein sequences

Strain	Protein	% Identity	Reference
Photorhabdus sp. strain K122	Pnp	86	Clarke and Dowds, 1994
K122	RpsO	86	Clarke and Dowds, 1994
K122	MalK	65	Clarke and Dowds, unpubl.
K122	MalE	75	Clarke and Dowds, unpubl.
K122	PdxJ	81	Wang and Dowds, 1993
X. nematophilus	OmpF	65	Forst et al., 1994
X. nematophilus	OmpR	70	Forst et al., 1994
X. nematophilus	EnvZ	50	Forst et al., 1994

4. Genetic Manipulation

The ability to genetically engineer a species requires that the species take up DNA, that cloning vectors are available that can be selected and maintained in the species and that the incoming DNA is not degraded by restriction enzymes of the host. Some preliminary work has been performed on the restriction-modification system and the endogenous plasmids and phages of *Xenorhabdus*. Transformation, conjugation and transduction of DNA into *Xenorhabdus* and *Photorhabdus* have been carried out successfully, using *E. coli* plasmids and phages as vectors.

There is evidence for a restriction-modification system in *X. nematophilus* (Akhurst et al., 1992; Xu et al., 1989) based on relative transformation efficiencies of plasmid grown in *E. coli* vs *X. nematophilus*, and the relative ineffectiveness of methylation-sensitive restriction enzymes at cutting DNA from this species. Furthermore, an isoschizomer of *Xho*1 has been purified from a *Photorhabdus* sp. (Akhurst et al., 1992)

Plasmids and phages of Xenorhabdus/Photorhabdus

There are several reports of plasmids and phages in *Xenorhabdus* and *Photorhabdus*. However, virtually no work has been done to characterize them or engineer them to generate cloning vectors. Reports noting the presence of plasmids in *Xenorhabdus* and *Photorhabdus* spp. include Couche et al., 1987; Poinar et al., 1989; Smigielski et al., 1990; Leclerc and Boemare, 1991 and Dowds, unpublished observations). Poinar et al. (1989) found a 50–56 kb plasmid in *Photorhabdus* and indeed most strains of *Xenorhabdus* and *Photorhabdus* were found to contain megaplasmids of 60 to 680 MDa (Smigielski et al., 1990). The functions of the genes encoded on these megaplasmids are not yet known but megaplasmids in other species are known to contain genetic information necessary for symbiosis, pathogenicity and conjugation, in different cases. In the case of smaller plasmids, Couche et al. (1987) reported that 7/10 *Xenorhabdus* strains contained plasmids and that they ranged in size from 3.6–12 kb and Leclerc and Boemare (1991) found between 0 and 3 plasmids per strain. Several studies have failed to find any difference in plasmid (small or large) content between the primary and secondary phases. However Frackman and Nealson (1990) found a plasmid in *Photorhabdus* strain Hm in phase 1 cells which could not be isolated from phase 2 although the DNA of phase 2 hybridized to the plasmid. The plasmids of *Xenorhabdus* and *Photorhabdus* have not been further characterized or engineered to generate cloning vectors e.g. it is not known whether they carry antibiotic resistance genes. In this lab, we have found that most strains of *Photorhabdus* are resistant to ampicillin and that this is true even for strains (e.g. K122) in which a plasmid was not detected, so it seems unlikely that this trait is plasmid-borne. Xu et al. (1989) also found that 3 strains of *Xenorhabdus* which lacked plasmids were resistant to ampicillin.

Poinar et al. (1989) discovered a bacteriophage in *Photorhabdus* sp. that lysed the primary but not the secondary phase cells; the lytic activity occurred without induction, suggesting that the phage was not lysogenic. The laboratory of Noel Boemare has studied the production of bacteriocins by *Xenorhabdus* and *Photorhabdus*. One group of these antibacterial agents includes phage tail-like particles, lethal phages and defective phages. Boemare et al., (1992) and Baghdiguian et al. (1993) searched for lysogenic phages by attempting to induce lysis with heat and mitomycin C treatments. Lysis of both phases of *Xenorhabdus* spp. was observed and phages and bacteriocin-like phage tail particles were observed in the lysates. Induction by mitomycin C increased the antimicrobial activity of the bacteriocin suspension up to 10,000 fold. A *Photorhabdus* sp. did not lyse, though low levels of bacteriocins were detected in uninduced cultures. Thus *Xenorhabdus* spp., at

least, harbour prophages which can be induced. However, further work has not yet been done to generate suitable cloning vectors from these phages.

Transfer of DNA into Xenorhabdus and Photorhabdus

DNA has been successfully transferred into *Xenorhabdus* and *Photorhabdus* by means of transformation, conjugation and transduction using *E. coli* plasmids, mobilizable plasmids and phage λ respectively. Xu et al. (1989) transformed *X. nematophilus* with a limited-host-range plasmid pBR325 and the broad-host-range plasmid pHK17 using the *E. coli* $CaCl_2$-$MgCl_2$ procedure. When the plasmids were grown in *E. coli*, the efficiency of transformation was 1–10 transformants per μg of DNA. However, when pHK17 DNA was prepared from the transformants and re-transformed into *X. nematophilus*, the efficiency rose 250 fold, suggesting that *X. nematophilus* contains a restriction-modification system. Optimization of the transformation conditions allowed the group to reach efficiencies of 10^5–10^6 transformants per μg of pHK17. Xu et al. (1989) also succeeded in transforming *Photorhabdus* sp. and other species of *Xenorhabdus* with a range of plasmids, and Frackman and Nealson (1990) reported the transformation of *Photorhabdus* strain Hm using competent cells prepared by a modification of the $CaCl_2$/RbCl method used for *E. coli*. In this laboratory, we have transformed *Photorhabdus* sp. using electroporation conditions designed for *E. coli* and reached a maximum efficiency of 10^4–10^5 transformants per μg of plasmid for strain HF transformed with pBR322 and pHK17 (Sommer and Dowds, unpublished). Strain K122 was transformed with pBR322 under the same conditions, yielding approximately 10^2 transformants per mg, indicating the specificity of the requirements for each strain.

Conjugational transfer of plasmids from *E. coli* to *Xenorhabdus* spp. has been successful. Xu et al. (1991) transferred 4 different plasmids into *X. nematophilus* in this way, the highest frequency of transfer being 5.8×10^{-2} for pRZ102 into the secondary form of the bacteria. They were also successful using *X. poinarii* as recipient but failed to obtain transconjugants with another strain of *X. nematophilus* and a strain of *Photorhabdus*. In this laboratory, chloramphicol resistant mutants of 2 strains of *Photorhabdus* were isolated for counter selection and plasmids RP4 and pSUP104 transferred by conjugation from *E. coli* into *Photorhabdus* spp. strain K122 but not into strain HF. Francis et al. (1993) obtained exconjugates of *X. bovienii* at a low frequency (10^{-8}–8×10^{-7}) after transfer of pTROY plasmids from *E. coli*.

Francis et al. (1993) have succeeded in transducing DNA into *X. bovienii*, using bacteriophage λ as vector. They did this by first introducing (by conjugation) a plasmid encoding the *E. coli* λ receptor protein LamB. One of the engineered strains expressed LamB on its outer membrane and adsorbed twice as

many phage particles as the parent strain of *X. bovienii*. The LamB expressing strain was then transduced with λ::Tn10 mutagenesis vectors to construct a bank of insertion mutants. In this laboratory, we have constructed a *Photorhabdus* strain carrying the *lam*B gene and found that it adsorbed phage λ better than the non-recombinant strain. However, tet^r transformants were not detected following attempted transduction with phage λ carrying this marker, suggesting that phage adsorption was not accompanied by DNA uptake (Sommer and Dowds, unpublished).

Transposon mutant banks

Two transposon mutant banks have been described in the literature, both using *E. coli* transposons in *Xenorhabdus* spp. Xu et al. (1991) conjugated various Tn5-containing plasmids with different replication origins into *X. nematophilus*. Only one plasmid yielded colonies that had gained the transposon (with its associated Kan^r gene) but lost the plasmid at a useful frequency (15–25%). The other problem with this system was the formation of cointegrates between the plasmid and the chromosome. Because the frequency of transposition was low from matings, they constructed a negative selection plasmid pHX1. This plasmid contains the *Bacillus subtilis* levansucrase gene which is lethal to many Gram negative bacteria when plated on sucrose. Transformants which were Kan^r but Tet^s and Suc^r had lost the plasmid but gained the transposon. All such colonies were assumed to be independent Tn5 transposon mutants. More than 250 mutants were isolated and characterized, including avirulent strains and variants affected in antibiotic production.

Francis et al. (1993) developed an alternative approach for transposon mutagenesis by transducing *X. bovienii* expressing the LamB protein with phage λ delivery systems containing modified Tn10. The transposon contained the Kan^r gene between the inverted repeats and the transposase gene outside the repeats so that transposition events could be selected and stable insertions were made. Insertions were mapped by Southern blotting and a variety of mutants were identified such as auxotrophs and strains with altered DNase, lipase and protease production.

The future for genetic manipulation of Xenorhabdus/Photorhabdus

So far, the genetic manipulation work on *Xenorhabdus* and *Photorhabdus* has concerned the development of methodology as outlined above. In order to use this technology to generate useful strains e.g. with a narrower insect host range or increased symbiont range, it will be necessary to greatly expand our knowledge in two ways. (1) We need to know more about biological processes

involved in useful traits such as pathogenicity and (2) we need to increase the efficiency of uptake of DNA by the three different means and to extend this work into other strains, particularly those of commercial importance. (See Burnell and Dowds, 1996 for a review of the prospects for genetic improvement).

Much biological information can be obtained by genetic manipulation. Some can be obtained by the transfer of a single gene into cells and this is already possible for most strains, but needs to be refined. For example, vectors which integrate into the chromosome at specific locations would be useful. Much information can be gained about the genes and their products involved in biological processes by performing transposon mutagenesis. In order to create transposon mutant banks, a high efficiency of uptake of DNA is necessary along with an efficient counter selection system so that single transposition events can be selected. Both of these processes need to be improved and extended to other strains for us to fully analyse such biologically unique events as phase variation as well as the commercially important processes such as symbiosis and pathogenicity.

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