CHAPTER I

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Genetics: Genetic analysis of microorganisms has contributed much to microbiology and genetics. No branch of science in the 20th century has contributed more to man's understanding of himself and the living world in general as has "genetics". Genetic discoveries have provided new insights into such fundamental problems as the origin of life, the structure of living matter and evolution. Genetics, in short has become the theoretical backbone of biology. One main lesson is, that many things are done in a simple way, in all living organisms, from the most simple, to the most complex. This is a reflection, in part, of the fact that all known forms of life are based on nucleic acids and proteins. A most striking example of this unity is the discovery, that except for minor differences, the same triplet groups of bases are used for coding, transcribing and translating genetic information in all living organisms examined.

Evidence for nucleic acids as genetic material and the general acceptance of their unique role in the transmission of hereditary characters was provided by a few important experiments. The first of these was demonstrated by Avery, Macleod and McCarty (1). They showed that the
active principle in transmitting genetic characters is DNA. Later on, this was shown more convincingly by Hotchkiss (2) also by Goodal and Herriot (3) and Lerman and Tolmach (4). Hershey and Chase (5) showed that upon infection by virulent bacteriophages to their host cells, the phage DNA enters the bacterium, whereas nearly all the phage protein remains outside. This showed that it was the phage DNA which "programmes" the infected cells to form progeny phage particles.

These and many other experiments spotlighted the nucleic acids as the repositories of genetic information, and paved the way for the elucidation of the physicochemical structure of DNA (6), which beyond doubt, has been the most important and provocative biological discovery of this century.

The history of bacterial genetics show that most workers have devoted their efforts to one strain of one species, Escherichia coli K-12, and the success of this strategy is obvious in the wealth of knowledge and the elegant experiments which continue to be associated with work on this bacterium. However, other species of bacteria have attracted the attention of geneticists and their work revealed to microbiologists the value of genetics to many problems involving microorganisms. Pseudomonas is one such
example. The growth of interest over the last few years in the genetics of the two species, \textit{Pseudomonas aeruginosa} and \textit{Pseudomonas putida} has been both an intellectual stimulant and a delight to the few veteran workers in this field.

Most genetic work with \textit{P. aeruginosa} has been done with two strains, originally described by Holloway (7). These are strain PA01 isolated from a patient in Australia in 1954 and strain PAT2 which was originally isolated by Don and van der Ende (8) in South Africa as strain L-III 3bi. Both are prototrophs and aeruginocinogenic. Fortuitously, these two strains have shown a wide range of different genetic properties, and their separate investigation has enabled a broader picture of the genetics of \textit{P. aeruginosa} to be drawn. The study of these two strains has been mainly done by means of laboratory produced markers. In more recent years, other strains have been used to study naturally occurring variation, particularly with respect to antibiotic resistance.

It is interesting that with both species, \textit{P. aeruginosa} and \textit{P. putida} these native variants which have particular interest involve plasmids, and it is clear that a complete understanding of the genetic organization of \textit{Pseudomonas} must involve the identification of plasmids, their genetic and physical structure transmission, phenotypic potential
and relationship with the genomes of other bacteria. An understanding of the genetic organization of *Pseudomonas* is clearly necessary for the continued success of biochemical, epidemiological and microbiological research programmes in this genus.

**Mutation**: The concept of DNA as the genetic material and the elucidation of its structure by Crick and Watson (6), offered for the first time, a firm foundation on which to build a rational theory of mutagenesis.

Most of our understanding of the mechanism of mutation is derived from the results of experiments in which mutations are induced by chemical agents whose mode of action is on DNA. Such mutagens include base analogues, which are incorporated into DNA in place of the natural bases (9,10, 11); nitrous acid, which deaminates the purine and pyrimidine residues of DNA resulting in transitions (12,9); proflavine, which intercalates between the stacked bases of DNA, stretching the distance between them (13-16); alkylating agents which modify the bases of the DNA itself by adding alkyl groups to the ring nitrogens resulting in depurination of DNA (17,18,19). Mere exposure of DNA to moderately low pH, removes purine bases indiscriminately without loss of pyrimidines, and has been shown to be mutagenic for bacteriophage T₄ (20).
Mutations give genetic markers which can be useful in mapping the structural gene of a protein under study.

Special classes of mutations are known, viz., nonsense, frameshift, deletion, temperature sensitive etc. Nonsense mutations are useful as with different suppressors they allow, the insertion of specific amino acids at the mutated site (21). Frame shift mutations can cause polarity, and are useful for sequencing altered proteins and to deduce mRNA sequences (22). For fine structure mapping and operon fusion studies deletion mutations are useful (23).

Temperature sensitive mutations have facilitated the study of DNA synthesis (24,25,26), ribosomes (27,28,29), mRNA breakdown (30) and amino acid activating enzymes (31). In most of these cases temperature sensitive mutants are those which grow at low temperature but not at high temperatures. Cold sensitive mutants have also been isolated in phage T₄ (32), phage T₁ (33), phage φ x 174 (34), and E. coli (35). The experiments on the chemical basis of mutation have been done almost exclusively with the bacterium E. coli and with a number of its bacteriophages.

**Bacteriocins**: Bacteriocins are high molecular weight extracellular antibiotic like substances, produced by a number of bacteria and are active against closely related bacteria in contrast to low molecular weight antibiotics which have a broad spectrum activity.
The study of bacteriocin dates back to 1925 when Gratia observed inhibition of *E. coli* by *E. coli* V (36). Although inhibition of one bacterial strain by other strain of bacteria had been observed earlier; it was the thorough investigation by Gratia and Fredericq (37) which actually led to our precise knowledge of bacteriocin.

Colicins of *E. coli* were the first bacteriocin to be studied in detail. The classification of colicins put forward by Fredericq (38) has been used as a model for classification of other bacteriocin.

The classification and hence nomenclature of bacteriocins has room for improvement. The existing names which are given on the classification of their bacterial host due perhaps to the high degree of specificity of bacteriocins, the name is almost always based on the species rather than the generic name of the host (Table 1).

**Table 1: Name of principal bacteriocins.**

<table>
<thead>
<tr>
<th>Host organism</th>
<th>Bacteriocin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Colicin</td>
<td>(36)</td>
</tr>
<tr>
<td><em>Aerobacter aerogenes</em></td>
<td>Aerocin</td>
<td>(39)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Marcescin</td>
<td>(39)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Aeruginocin or</td>
<td></td>
</tr>
<tr>
<td><em>P. pyocyanea</em></td>
<td>Pyocin</td>
<td>(40)</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Monocin</td>
<td>(41)</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Cerecin</td>
<td>(39, 42)</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp.</td>
<td>Staphylococcin</td>
<td>(38)</td>
</tr>
<tr>
<td><em>Pasteurella pestis</em></td>
<td>Pesticin</td>
<td>(43)</td>
</tr>
</tbody>
</table>
The synthesis of bacteriocin is determined by genes encoded on bacterial plasmid. Although such bacteriocinogenic strains possess the stable genetic ability to produce a bacteriocin, they do not do so all the time, or under all conditions. It is only upon induction that bacteriocins are produced (44).

However, a small amount of bacteriocin is present in normal culture of the organism, it is produced presumably by the spontaneous lysis of a few cells by some unknown factor. The production of a large quantity of a bacteriocin can be conveniently achieved by causing a logarithmic culture of the host bacterium to lyse by means of an inducing agent such as ultraviolet light (45), mitomycin C (46,47), temperature shock (48), chloramphenicol (49) or by certain mutagen or carcinogen such as aflatoxin. In some cases induction is accompanied by extensive replication of col+ factor (50), but it is not essential for bacteriocin production (51).

Induced bacteriocinogenic cultures synthesized upto $3 \times 10^3$ molecules of bacteriocin per cell and after induction, large amount of bacteriocin remains bound to cell surface (52). Purification of bound bacteriocin is often greatly facilitated by salt extraction without prior disruption of bacteria (47).
Bradley (53) proposes a classification of bacteriocin in to two types.

1. S type bacteriocin
2. R type bacteriocin

Some of their respective properties, which admittedly have been studied in only a few cases are given in Table 2. The difference in trypsin sensitivity and thermostability are somewhat speculative, though few known examples fit into the scheme. If this division can be generally applied, it would contribute a useful basis for classification.

Table 2 : Properties of two groups of bacteriocins.

<table>
<thead>
<tr>
<th>S type bacteriocins</th>
<th>- R type bacteriocins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Low molecular weight</td>
<td>High molecular weight</td>
</tr>
<tr>
<td>2. Not sedimentable</td>
<td>Sedimentable</td>
</tr>
<tr>
<td>3. Sensitive to proteolytic enzymes</td>
<td>Resistant to proteolytic enzymes.</td>
</tr>
<tr>
<td>4. Thermostable</td>
<td>Thermolabile</td>
</tr>
<tr>
<td>5. Can not be resolved in electron microscope.</td>
<td>Visible in electron microscope as phage like objects or components such as tail assembly.</td>
</tr>
</tbody>
</table>

Action of bacteriocin to sensitive bacteria is
considered to be bactericidal and not bacteriostatic. The reaction to bacteriocin with a sensitive cell may be divided into two phases.

1. The first consists of a rapid normally irreversible adsorption to specific receptors at the cell surface of sensitive bacteria (Adsorption phase).

2. This is followed, some measurable time later, by the appearance of pathological changes leading eventually to death of the cell which is called as lethal phase (54,55).

Bacteriocins produced by *P. aeruginosa* were first identified by Jacob (40) and given the name pyocin (after the valid species name *P. pyocyanea*). In a view of acceptance of the species name *aeruginosa*, the aeruginocin is more correct. Aeruginocins are bacteriocins produced by certain strains of *P. aeruginosa* that have killing action against other strains of same family (56). It has been repeatedly shown that aeruginocinogeny is very common among the strains of *P. aeruginosa* (57,39,58).

In general, aeruginocins show the same range of properties as the more extensively studied colicins (54,59). Kageyama and his associates have studied aeruginocins of R variety and shown this type of aeruginocin to be a bacteriophage tail like particles, with a single protein type having molecular weight about $1 \times 10^7$ daltons (56).
All the other R type aeruginocins are found to be very similar to each other in morphology, mode of action and immunological properties (60,61). The morphology of the aeruginocin R is that of double hollow cylinder, 1200Å long in diameter with a core and a contractile sheath around the core (62). Aeruginocin 28 (63) is rod shaped, in contrast to the R type aeruginocins, although the properties of this bacteriocin seem to be similar to R type (64).

The S type of aeruginocins are less well known in P. aeruginosa. Fortuitously the well characterized strain PA01 of P. aeruginosa can produce both R and S aeruginocins (65,61). Like the R type aeruginocin, S type can be efficiently induced by mitomycin C or ultraviolet irradiation.

Aeruginocin do not pass through cellophane, but they diffuse in agar gel. Their sensitivity to heat, temperature and proteolytic enzymes varies with the individual strains which produce them (66). Aeruginocin has high antigenicity and antiserum is easily obtained (67).

The apparent mode of action of aeruginocin seems to be different, depending upon the kind of aeruginocin. It exerts bactericidal effect on the sensitive cells. Adsorption of aeruginocin on sensitive bacteria do not
have an instantaneous killing effect. First the multiplication of aeruginocin sensitive cell is inhibited followed by decrease in respiration and finally leading to the death of cells (66,68). It either inhibits the synthesis of DNA, RNA of sensitive cells, following its adsorption on the surface of the sensitive bacteria (68). In one case a strain of \textit{P. aeruginosa} is found to be susceptible to the aeruginocin that it releases, a result which stresses the difference between self immunity to bacteriophage lysis which is a replicative function, and immunity to bacteriocin killing which is a surface or membrane function (69).

\textit{P. aeruginosa} is a pathogen of considerable significance being involved in burns, wounds, urinary tract infections and is bronchopneumonia. \textit{P. aeruginosa} is particularly menacing due to its well known resistance to chemotherapeutic agents. Epidemiological studies of hospital infection with \textit{P. aeruginosa} requires the use of typing system for differentiation of strains, such system involves serotyping, pyocin typing and phage typing (70).

In pyocin typing \textit{P. aeruginosa} strains can be typed on the basis of pyocinogenicity of the strains to be typed or their sensitivity to pyocin (or aeruginocin). Reports on pyocin typing have been published by Darrell and Wahba (71), Osman (72), Gillies and Govan (73), Govan and Gillies (74),
Zabransky and Day (75), Farmer and Herman (76) and Tripathy and Chadwick (70). Recent technical developments especially the preparation of phage-free pyocin extracts (77) give promise of improved bacteriocin sensitivity typing of \textit{P. aeruginosa}.

**Bacteriophages:**

The most exciting viruses from the viewpoint of the geneticist are the bacterial viruses or bacteriophages. Of the many angles used to unravel the most fascinating mysteries of biology, none have been more exciting than the experiments with bacteriophages.

In the study of genetics, everything hinges upon the choice of the organism. When one works with fruit-flies, one deals with at the most a few thousand individuals, and each generation takes roughly 20 days. If one works with a microorganism, such as a bacterium or better still a bacteriophage, one can deal with billions of individuals and a generation takes only minutes. One can, therefore, perform in a test tube within 20 minutes an experiment yielding a quantity of genetic data that would require, if humans were used, the entire population of the earth.

Throughout the past 25 years, the study of these organisms has yielded information and insights concerning
the basic nature of life (especially the molecular nature of heredity) and thus nourished the science of molecular biology.

In recent years, the phages have occupied a focal point in the study of basic problems in molecular biology. It was this system that was effectively used for the classical investigations to decipher the genetic code to split the gene and to map it down in detail to the molecular limit of its structure, to study the mechanism of transmission of information for protein synthesis and so on.

Most of our knowledge of the genetics of bacterial viruses is largely a result of the work on E. coli and its bacteriophages. The work done so far on bacteriophages is voluminous and has been the subject of many books (78, 79, 80). Twort in England and d'Herelle in France independently showed the existence of bacteriophages, nearly half a century ago, and after a great controversy, bacteriophages are categorised on the basis of their interaction with host bacteria as virulent or temperate.

The character of virulent phages is invariably to lyse the bacteria they infect. Temperate phages, on the other hand, do have an alternative; each infecting particle has a choice; following infection; either of entering the lytic cycle like a virulent phage, or of establishing a kind of symbiotic relationship known as lysogeny.
Temperate phages have been of great importance in the development of bacterial genetics. The inducible nature of the prophage (81), was an evidence for the model of the regulation of the lactose Operon (82). Temperate phages have provided a model for studies in macromolecular synthesis and recombination (83).

The infective process:

The process of phage infection may arbitrarily be divided into a number of stages. These stages are:

A. Adsorption of phage particles to the host cell.
B. Penetration of the phage DNA into the bacterium.
C. The intracellular multiplication of the phage.
D. The lysis of the host cell and release of the phage progeny.

A. The adsorption of phage: The first step in bacteriophage infection is the adsorption of the phage to a receptor on the bacterium. Almost every structure on the surface of a bacterial cell or extending from it, can act as (or include) phage receptors. Phages adsorb to structures as varied as flagella, pili, capsules, lipopolysaccharide, teichoic acid peptoglycan complexes, surface proteins etc. (84). Thus the external surface of the bacterium act as a mosaic of receptors for different phages.

Phages vary widely in size and structure. A morpholo-
Phage adsorption to its receptor is a highly specific process. The adsorption steps will be illustrated by four different models.

(i) Adsorption of small \( \phi X 174 \) like phages where the receptor is found in the lipopolysaccharide (LPS) of rough mutants of enterobacteria.

(ii) Adsorption of phages where the receptor is found in the exopolysaccharide (capsule or slime) of the bacterium.

(iii) Adsorption of phages where the receptor is found in the O-antigenic polysaccharide portion of the LPS of enterobacteria, or the teichoic acid - peptidoglycan complex of Gram-positive bacteria.

(iv) Adsorption of large complex phages like T4 where the
receptor is found in the LPS of rough mutants of enterobacteria.

The term "adsorption" as it is used in the literature implies two different steps, binding and eclipse. Binding is the process by which the receptor is recognised, but the interaction between phage and receptor does not result in loss of infectivity of the phage. Eclipse results, however, in loss of phage infectivity and is characterized by a conformational change of the phage whereby the phage nucleic acid becomes accessible to nuclease.

Uptill now most of the work on adsorption was done using E. coli phages. The adsorption of T4 to E. coli and the subsequent penetration of its nucleic acid into the cell can arbitrarily be subdivided into five discrete steps according to Benz and Goldberg (85).

1. Surface binding - reversible binding to surface receptor by long tail fibres.
2. Pinning - irreversible binding to receptor through tail pins and short fibres.
3. Contraction - tail sheath contracts.
4. Penetration - the inner tube of the tail "needle" penetrates the cell envelope layers and reaches the cytoplasmic membrane.
5. Unplugging - "unknown" membrane receptor triggers unplugging of needle and injection of the DNA ensues.

The long tail fibres, tail pins and short tail fibres anchor the phage to the T4 receptor found in the LPS of the outer membrane, triggering and controlling the contraction, penetration and unplugging process (86-88). A tentative model of adsorption of T4 to its receptor was given by Yamamoto and Uchida (88).

Surface binding occurs by interaction between the distal (carboxyl terminus) part of the long tail fibres (89) and a lipopolysaccharide containing as a minimal oligosaccharide structure in a micellar complex with lipid A (90,91).

\[
\text{Glu} \rightarrow 3 \text{ Hep} 1 \rightarrow 3 \text{ Hep} 1 \rightarrow (\text{KDO})_3
\]

This interaction is apparently necessary for tail contraction to be initiated (88). Whether the tail fibre recognises the oligosaccharide or the parts of lipid A are involved has not been investigated. Nor do we know whether one T4 tail fibre binds to one oligosaccharide/lipid A chain only or if repetitive sequences interact with several oligosaccharide lipid A chain in the LPS. The observation that isolated long tail fibres of T4 agglutinate bacteria (92) does suggest that the long tail fibre has more than one binding site. Even if the correct oligosaccharide sequence is found in the core, the presence of long O-polysaccharide chains as
in smooth *Shigella flexneri* strains may interfere with the binding (93). The binding between the long tail fibres and the receptor apparently represents the reversible step in phage T4 adsorption; phage T4 mutants lacking only the short tail fibres (12-particles) bind to the receptor and tail contraction is initiated but the long tail fibres cannot hold the phage to the receptor (94,95).

Pinning is thought to occur by interaction between tail pins and short tail fibres as phage organelles, with again LPS from the bacterium as receptor. This step is not at all well understood and has not been studied in isolated experimental systems. It may not even be accessible to a simple study since available data clearly indicate that the long tail fibres play an essential role in the control of tail contraction (88) and may thus be necessary for induction of conformational changes in the baseplate. The interrelationship, if any, between the short tail fibres and the tail pins is not known. It was once postulated that the tail fibres anchored the phage to the receptor irreversibly (86). Kells and Haselkorn (95) opposed this idea since they observed both tail pins and short tail fibres on unadsorbed T4 phages. This observation, which was not incorporated into the model of Yamamoto and Uchida (88), is the only basis for assuming that both tail pins apparently make the first contact with the receptor during "pinning"; the interaction
between short tail fibres and receptor should then be a subsequent step (86, 94). This interaction also appears to be controlling element in tail contraction, since certain mutants in the short tail fibre gene 12 have a blocked tail contraction (88).

It is not known whether the tail pins and short tail fibres interact with the LPS molecule; however, if they bind to the same or different structures, nor do we know the importance of oligosaccharide determinants (LPS layer) as opposed to lipid A, 2 ketodeoxyoctonate, phosphorus groups etc. However, available literature (88) stresses that all gene products of the T4 base plate can be replaced by gene products from the T2 base plate. The T4 phage is inactivated by LPS, the T2 phage by a protein fraction from the outer membrane, which appears contradictory.

Urea treated phage T4 contracted phage particles infect spheroplasts but not whole bacteria (96). This experimental model bypasses the need for long tail fibres (96), the "outer proteins" P5, P6, P11 and P12 of the base plate (85) as well as the protein of gene 10 (which is required for spheroplast infection by urea-treated T4 phage. The lytic spectrum of urea-treated phage, as assayed on spheroplasts, is also much wider than for the intact T4 phage. Thus when the LPS receptor recognition step is bypassed T4 can multiply in strains of \textit{Salmonella}, \textit{Serratia},
Enterobacter, Erwinia and Proteus (96, 85). Similarly reversibly adsorbed gene 12 mutants, which have a contracted tail sheath, infect spheroplasts with a broad host range (85).

Surface binding and pinning occurs during the early stages of adsorption. During the steps subsequent to pinning, e.g. contraction, penetration and unplugging, at least one other receptor is required, probably located in the cytoplasmic membrane (99, 85).

B. Penetration by the phage: The famous experiment by Hershey and Chase (5) showed that only the phage DNA penetrates the infected bacterium, leaving the protein coat outside. During phage adsorption the phage tail makes contact with the cell wall and so is brought into contact with zinc protein complexes of the bacterial cell wall triggering the unwinding of the tail fibres which then adhere specifically to certain molecular grouping of the cell wall. At the same time, the phage enzyme, is released after the rupture of the thiol ester bond present in viral tail, which bores a hole through the rigid layer of the bacterial cell wall (100, 101). The products of this lytic activity finally trigger contraction of the sheath, leading to penetration of the cell wall by the core and to discharge DNA into the space between the cell wall and the inner cell membrane. The tail tube does not penetrate this membrane. The high proline content of the tail tube in some way aids
the passage of the DNA through the tail tube. Bayer (102) has presented the evidence that the cytoplasmic membrane of \textit{E. coli} has several channels which reach almost to the surface of the cell. It is conceivable that the viral DNA passes through one of these channels into the cytoplasm of the cell, where the replication takes place.

C. The intracellular development of the phage: The first direct study of the intracellular events which follow phage infection was done by Doermann (103). After infection, during the eclipse period of phage and bacterial cell merge to become briefly a new organism (104). All host processes including DNA synthesis are stopped (105-109), but soon phage specific DNA is synthesized (110-113). The protein synthesizing machinery of the host is used for the synthesis of phage proteins. As phage DNA is different from the host, some enzymes are induced and they initiate new DNA synthesis (114-119). The action of the early enzymes (120-123) establishes the new metabolic pathway providing precursors for phage type DNA synthesis.

Once phage specific DNA is synthesized, it is followed by RNA and protein synthesis. So it is observed that the phage develops intracellularly in the form of precursor pools of DNA and protein, which increase linearly with time. The morphogenesis proceeds along a branched pathway. The
head, tail and tail fibres are formed independently and only when essentially complete, do these structures combine, and that too in a strict sequential order (124).

D. The lysis of the host bacteria: The discovery that phage tails contain an enzyme which can breakdown the bacterial cell wall, and the earlier observation that at a high multiplicity of infection by T-even phages produce almost immediate lysis from without (125), suggested that normal lysis might result from synthesis, directed by the phage genome, of a lytic enzyme within the infected cell.

Lysogeny: An important aspect in the classification of bacteriophages is the property of genetic integration into the bacterial genome to establish lysogenic bacteria. The decision between lysogenic and lytic pathways made by infecting temperate phage is finally balanced and modulated by a number of phage and host gene products. The critical event in the establishment of lysogeny appears to be the early and abundant synthesis of repressor (126). Non-hereditary factors such as temperature (127), age of the cells (128, 129); growth medium (130), multiplicity of infection (131, 127), affect this modulation. The introduction into the medium of agents such as chloramphenicol, which specifically inhibit protein synthesis, leads to virtually 100 per cent lysogenization (132).
That lysogenic bacteria do not harbour infectious phage particles was first demonstrated by Burnet and McKie (133) and this concept was later established by Lwoff and Gutman (134).

Lysogenic bacteria are capable of producing and releasing phage particles and they are immune to lytic response by same or closely related phages (128). In addition, lysogeny is not limited to the carriage of a single type of phage by each bacterium, many cases of double or triple lysogeny have been described while one strain of *Staphylococcus* has been reported to carry as many as five different phage types (135).

It was discovered that the exposure of lysogenic strain of *B. megaterium* to the doses of ultraviolet light resulted in the lysis of the entire population (after a latent period of one or two divisions) causing liberation of 70 to 150 phage particles per bacterium (136). Many other effective inducing agents have been found which include X-rays, $\gamma$-rays, nitrogen mustard, hydrogen peroxide, organic peroxides (137), 6-azauracil (138), fluorodeoxy uridine, mitomycin C (deprivation of thymine) (139) and aflatoxin B1 (140, 141).

All lysogenic bacteria are not inducible. The property of inducibility appears to be a function of the genetic
constitution of the phage rather than that of the bacterium. Among a series of phages lysogenic for \textit{E. coli} K-12, isolated at random from 500 strains of \textit{E. coli} derived from human faeces, some were inducible and some were not\cite{142}.

The bacteriophage which is lysogenic for \textit{E. coli} K-12 has been extensively studied\cite{143,144}. A large number of \textit{P. aeruginosa} strains have been found out to be lysogenic. The frequency of lysogeny in \textit{P. aeruginosa} strains is probably close to 100 per cent\cite{145,39,146,58}, in contrast to \textit{E. coli} where only about 10\% of strains are lysogenic\cite{82}. Polylysogeny also appears to be common in \textit{P. aeruginosa}\cite{147-150} including one example of a strain harbouring 8 and possibly 10 different prophages\cite{151}. Lysogeny among other species of \textit{Pseudomonas} appears to be very rare or undetectable\cite{39,58}. Although Patterson\cite{58} found 3 out of 8 \textit{P. fluorescens} strains to be lysogenic. However, Hamon and his colleagues\cite{39} did not find any amongst 28 \textit{P. fluorescens} strains tested. In fact, they found only one lysogenic strain amongst a collection of 19 \textit{P. stutzeri}, \textit{P. putida} and phytopathogenic \textit{Pseudomonas} strains\cite{152}.

The genome of both the phage and the sensitive bacterium can influence the establishment of lysogeny. Clear plaque mutants (C mutants) can be readily isolated from most temperate phages and these show a reduced
ability to lysogenize. A genetic analysis of such mutants in the *P. aeruginosa* phage D3 (153) showed that three loci were involved, analogous to the pattern of genetic control of lysogeny in coliphage lambda (154).

**Pseudomonas bacteriophages**

A large variety of temperate and virulent phages of *P. aeruginosa* are known to date. Many, if not all, strains of *P. aeruginosa* are lysogenic. However, other species of *Pseudomonas* are not commonly lysogenic. For *P. aeruginosa* and other species virulent (or intertemperate) phages may be readily isolated from soil or sewage. Bacteriophages of *P. aeruginosa* have not been described as widely as those of *E. coli*. The properties of a few *P. aeruginosa* phages are shown in Table 3.

Morphologically, the range of structures is as diverse as in other bacterial genera. Phages containing long contractile tail, short non contractile tail and contractile tail have been reported (155, 156, 157, 150). Phages similar to lambda, filamentous phages, and spherical phages have also been reported (158, 159, 53, 160, 161). By comparison with other phages, phage PM2, which plates on a marine *Pseudomonas* and phage φ 6 of *P. phaseolicola* have an unusual morphology. Phage PM2 has an internal nucleocapsid surrounded by a phospholipid bilayer bridged by
<table>
<thead>
<tr>
<th>Phage</th>
<th>Morphology</th>
<th>Nucleic acids</th>
<th>Virulent or Temperate (min)</th>
<th>Burst size</th>
<th>Mol. wt. (X10^6) daltons</th>
<th>Other characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ϕMC</td>
<td>Icosahedral (55 nm diam. head), short tail</td>
<td>Double stranded DNA</td>
<td>Temperate</td>
<td>45</td>
<td>42</td>
<td>Phage adsorbs to terminal ends of P. aeruginosa</td>
<td>(164,165)</td>
</tr>
<tr>
<td>SD1</td>
<td>Hexagonal head (50 nm diam.) and tail (108 nm x 6.2 nm)</td>
<td>Double stranded DNA</td>
<td>Virulent</td>
<td>-</td>
<td>-</td>
<td></td>
<td>(166)</td>
</tr>
<tr>
<td>B3</td>
<td>Octahedral (52 nm diam. head) with almostless tail (163 nm x 8 nm), 3 front tail fibres and tail plate</td>
<td>Double stranded DNA</td>
<td>Temperate</td>
<td>-</td>
<td>20-25</td>
<td>Ultraviolet noninducible, Transducing shows HEM; DNA is linear and has no cohesive ends.</td>
<td>(166,167,168)</td>
</tr>
<tr>
<td>F116</td>
<td>Octahedral (50 nm diam. head) with loosely structured and heavy tail (79 nm x 8 nm)</td>
<td>Double stranded DNA</td>
<td>Temperate</td>
<td>-</td>
<td>35</td>
<td>Ultraviolet inducible; Transducing does not show HEM; DNA is linear and has no cohesive ends.</td>
<td>As for phage B3</td>
</tr>
<tr>
<td>G101</td>
<td></td>
<td>Double stranded DNA</td>
<td>Temperate</td>
<td>-</td>
<td>39-41</td>
<td>Ultraviolet noninducible; Transducing shows HEM; DNA is linear and has no cohesive ends.</td>
<td>As for phage B3</td>
</tr>
<tr>
<td>ϕ3</td>
<td>-</td>
<td>Double stranded DNA</td>
<td>Temperate</td>
<td>-</td>
<td>-</td>
<td>Phage recombination has been demonstrated and also mediates phase conversion of bacterial surface antigens; shows HEM.</td>
<td>(148,153,169)</td>
</tr>
<tr>
<td>R79</td>
<td>Octahedral (66 nm diam. head), contractile tail (150 nm x 17 nm) with six tail fibres and tail plate</td>
<td>Double stranded DNA</td>
<td>Virulent</td>
<td>-</td>
<td>120 (estimate)</td>
<td>-</td>
<td>Non-transducing does not show HEM</td>
</tr>
<tr>
<td>Pf1,Pf2</td>
<td>Filamentous</td>
<td>Single stranded DNA</td>
<td>Virulent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not sex specific (FP2') in P. aeruginosa as with similar phages in E. coli (Hoffmann-Berling, Keeser, Knappe, 1967). Narrow host range.</td>
</tr>
<tr>
<td>75</td>
<td>Spherical (25 nm diam.)</td>
<td>Single stranded RNA</td>
<td>Virulent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Isolation of RNA phage from lysogenic P. aeruginosa is unusual; sensitivity of free phage to RNases is unusual. Phage multiplication in bacterial host leads to lysis before phage release. Not sex specific (FP) in P. aeruginosa. Plates on wide range of grief containing R factor R1822.</td>
</tr>
<tr>
<td>77</td>
<td>-</td>
<td>Single stranded RNA</td>
<td>Virulent</td>
<td>-</td>
<td>36000 (estimate)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PRK1</td>
<td>-</td>
<td>RNA</td>
<td>Virulent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Plates on wide range of grief containing R factor R1822.</td>
</tr>
<tr>
<td>Twelve phages</td>
<td>Octahedral (66 nm diam. head), contractile tail (15 nm x 17 nm), with six tail fibres and tail plate</td>
<td>Double stranded DNA</td>
<td>Temperate</td>
<td>-</td>
<td>35</td>
<td>Ultraviolet inducible transducing</td>
<td>As for phage B3, DNA is linear and has no cohesive ends.</td>
</tr>
</tbody>
</table>
spikes and a final outer icosahedral shell (162). Phage $\phi 6$ has a polyhedral head surrounded by a membranous compressible envelope which appears to elongate on attachment to pili (163). These two phages are the only phages reported to contain phospholipid and glycoprotein components characteristically found in many plant and animal viruses.

P. aeruginosa phages utilize a diverse of structures for adsorption on their host. A tailless small RNA phage and filamentous phages utilize pili for adsorption and infection (157). A few cell wall specific phages of P. aeruginosa have been reported. These include phage 2 (175), $\phi$ PLS-1 (176), phage 16,44,109, and $E8$ (177), 68 and PB1 (178), $E79$, $B_3$, $D3$, and G101 (179,180).

The nucleic acid of Pseudomonas phages is usually of the double stranded DNA ranging in size from 6-120 X 10$^6$ daltons. The DNA of PM2 which plates on a marine Pseudomonad is of particular interest. The double stranded molecule is covalently closed and consequently is supercoiled having $51\pm3$ superhelical turns per phage molecule. This property has been used to study the relative contributions of single-strand breaks, double strand breaks and nucleotide damage in DNA produced by gamma radiation (181).

Several RNA phages also have been found (171,156,174) for Pseudomonas. Unlike the RNA phages of E. coli, only one
RNA phage (PRR1) shows some degree of host specificity (174). This PRR1 phage not only plates on *Pseudomonas* but also to those bacterial strains possessing a particular RP plasmid, for example *Enterobacteriaceae*, soil saprophytes, *Neisseria perflava* and photosynthetic bacteria. The nucleic acid of phage φ6 is unusual in that it is the only known phage with double stranded RNA (182, 163), a characteristic only shared by some animal and plant viruses. Moreover, φ6 phage RNA exists in a triple-segmented form.

The host range of most phages are usually specific, plating only on one species. Some exceptions to this rule occur in case of some phages, showing plating efficiency on such varied species like *P. aeruginosa*, *P. putida*, *P. fluorescens*, *P. geniculata* and *P. stutzeri* (183, 184).

**Bacteriophages binding to exopolysaccharides:**

Numerous bacterial species produce exopolysaccharides which are not structural components of the cell envelope, but lie outside it or are released into the environment. Depending on their relationship to the bacterial cell, they have been termed either "capsule" or "slime". The capsule is thought to have a binding site in the cell envelope, whereas the slime should have no, or few, connecting points in the envelope.

**Attack of bacteriophages on exopolysaccharide**
producing bacteria is often revealed by the fact that the plaques are surrounded by a halo (185-189). Within the halo the bacterial lawn is decapsulated as evidenced by a diminished volume of the plaques (188,189,190). These haloes are at least partly, the result of diffusion of a phage induced enzyme which hydrolyses the capsule without killing the bacteria. The finding of haloes almost created the concept that phages active on strains producing exopolysaccharides always are surrounded by haloes. This is however, not always the case.

Sertic and Gough (191) isolated a phage mutant active on capsule producing bacteria, where the plaque was not surrounded by a halo. There are a number of subsequent reports in the literature describing the same phenomena (192-195).

Bacteriophages active on exopolysaccharide producing bacterial strains are generally exopolysaccharide specific. Non-capsulate or non-slime producing mutants are resistant to the phages (192,196). Interaction between phage and exopolysaccharide does not trigger the ejection of the phage nucleic acid, while the presence of the specific exopolysaccharide does not always correlate with the phage sensitivity (197,198,199). Evidently the phage has to interact with a second unknown receptor for triggering of nucleic acid ejection.
Morphological examination of exopolysaccharide specific phages has revealed that most of them belong to group C (possess an isometrical head containing double stranded DNA, and a short baseplate with spikes attached to it). Bacteriophages representatives of group A (long tails with a contractile tail sheath) and group B (long tails without the contractile tail sheath) are, however, also found (194). Vi phages I, II and III, all attacking Vi-polysaccharide producing *Salmonella typhi* and deacetylating the poly-N-acetyl galactosaminuronic homopolysaccharide, represent the morphological groups A, B and C, respectively (200,201,202). One common feature to all phages active on exopolysaccharides is that the baseplates, as seen in the electron microscope, are provided with spikes and that no tail fibres are seen. Because of the relative simplicity of the group C phage in terms of number of phage proteins present, as compared to group A and B phages, most of the work in characterization of phage associated depolymerases has been done with group C phages. Morphological studies on ten such phages by Stirm and Freund-Mölbert (194) revealed that the heads are isometric. A structure clearly visible as a baseplate was seen only in the largest phage (head diameter 63 nm) (194). Later studies on one of the smaller phages, the *E. coli* K 29 phage, showed that it too had a baseplate (203). The baseplate looked like a six pointed star of 14 nm. All phages have
tail spikes linked to the baseplate, probably six or
twelve per phage. The spikes appeared as hollow tubes:
15 nm long and 5 nm in diameter in phage E. coli K 29 (203),
12.5 nm in Klebsiella phage 11 (204) and 11 nm by 4 nm in
Vi-phage III (205).

The phage-associated depolymerases termed as capsule
or exopolysaccharide depolymerases are only detected in
cultures infected by specific bacteriophages (206,207).
Recent attempts to isolate and purify the exopolysaccharide
depolymerases have been based on two approaches: to
isolate the free enzyme from supernatants of phage lysates,
or to isolate the bound enzyme by disruption of a purified
phage particle. The first approach involves differential
centrifugation of the phage lysate, salting out of the
protein, gel filtration and ion-exchange chromatography.
This procedure is economical since, in most systems studied,
free enzyme is produced up to twenty fold excess over phage
bound (208,209) enzyme.

Until now a few free exopolysaccharide depolymerases
have been isolated and purified to homogeneity which have
been shown to have molecular weights in the range of
150,000 to 380,000 daltons. Electron micrographic investi-
gations revealed that most of these enzymes are cylindrical
and of approximately the same size and shape as the tail
spikes seen on the baseplates of the corresponding
bacteriophage particle (210,209). The amino acid composition of isolated depolymerase tail spikes (free or obtained after disruption of phage) has been determined for the Enterobacter aerogenes phage 2 (211), Klebsiella phage 11 (210) and E. coli phage 29 (203). Aspartic acid, glycine, serine and alanine were the predominant amino acids found. No amino sugars or carbohydrates were detected.

Characteristics of few isolated exopolysaccharide depolymerases have been shown in table 4.

Data on the composition and structure of exopolysaccharides produced by Gram-negative bacteria outside the Enterobacteriaceae family are fragmentary. Pseudomonas phages specifying the synthesis of exopolysaccharide depolymerases have been described in P. putida (193) and in P. aeruginosa (198,175,214,215). In the latter case 6 apparently distinct depolymerases specified by 6 different phages have been characterized and shown to possess at least 2 distinct substrate specificity groups on the basis of enzymic activity on different exopolysaccharides (215). One of the phages, phage 2, and its interaction with slime polysaccharide B have been investigated. Phage 2 belongs to group B of Bradley (53) morphologically and has a long tail with a prominent Knob-like structure at the tip (175). This phage produces a depolymerase in excess when grown on the exopolysaccharide producing strain. The free depolymerase
Table: 4. Phage Depolymerases hydrolysing exopolysaccharides.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Phage Morphological type - Bradley</th>
<th>Estimated MW of spike tail unit with enzymatic activity</th>
<th>Enzymatic specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) <em>E. coli</em> K29</td>
<td>29</td>
<td>245,000</td>
<td>57,000</td>
<td>Endoglucosidase</td>
</tr>
<tr>
<td>2) <em>E. coli</em> &quot;colanic acid&quot;</td>
<td>m59</td>
<td>140,000</td>
<td>?</td>
<td>Endofucosidase and endoglucosidase</td>
</tr>
<tr>
<td>producing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) <em>Klebsiella pneumoniae</em></td>
<td>11</td>
<td>155,000</td>
<td>62,500</td>
<td>Endoglucosidase and endogalactosidase</td>
</tr>
<tr>
<td>4) <em>Aerobacter aerogenes</em></td>
<td>K-2</td>
<td>379,000</td>
<td>63,000 or 36,000</td>
<td>Endogalactosidase</td>
</tr>
<tr>
<td>5) <em>Salmonella typhi Vi</em></td>
<td>ViIII</td>
<td>?</td>
<td>91,000</td>
<td>Deacetylase</td>
</tr>
<tr>
<td>6) <em>Pseudomonas aeruginosa</em> BI</td>
<td>2</td>
<td>180,000</td>
<td>?</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
was isolated from phage 2 lysate and purified by high speed centrifugation, ammonium sulphate precipitation and gel chromatography. The molecular weight of this depolymerase was estimated to be 180,000 daltons (213).

The specificity of phage 2 depolymerase is not known because little information is available on the structure and quantitative composition of exopolysaccharide (216). Two interesting features were, however, observed when the interaction between phage 2 and slime and LPS from the susceptible host P. aeruginosa was studied (175). First, phage tail fibres were seen when the phage-receptor reaction was studied in the electron microscope. Secondly, the interaction between the slime and the phage triggered DNA ejection, whereas in all other instances the exopolysaccharide has failed to eclipse the phage. Phage 2 ejection was also triggered by isolated LPS; thus it may bind to and be eclipsed by both exopolysaccharide and LPS from Pseudomonas aeruginosa BI. Phage 2 was reported to cause lysogenic conversion of P. aeruginosa with concomitant loss of phage 2 receptor activity (217).

Present Investigation:

Apart from the plasmids which have been elaborated, to certain extent in Pseudomonas aeruginosa two other plasmid related structures merit special consideration (Bacteriocin
and Bacteriophages). Both are concerned with important aspects of the phenotype of this bacterium. The genetic determinants of both constitutes an important component of the genome which play an important role in the genus *Pseudomonas*. Therefore, in the present investigation studies were carried out in detail to understand the role of bacteriocin and bacteriophage in the morphological and functional analysis of the genus *Pseudomonas* in the following aspects:

1. Cultural conditions favouring the production of aeruginocin A10 from *P. aeruginosa* AIIMS 10.
2. Investigation of aeruginocin A10 receptors on *P. aeruginosa* PA01.
3. Isolation and characterization of phage PIK specific for *P. aeruginosa* PA01.
4. Adsorption properties of phage PIK with respect to pigmentation.
5. Isolation, purification and characterization of phage PIK receptors on *P. aeruginosa* PA01.
6. Isolation, purification and characterization of enzyme exopolysaccharide depolymerase from phage PIK infected *P. aeruginosa* PA01 and its role in phage PIK infection and release.
References


