Bacteriocins of Vibrio cholerae in relation to defective bacteriophage
by John Vander Schalie

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:
Defective bacteriophage of Vibrio cholerae were studied, A mutant phage which retained its viability was found by electron microscopy to have an aberrant structure, Phage-associated particles released from a vibrio host upon mitomycin C induction were isolated and partially characterized. These particles had activity similar to bacteriocin of V. cholerae called "vibriocin," and exhibited serological homologies with several choleraphages. Upon mitomycin C induction, phage were isolated from two strains of non-agglutinable V. cholerae.

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Date December 4, 1970

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ABSTRACT

Defective bacteriophage of Vibrio cholerae were studied. A mutant phage which retained its viability was found by electron microscopy to have an aberrant structure. Phage-associated particles released from a vibrio host upon mitomycin C induction were isolated and partially characterized. These particles had activity similar to bacteriocin of V. cholerae called "vibriocin," and exhibited serological homologies with several choleraphages. Upon mitomycin C induction, phage were isolated from two strains of non-agglutinable V. cholerae.

INTRODUCTION

Bacteriocins were discovered in 1925. Since then, many genera of bacteria have been found capable of producing bactericidal or bacteriostatic substances. Certain agents, originally called bacteriocin, have subsequently been shown not to satisfy the formal definition of "bacteriocin." Even now, there remains a heterogeneity in the types of substances included in this category. It has been suggested that one of the two general bacteriocin types is a form of lysogeny for a bacteriophage that is defective.

This investigation involved a study of defective phage of Vibrio cholerae and a substance released from vibrio hosts which satisfies the formal definition of bacteriocin. The purpose of this investigation was to search for existing correlations between the defective cholera phages and a bacteriocin of V. cholerae called "vibriocin." These studies also define the first reported isolation of bacteriophage from the non-agglutinable vibrios, and reveal the aberrant structure of a viable mutant choleraphage.

REVIEW OF THE LITERATURE

Historical Observations

The discovery of bacteriocins and the development of knowledge on the relationships of these substances to the bacteria which they affect has followed quite closely the study of bacterial viruses. The initial association between these two fields was due to the similar methods used in detecting phage and bacteriocins and to similarities in the gross effect on the bacterial cell. As the bacteriocins have become better defined, the rationale for studying them in association with bacteriophages seems well justified.

Gratia (1925) was the first to isolate and define the class of substances known as bacteriocins. He found that a substance produced by one strain of Escherichia coli would inhibit another strain. The inhibition which he observed was similar to that of a bacteriophage with one essential difference; it did not reproduce in the sensitive strain. The term "colicin" was coined by Gratia to designate this class of inhibitory substances. Jacob et al. (1953) defined the protein nature of colicins, showed that biosynthesis by a colicinogenic strain was lethal for that strain, revealed the involvement of receptors on the bacterial cell, and introduced the general term "bacteriocin" to the antibacterial substances produced by various species of bacteria.

Many genera of bacteria have now been shown to possess the genetic capacity to produce bacteriocins. It seems clear that bacteriocinogeny will become as general a phenomenon as lysogeny.

Bacteriocin Characteristics

Bacteriocins are a natural class of antibiotics or bactericidal substances which are as efficient as they are unique in their mode of action. The narrow specificity of their action and their protein nature distinguish them from most of the other "classical" antibiotics. They affect sensitive bacteria by adsorption to a specific receptor site (Maeda and Nomura, 1966), which may also serve as a receptor for phage (Fredericq, 1948, Fredericq and Gratia, 1949), and act while remaining at the cell surface (Nomura, 1963). Adsorption of a single molecule is sufficient to kill a sensitive bacterium (Jacob et al., 1952). Bacteriocins are active on other bacterial strains of the same or closely related species. The producing cell, called bacteriocinogenic, is resistant or immune to the action of the bacteriocin it produces, or homologous "external" colicins (Nomura, 1967). Bacteriocins, unlike phage, contain no nucleic acid (Kageyama and Egami, 1962) and do not multiply in the cells they kill. Extensive studies

done on colicins have revealed unique features showing the close relationship of bacteriocins to bacteriophages on one hand (Endo et al., 1965, and Sandoval et al., 1965), and to F factors on the other (Bhaskaran, 1964, and Hayes, 1968), and testify that the classification of bacteriocins as a unique class of antibacterial substances is appropriate.

Bacteriocin Types

There are two distinct basic types of bacteriocins. The properties of the two types are summarized in Table 1 (from Bradley, 1967). Low molecular weight bacteriocins can be released with the absence of cellular lysis, whereas following the induction of high molecular weight bacteriocins, which resemble bacteriophage tails, release follows lysis of the host cell. The common feature of the two types is their protein nature and use of specific receptor sites on the host cell (Reeves, 1965). The two bacteriocin types are not necessarily associated with different bacterial species. Both types may be found in the same species. For example, colicin V of E. coli forms a large clear area on agar and is dialyzable and sensitive to trypsin. These properties make it a member of the first bacteriocin type. Colicin 15, also produced by an E. coli strain, has a molecular weight exceeding 2×10^5, is sedimentable, and

TABLE 1. Properties of the two groups of bacteriocins*

Low molecular wt. bacteriocins	High molecular wt. bacteriocins
Not sedimentable	Sedimentable
Trypsin-sensitive	Trypsin-resistant
Thermostable	Thermolabile
Cannot be resolved in electron microscope	Visible in electron microscope as phage-like objects or components

* From Bradley, D. E., 1967. Ultrastructure of bacteriophages and bacteriocins. Bact. Rev. 31:230-314.

can be observed in the electron microscope as phage-like components (Bradley, 1967). Campbell (1969) states that the high molecular weight structures are "obviously a form of lysogeny for a phage that is defective" and that their inclusion with other types of bacterioinogeny is a "semantic accident."

There are instances where bactericidal substances released from a cell were termed bacteriocins, and later were shown to be erroneously classified as such. Megacin A, produced by B. megaterium, is an inducible bacteriolytic substance which has since been shown to be a simple hydrolytic enzyme, with phospholipase A activity (Nomura, 1967). Nomura states that it is likely that this enzyme may be the product of a highly defective lysogenic strain which, after induction, produces no particle with visible phage structure, but produces several proteins, one of which is phospholipase A which can be detected by its killing action.

Every group of the Enterobacteriaceae has been shown to produce bacteriocins except Proteus-Providencia (Hamon and Peron, 1963). Bacteriocins have been discovered in other bacterial families including a few Gram positive organisms such as Staphylococcus, Streptococcus, and Bacillus. A partial listing of bacteriocins and the producing genera is contained in Table 2 (from Reeves, 1965).

TABLE 2. Various families of bacteriocins*

Bacteriocin name	Producing genus
Colicins	*Escherichia*
	Paracolobactrum
	Shigella
	Salmonella
	Aerobacter
Alveicins	*Hafnia*
Caratovoricins	*Erwinia*
Arizonacins	*Paracolobactrum*
Cloacins	*Enterobacter*
Marcescins	*Serratia*
Pneumocins	*Klebsiella*
Aerocins	*Aerobacter*
Pyocins	*Pseudomonas*
Fluocins	*Pseudomonas*
Pesticins	*Pasteurella*
Megacins	*Bacillus*
Monocins	*Listeria*
Cerecins	*Bacillus*
Enterococcins	*Streptococcus*
Staphylococcins	*Staphylococcus*
Vibriocins	*Vibrio*

* Compiled from Reeves, P. 1965. The bacteriocins. Bact. Rev. 29:24-45.

Some explanation of the nomenclature of bacteriocins should be included here. It is obvious, and stated by Bradley (1967), that the classification and hence the nomenclature of bacteriocins has room for improvement. The existing names are given to the various bacteriocin families based on the specific rather than the generic name of the host organism. This is due perhaps to the high degree of specificity of bacteriocins. Thus, colicins are bacteriocins of E. coli, monocins of Listeria monocytogenes, and so on. Fredericq (1948a) based a classification of colicins on the spectrum of resistance of various mutants of E. coli. From this code, nomenclature is used based on the number of the producer strain, followed by the original letter designation given by Fredericq (1948b). For example, colicin CA23-D is a type D colicin produced by E. coli strain CA23.

Mode of Action

Bacteriocins, as with bacteriophages, adsorb to specific host receptors located within the cell wall. The first stage of killing is the specific, irreversible (in most instances), adsorption of the bacteriocin. With most low molecular weight bacteriocins, trypsin "rescue" is possible for a short time following adsorption by the addition of trypsin to the medium (Bradley, 1967). Nomura and Nakamura

(1962) found that the complete inhibition of macromolecular synthesis by a small molecule type colicin was reversible by trypsin treatment. This suggests that some bacteriocins are bacteriostatic rather than bactericidal. It also suggests that bacteriocins exert their action while attached to the outside of the cell. This has been shown in several instances (Nomura, 1963). Trypsin added to the growth medium in which the host cells are suspended will also effectively prevent the adsorption of low molecular weight bacteriocins (Ozeki, 1968).

The biochemical effects produced by colicins of E. coli have been studied in the most detail. Table 3 summarizes these effects as listed by Nomura (1967).

A theoretical explanation for how a bacteriocin present on the outside of the cell can arrest the synthesis of macromolecular components was given by Nomura (1963). He postulated a functional alteration of some specific element of the cytoplasmic membrane caused by the attachment of the bacteriocin. The bacteriostatic or bactericidal effect is then exerted through hypothesized "amplification" mechanisms residing in the cell envelope. Luria (1970) suggests this amplification mechanism may be mediated by conformational changes of the cell membrane.