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Bacteriocins: The Lure and the Reality

Agents to control plant pathogens should be specific; harmful organisms should be attacked without destroying beneficial ones. Ideally, the disease control agent should be easy to produce and store, cheap to make and use, and safe for the user and the environment. Many of these properties are found in some specialized antibiotics called bacteriocins, or bacteria-killers.

What Are Bacteriocins?

Historically, the term "bacteriocin" was applied to antibioticlike compounds with specificity primarily restricted to bacterial strains related to producer strains; this can be thought of as microbial "murder" of one's relatives. These bacteriocins contained protein as an essential constituent. Their specificity and chemical composition served to distinguish them from so-called classical antibiotics, such as streptomycin, a broad-spectrum, low-molecular weight aminoglycoside. And "Despite the impossibility of defining the limits of 'closely related'. . . the term bacteriocin is readily justifiable to encompass this distinctive group of antibiotics" (15).

The historical definition of bacteriocins applies to the most widely studied group, the colicins, which are produced principally by the gut inhabitant *Escherichia coli* (6,10). Killing activity of colicins is directed primarily against other strains of *E. coli* and close relatives. Colicins have no activity against gram-positive bacteria or against most other gram-negative bacteria. Substances analogous to colicins are produced by many groups of bacteria, as well as by some yeasts and protozoa. Because of the wide variety of inhibitors produced by many bacteria and their general lack of chemical characterization, it is difficult to decide which substances can usefully be called bacteriocins. The problem of definition becomes more acute with the well-studied, specific, *nonprotein* antibiotic agrocin 84, produced by *Agrobacterium radiobacter* strain 84 (9); strain 84 is used as an effective biological control agent for crown gall in many plant species. (*A. radiobacter* is in the "Approved Lists" but the most acceptable classification is the saprophytic, nonphytopathogenic state of *A. tumefaciens*.)

There is, therefore, no commonly acceptable definition or classification of bacteriocins. About a hundred bacteriocins have been characterized; these are very heterogeneous chemically, ranging from low molecular weight compounds to large macromolecules. All these compounds, however, have the primary property of restricted biological specificity, the basic characteristic used to delimit bacteriocins from other antibiotics.

Discovery and naming of bacteriocins. Specific antagonism between related bacteria was reported in the 1920s, but the term "colicin" was not coined until 1946 and the more general term "bacteriocine," in 1953 (10,14); the terminal "e" is usually omitted in current writing.

A uniform system of naming bacteriocins does not exist (10); names are generally derived from the producing genus or species. One prevalent method is to use the foot of the species plus "cin," followed by a strain designation, eg, syringacin 4-A, which conveys the information that strain 4-A of *Pseudomonas syringae* pv. *syringae* produces a bacteriocin.

Prevalence and detection of bacteriocins. Bacteriocinogeny, or the ability to produce bacteriocins, is widespread. Species in over 30 bacterial genera, including many plant pathogens (18), have been reported to produce substances antagonistic to related bacteria (10). Such apparently specific antagonisms, however, can be due to nonspecific compounds, including hydrogen peroxide, lactic acid, and ammonia (10). A good criterion for specific bacteriocin production is to determine whether the producing bacterium is insensitive or immune to generally effective concentrations of its own bacteriocin. The producer is rarely sensitive to its own bacteriocin.

Within a species, strains originating from different locations and environments collectively may produce from few to many bacteriocins. For example, many pathovars of *P. syringae* produce different, but strain- or species-specific, inhibitory substances (18,19). Individual strains may produce from one to several different bacteriocins, often dependent on growth conditions. Strains that produce more than one bacteriocin at a time are rare, however.

Indicator strains are required to detect a particular bacteriocin. As with other antibiotics, bacteriocin activity is detected by looking for a zone of growth inhibition (Fig. 1). Zone size and clarity can be affected by different indicators, as well as by indicator cell concentration. Various detection schemes are used (11). One of the simplest, least ambiguous methods is growing the prospective producer strain on a rich solid medium until growth is stationary, then killing the producer with chloroform vapors and pouring or streaking an indicator over the producer. Further incubation shows the presence or absence of bacteriocins.

Bacteriocin production is affected by a variety of conditions, most notably the growth medium and temperature (10,11). The growth phase of a producer also frequently affects production. So, we can say that a particular strain does or does not produce a specific bacteriocin only under the particular conditions tested. We cannot infer that a bacterium will produce a bacteriocin without doing the appropriate tests. A possible exception to such inference is to treat cultures with an inducing agent (one that can increase

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production in many strains), such as mitomycin C, and examine culture lysates by electron microscopy for so-called particulate bacteriocins; these resemble parts of bacterial viruses. If such particles have no biological activity against any test strain, however, they are considered defective bacterial viruses rather than bacteriocins. Because of the occasional serological relatedness between bacteriocins and bacterial viruses, many microbiologists believe these particles represent a continuum of evolution.

How Do They Attack?

Several bacteriocins require specific sites on the bacterial cell wall, called receptors, for attachment and killing. The low molecular weight agrocin 84 relies instead on a specific binding protein of the periplasmic space in *A. tumefaciens*; the specific sensitivity is determined by the Ti (tumor-inducing) plasmid of the target bacterium (12).

Particulate bacteriocins can be seen with the electron microscope as viruslike components, often attached to cells. If the cell wall is stripped away with enzymes or chemicals, however, the specificity can be lost. A number of bacteriocins then can bind to the cytoplasmic membrane and damage it in different ways. Such activity has led to the recognition that many bacteriocins have two stages of adsorption: one dependent on a specific receptor or attachment site in the outer membrane or wall and one independent of a specific attachment site. This suggests that bacteriocins could be as effective against wall-less bacteria, such as mycoplasmalike organisms, as against those with walls.

In tissue culture of animal origin, ie, without any physical barrier, some bacteriocins appear to show a selective toxicity toward some tumor cells (20). If these reports are confirmed, some bacteriocins may be of chemotherapeutic use in human and animal medicine. Figure 2 shows the similarity of agrocin 84 to ara-C, an effective anticancer drug, other biologically active adenine derivatives, and their relationship to the normal nucleotide, adenine.

Fig. 1. Bacteriocin production by *Corynebacterium* species, showing differences in size and clarity of the zones and some nonproducers. Each strain is replicated three times on a plate.

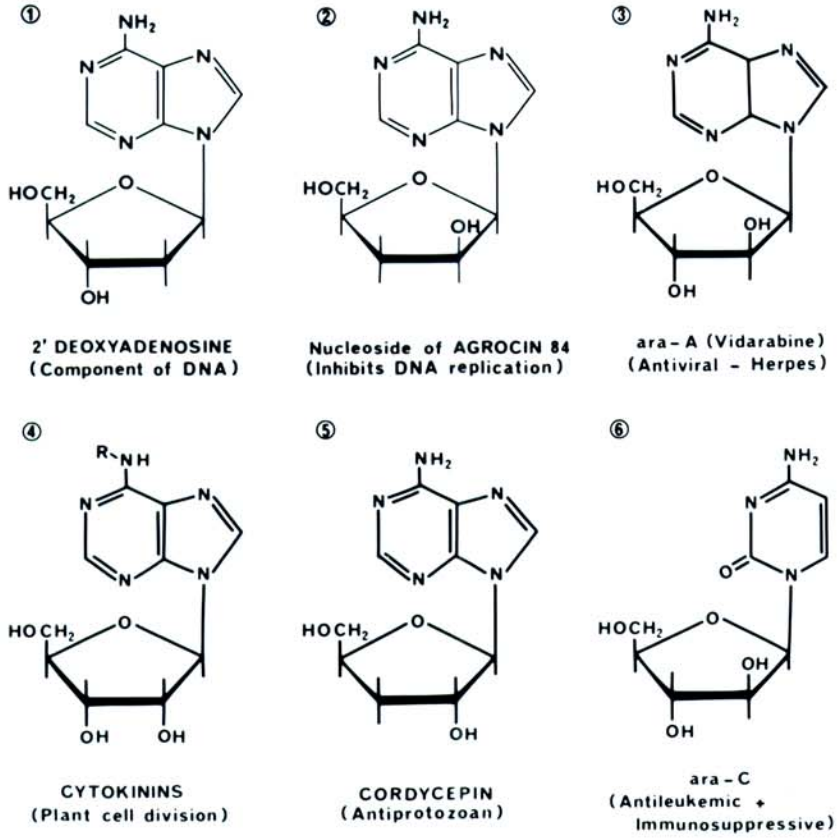

Fig. 2. Some biologically active nucleosides.

What Do They Do?

Bacteriocins generally kill bacteria in any of several different ways, reflecting the heterogeneity of these compounds. Most affect protein synthesis, DNA stability, energy flux, or membrane integrity. For bacteriocins effective against plant pathogens, only the mode of action of agrocin 84 and a carotovoricin (produced by *Erwinia carotovora*) have been studied. Agrocin 84 specifically inhibits DNA synthesis (4), which is not surprising in view of its being a structural analog of adenine (Fig. 2). Carotovoricin has phospholipase activity and thus acts on the cytoplasmic membrane (7).

Genetics. Bacteriocin production of the most widely studied bacteriocins, including agrocin 84, is controlled by the presence of extrachromosomal elements called plasmids. Bacteriocin production is also plasmid-determined in a saprophytic strain of *E. herbicola* (S. V. Beer, *personal communication*). Sensitivity to bacteriocins may also be a plasmid-determined property, as in *A. tumefaciens*. In some bacteria, chromosomal genes code for bacteriocin production. The genetics of chromosomal genes is more difficult to study than that of plasmid-coded ones.

Uses of bacteriocins. Bacteriocins have potential for pathogen control (Fig. 3). They are used to follow the spread of bacteria (epidemiology) and to identify some bacteria (taxonomy). Broad-

spectrum antibiotics have been so effective in medical microbiology that bacteriocins have been tested only for use in poorly healing surface infections. Bacteriocins are used in epidemiological investigations to type or differentiate strains that are otherwise indistinguishable. For example, the source of hospital-acquired infections is frequently traced by isolating the bacteria from each patient and performing bacteriocin typing (usually in combination with other typing systems [1]). Strains usually are tested for production of the same pattern of bacteriocins. If different bacteriocin types are isolated, the bacteria are usually from more than one source; if a single bacteriocin type is isolated, the source or carrier can often be traced. Bacteriocins are also useful in identifying some bacteria, because production of particular groups of bacteriocins tends to be restricted to certain taxonomic groups.

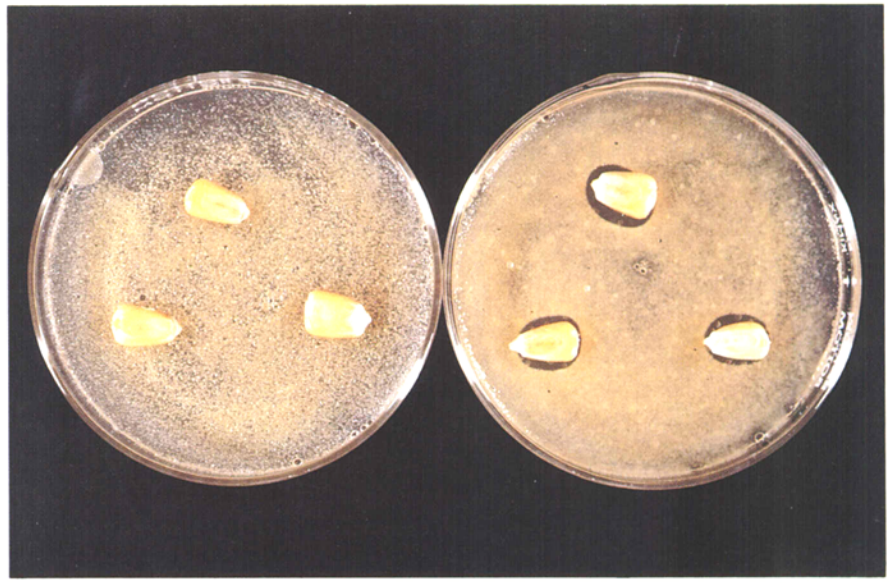

Fig. 3. Protection of corn seed from contamination by *Corynebacterium michiganense* subsp. *nebraskense*. Seeds were dipped into buffer (left) or partially purified bacteriocin (right), dried, and then placed on a plate seeded with bacteria sensitive to the bacteriocin.

Bacteriocins in nature. Bacteriocins may play a role in successful intraspecies and interspecies competition in nature, but definitive evidence is lacking. In medical microbiology, bacteriocins may be effective antagonistic agents in mixed infections; the detection of bacteriocins in blood sera of humans and other animals suggests that such interactions occur (10). In phytobacteriology, bacteriocin production by *A. radiobacter* strain 84 has yet to be shown to occur on plant surfaces or in wounds. Bacteriocin production on treated plants might occur transiently, continuously, or not at all; even if bacteriocin is produced, the quantity may be necessary but by itself insufficient to account for crown gall control. For example, transfer of the plasmid determining agrocin 84 production to a nonbacteriocinogenic strain was insufficient to effect control equivalent to that with strain 84, suggesting that an additional factor(s) was necessary (5).

In fire blight, *E. amylovora* infection of blossoms could be reduced by treatment with bacteriocin-producing antagonistic *E. herbicola*, a close taxonomic relative. However, a nonbacteriocinogenic strain, detected in the laboratory, was as effective in control as the bacteriocinogenic strain (2). A *P. syringae* pv. *syringae* bacteriocin-producing strain effectively suppressed a bacteriocin-sensitive pathogenic strain in infected bean hypocotyls at certain cell concentrations, and bacteriocin activity could be detected in extracts from plant tissues (17). No growth suppression occurred when a bacteriocin-resistant mutant of the sensitive strains was used. These are examples of current research in the use of bacteriocin-producing strains to control certain plant-pathogenic bacteria.

The chief advantage of using bacteriocin-producing strains in biological control is the presumed perpetuation in the same ecological niche as the pathogen of interest. The current limitations of such use include: 1) the inability to control distribution and genetic changes of a live organism after widespread release, 2) the improbability of obtaining from a single bacterium multiple bacteriocins with desired bactericidal activity, and 3) the possible transfer of production ability from the producer to the target bacterium. Some of these limitations might be partially overcome, however, by use of genetic engineering techniques (8) to combine genes for bacteriocin production, immunity to such bacteriocins, and production control (regulatory genes).

The use of live bacteria can be avoided altogether. Bacteriocin preparations themselves could be used as control agents, analogous to antibiotics. The screening methodology and large-scale production practices are available, and the concentrations, formulations, and applications can be manipulated to achieve the desired control effect. Thus, the manufacturer, distributor, and user remain in control of the product.

Commercial considerations and potential. Effective and commercial use of bacteriocins requires consideration of many questions, including assessment of disease severity, market potential, availability of desired bacteriocins, sensitivity of a majority of test strains, inducibility (for maximizing yields), cloning potential, compatibility of bacteriocin mixtures, useful lifetimes, and potential additives to maintain or prolong biological activity.

There are few answers to most of these questions. And there are only partial answers to some other questions, such as the estimated material costs of bacteriocin treatment. Treating bean seed with bacteriocins effective against the halo blight bacterium, *P. syringae* pv. *phaseolicola*, is estimated at 10¢ per pound; the estimated cost for treating rootstock with agrocin 84 is prohibitive (A. Kerr, *personal communication*). Also, bacteriocins have little curative or therapeutic potential for plant diseases caused by bacteria and therefore need to be used as a prophylactic measure. However, bacteriocins have the potential to limit secondary spread of some pathogens.

State of the Art and the Challenge

Probably less than $0.5 million has been spent on research on bacteriocins of plant-pathogenic bacteria. From 10 to 100 times as much has been spent on basic research of bacteriocins of other bacteria, as judged by the extensive literature on this subject. In addition, few people are working with bacteriocins of phytopathogenic bacteria. Therefore, it is not surprising that there has been relatively little progress with plant pathogens. This could be remedied by bacteriologists and plant pathologists becoming involved in this type of research with university, government, and industry encouragement and support. For industry, the challenge is even greater. It is an unattractive venture to use such specific antibioticlike compounds because the profit potential is perceived as small after product development, total market, and the regulatory climate are taken into account. Yet, at least two major pathogens—*P. syringae*, including its pathogenic variants, and *E. amylovora*—usually appear in the United States each year in varying degrees of severity. The former causes diseases from citrus blast to bacterial speck of tomato, and the latter causes fire blight of rosaceous plants, notably pears and apples. In other parts of the world, some of which could be a potential

market, other bacterial pathogens are more important economically.

There are few acceptable alternatives at present for controlling bacterial diseases of plants. Antibiotics are commonly used in the United States, but it is the only country in the western world that allows the same antibiotics to be used in human and plant disease control. This practice is not likely to continue, as there is belated recognition throughout the scientific community and regulatory agencies that nonspecific antibiotics are detrimental to nontarget microorganisms, both saprophytic and beneficial bacteria. Antibiotic alternatives, such as relatively expensive inorganic compounds, mainly copper derivatives, have poorly documented effectiveness against bacterial diseases; a possible exception is combined heat and copper treatment to eradicate *Xanthomonas campestris* pv. *campestris* from cabbage seed (16). Other bactericides are not forthcoming (3), except possibly for a novel systemic bactericide effective against the rice bacterial leaf blight bacterium, *X. campestris* pv. *oryzae* (13). Finally, as production and consumer costs of crops continue to increase, disease losses will become less acceptable. Thus, bacteriocin research and development are warranted.

The lure of bacteriocins, based on environmentally desirable specificity, faces the present reality of economic risk. There is no guarantee that bacteriocins will ever be commercially useful plant disease control agents, but all living things would benefit from their success.

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