Bacteriophage biocontrol and bioprocessing: Application of phage therapy to industry

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Bacteriophages are the viruses of prokaryotes. Although they were discovered during the second decade of the 20th century, a clash of great personalities conspired to prevent, for the next 20 years, a widespread appreciation of their viral character. Irrefutable proof would come only with the invention of the electron microscope. Key to early confusion was the diversity of bacteriophages and, especially, conflicting interpretations of lysogeny. Lysogeny, as originally defined, is the ability of some bacterial cultures, seemingly rendered virus free through the application of anti-viral serum, nevertheless to seed cultures of similar bacterial strains with virus (i.e., lysogeny ≈ lysis genesis). Clearly, to one camp, the bacteriophage therefore was a bacterium-produced enzyme, somewhat equivalent to what today we might describe as a bacteriocin. The ability of this "enzyme" to propagate exponentially, however, required some serious hand waving on behalf of this camp's proponents; perhaps from a modern vantage this would be a prion-like protein whose presence stimulates previously naive bacteria to generate more of the same. The other camp espoused from the very start the viral—or, at least, "invisible microbe"—nature of what at times was described instead as the "bacteriophage phenomenon." This camp was led by the French Canadian co-discoverer of bacteriophage, Felix d'Herrele, who today is credited as the first to appreciate the potential of bacteriophage to do great things (Summers, 1999).

What was uncontroversial about bacteriophages was their ability to lyse cultures of bacteria. This ability would become their defining feature, with the Greek word phage (φαγειν) meaning to devour, perfectly capturing the ability of bacteriophages to seemingly consume the turbidity of a dense bacterial culture. Indeed, today it is common shorthand to describe bacteriophage simply as phage. d'Herrele, a man with no small interest in the treatment of disease, immediately recognized the importance of this lytic aspect of phage biology: Phage "devour" bacteria, bacteria cause disease, and therefore phage may be harnessed to fight infectious disease. d'Herrele's first published experience with phages was, in fact, in the context of patient recovery from bacillary dysentery.[1] The promise of this "phage therapy" against bacterial disease faded over the coming decades, however, a victim of poorly designed trials, the ongoing lytic-lysogeny controversy, and a poor appreciation of phage biology, such as the virion potential for lability. It was the sudden availability of effective chemical antibiotics in the 1940s, however, that truly marginalized the practice of phage therapy in the West. Phages instead would go on to play key roles in founding the disciplines of molecular genetics and molecular biology.

d'Herrele's phage-therapy legacy nevertheless lived on in Europe, principally in the Soviet block, where antibiotics and prejudice against the use of phage to treat infectious disease were both in short supply. A trickle of research, published in second-tier journals, kept the promise of phage therapy alive through these decades even in the West. Recent world-wide revival of interest in phage therapy, however, can be attributed to a confluence of factors: declines in antibiotic efficacy, an AIDS-led rise in modern appreciation of the horrors of infectious disease, the fall of the Soviet Union which allowed greater East-West scientific collaboration, a rise of interest in the U.S. in unconventional approaches to medicine, and the ready availability of venture capital during the 1990s. In addition to what one can find on phage therapy in the popular media,[2] numerous reviews are now available describing the potential for—and caveats associated with—the employment of phage to treat bacterial infections, especially in clinical settings (e.g., Chanishvili et al., 2001; Duckworth and Gulig, 2002; Krylov, 2002; Merril et al., 2003; Sulakvelidze et al., 2001; Summers, 2001). Numerous companies are also vying for an opportunity to sell phage therapeutics to a willing public.[3]

Here we take a slightly different tack from the mostly clinical considerations of phage therapy, emphasizing instead the role of phages as a means of selectively reducing bacterial loads in non-clinical settings. Since the phrase phage *therapy* carries a connotation

of medical doctors administering phages as living drugs to suffering patients, we instead employ the alliterations *bacteriophage biocontrol* and *bacteriophage bioprocessing* to describe, as we review here, the more generalized application of phages as everything from biocontrol agents on the farm to the bioprocessing of certain foods. We also provide a primer on phage-based methods of bacterial diagnosis.

Phage biology

All phages are composed of nucleic acid encapsulated by a protein coat; the genome and the capsid, respectively. Phage genomes can be double-stranded DNA, single-stranded DNA, or single-stranded RNA. Capsids come in many forms, ranging from small hexagonal structures, to filaments, to highly complex structures consisting of a head and a tail.[4] These virion particles, like their eukaryotic counterparts, are metabolically inert. This all changes upon bacterial infection which begins, in a process called adsorption, with capsid binding to the cell surface and subsequent genome uptake into the cell cytoplasm.

Upon infection phages can exhibit one of two distinct life cycles: (i) active infection, in which phage virion particles are assembled within the cytoplasm of an infected bacterium, or (ii) lysogeny, in which the phage genome integrates (as a prophage) into the bacterial chromosome as a giant gene complex. To produce virion progeny the prophage, which together with the host bacterium is described as a lysogen, must be induced. Induction can occur as a stochastic process, or may be forced via the application of DNA damaging agents. Many phages, described as temperate, are capable of displaying either active infection or reduction to lysogeny upon bacterial infection. Other, non-temperate phages are obligated to actively produce phage progeny upon infection of actively metabolizing bacteria.

Active phage infections, whether immediately active upon phage adsorption or the product of lysogen induction, may be further distinguished by their means of progeny-phage release from the infected bacterium, with release occurring, depending on the phage, either by lysis or by extrusion. Lysis is by far the most common and involves the destruction of the bacterial cell envelope by so-called lytic phages to allow intracellularly matured phage progeny to leak into the extracellular environment (Abedon et al., 2001). Lysis kills the infected bacterium and terminates the phage infection. By contrast, extrusion—which is the means of movement of filamentous virions out of cells—allows phage-progeny release without causing bacterial death. We can also differentiate these various phage infection strategies in terms of their utility to us: Temperate phages (particularly the *E. coli* phage λ) are used for cloning;, filamentous phages (such as the coliphage M13) are ubiquitously employed as platforms for protein display; and obligately lytic phages (often described as virulent) are the phages of choice for bacteriophage biocontrol and bioprocessing.

Phage therapy

Phage therapy is the application of bacteriophages to bacterial infections of humans (or other animals) with the goal of reducing bacterial load. There are a number of means by which we can distinguish phage therapies. First, phages can be delivered topically, orally, directly into body tissues, or systemically. A second way in which therapies differ also is in terms of delivery, with free phages the usual route of delivery to infections or, instead, phage-infected bacteria may be employed as a means (still very much experimental) of delivering phages to intracellular pathogens (Broxmeyer et al., 2002). Finally, the natural ability of lytic phages to kill infected bacteria may be exploited, as is usually the case with phage therapy, or instead phages may be engineered to deliver non-phage genes coding for antibacterial agents (Westwater et al., 2003).

Regardless of the approach, phage-therapy success is a function of basic principles of phage-bacterial interaction. One first must identify a phage that can infect a given bacterium, and the more effective that phage is at bacterial adsorption, bacterial killing, and subsequent phage amplification, the more effective a phage therapeutic we expect it will be. Secondly, if treatment is initiated during early stages of infections it may be necessary to compensate, by employing a larger phage dosage, for the inability of too-few bacteria to successfully expand phage populations (Kasman et al., 2002). Third, one must catch infections on time, before bacterial numbers are so great that the very process of bacterial eradication becomes harmful to the patient. Key to phage therapy success, therefore, is proper phage choice, quantity of delivery and timing of treatment, all of which are criteria that also apply for chemical antibacterials. What, then, distinguishes phage therapy from antibacterial chemotherapy?

The phage-therapy advantage

Phage therapy has specific advantages compared with chemical antimicrobials. The first advantage is that phages are not small molecules capable of modifying and thereby degrading animal metabolisms but, instead, exert their effects on the animal host through bacteria. The second advantage is that phages are self-replicating. As a consequence, a given phage dosage can self-amplify over the course of treatment, greatly increasing efficacy. The third advantage is that this self amplification can allow phages to burrow into bacterial infections, one infected bacterium at a time, resulting in greater penetration than chemical antimicrobials, even though the small size of the latter would seem to bestow a diffusion advantage.

The fourth advantage comes from the ubiquity and diversity of phages. Phage workers now commonly argue that the Earth may support as many as 10^{31} phage particles, with upwards of 10^{8} phage particles found per gram or ml of soils, sediments, or nutrient-rich aquatic systems.[5] Thus, while bacteria can readily mutate to phage resistance, the natural environment typically can supply numerous alternative phages, differing in host range, to attack a diversity of bacterial infections. Typically these phages may be employed in cocktails—the phage equivalent of multi-drug therapy—so that phage resistance is countered right from the start of therapy.

The fifth advantage of phage therapy stems, nevertheless, from the narrow spectrum of phage activity, particularly as compared to many chemical antimicrobials. Not only do many phages display monovalence, i.e., a propensity to infect only a single species or even only a small selection of strains within a given species of bacteria, but common phage-enrichment schemes likely select for phages that are specialized for propagation on a small range of bacterial types (Jensen et al., 1998). Even when they are multivalent, and thereby able to infect numerous bacterial types, one would still classify phages as relatively narrow-spectrum antibacterials. In fact, even given the employment of cocktails of numerous phage types, the spectrum of activity remains narrow due to the narrow and overlapping spectrums of activity of the individual phages making up a cocktail. Phages consequently can be very selective in the bacterial populations that they attack, reducing the likelihood of superinfection and other complications of normal-flora reduction that can often result following treatment with chemical antibacterials.

Hurdles to success

Despite the many advantages of phage therapy over chemical antibacterials, these advantages are not necessarily sufficient for phage therapy to represent a viable alternative to the current standard of care. Here we consider obstacles to widespread phage-therapy employment. In particular, phages encode bacterial toxin genes or, in the course of lysogeny, can otherwise transform harmless commensal bacteria into pathogens (as reviewed by Wagner and Waldor, 2002). Since not all bacterial virulence factors have been identified, even in this modern age of whole-genome sequencing, the demonstration of a phage's *inability* to increase bacterial virulence upon infection is by no means a trivial process. One approach towards bypassing this concern is to avoid phages that are capable of displaying lysogeny, or even to avoid phages which contain genes that are consistent with an ability to display lysogeny. Still, if one is to use chemical chemotherapeutics as the regulatory model, proper regulation would involve rigorous safety assessment of all phage strains to be employed therapeutically in humans.

Ways around this concern can involve employing phages that one already fully understands and/or employing phages that neither replicate nor carry many phage genes, but instead serve as vectors for delivery of antibacterial genes. The first approach, however, can neutralize the ubiquity advantage otherwise ascribed to phages and phage therapy. The second approach (see Westwater et al., 2003) additionally can eliminate exponential phage growth, associated phage penetration into colonies of bacteria, and resulting bacteria–killing potential. With genetic engineering there does exist a possibility of taking specific well-characterized phage platforms (such as phage λ) and manipulating these phages to extend host ranges. Not all bacterial pathogens, however, possess extremely well-characterized phages. Furthermore, such a phage-platform approach could fail to take full advantage of phage therapy's theoretical flexibility, which at best might involve local phage isolation, local characterization, and then subsequent treatment of local nuisance strains of bacteria.

Further problems arise within the clinical setting, particularly given late-presenting systemic infections and an absence of local laboratory

support for phage therapy. That is, while the narrow spectrum of activity ascribed to phages may be a boon towards phage-therapy safety, it also puts a strong onus on effective pathogen identification, particularly from the perspective of phage susceptibility. Alternatively, one could create phage cocktails that simultaneously can defeat multiple bacterial etiologies, but such efforts would not only increase the complexity of the phage product, and therefore the costs of regulatory approval, but also would begin to move phage therapy away from the relative advantages stemming from narrow spectrums of activity. Ideally, then, one's local microbiology diagnostic laboratory would not only identify bacteria but also would determine spectrums of phage susceptibility, just as one can determine spectrums of antibiotic susceptibility. In any case, and despite the emphasis on the treatment of bacteremias in modern phage-therapy literature, it remains to be determined whether phage therapeutics may be developed that are clinically effective against late-presenting systemic infections, or whether there is significant money to be made using phages to fight chronic infections against which chemical antibiotics have been found to be ineffective. However, despite these various caveats, we believe that phages can (and do!) make for effective antibacterials, useful in the treatment of human disease.

Bacteriophage-mediated biocontrol

Biocontrol is the introduction of specific organisms, often predators or parasites, into ecosystems such as croplands in order to combat species that have become pests. Clearly with bacteria the pests, our bodies the ecosystem, and phages the predators, phage therapy represents a form of biocontrol.Moreover, we are aware of a handful of studies that have employed the term *biocontrol* to describe phage-mediated "therapy" of bacteria found in association with plants, fungi, or their products (Flaherty et al., 2001; Leverentz et al., 2001; Munsch and Olivier, 1995; Randhawa and Civerolo, 1985; Schnabel et al., 1999; Westwater et al., 2003). In this section we consider use of *bacteriophage* biocontrol in the great outdoors, particularly phage application to cropland.

The major problem with bacteriophage biocontrol is one of transition from laboratory or field-test success into farmer-mediated control of plant pathogens. In part this is a problem of priorities, with insects, weeds, fungi, and plant-specific viruses already demanding significant outlay for control by farmers. A second problem is regulatory. It is clear that application to crops of phages specific for bacterial plant pathogens should be less of a regulatory concern than, for example, application to humans of phages specific for human pathogens. Nevertheless, concerns over phage spread beyond the point of application, whether to downstream ecosystems or "upstream" to the human dinner table, have limited phage application as biocontrol agents in the U.S. Yet for a variety of reasons—centered around the already ubiquitous presence of phages in the human environment, the presumed low specificity of plant-pathogen phages for members of the human normal flora, the high lability of phages following their application to plants in the field, and the already common practice of washing and even disinfecting agriculture products post harvest—we believe that regulatory efforts to limit phage application in agriculture settings are unfortunate. This is particularly so when the obvious alternatives to bacteriophage biocontrol are chemical antimicrobials.

Circumstances in which bacteriophage-mediated biocontrol of plant pathogens has been successfully attempted include use against *Xanthomonas pruni*-associated bacterial spot of peaches, to control *Xanthomonas* infections of peach trees, cabbage, and peppers; to control *Ralstonia solanacearum* infection of tobacco; to control soft rot and fire blight associated with *Erwinia*; and against bacterial leaf spot of mungbeans. In studies notable for the employment of phage host-range mutants, phage biocontrol has also been employed against bacterial spot of tomatoes caused by *Xanthomonas campestris*. Phages also have been employed to disinfect *Streptomyces scabies*-infected potato seed-tuber and have been successfully tested as agents of biocontrol against bacterial blotch of mushrooms caused by *Pseudomonas tolaasii*. Bacteriophage biocontrol may also be employed as a means of manipulating plant bacterial flora beyond simply killing off unwanted pathogens. For example, phages that are ineffective against a preferred inoculum of *Bradyrhizobium japonicum* but effective against naturally occurring competitors can be coated on seeds which thereby enhances nitrogen fixation in resulting plants. For references to these various bacteriophage biocontrol studies see Gill and Abedon (2003). In addition, a regularly updated list of articles—including references to the studies

outlined above—that address bacteriophage biocontrol, broadly defined, can be found at *www.phage.org/bib_pt.htm*.

Another role for bacteriophage biocontrol is in the management of nuisance bacteria in non-food settings. Use of phages in such settings should occur without concern of phage contaminating the human food supply. Phages, for instance, have been presented as a means of biocontrol of the bacterial blight of geraniums as caused by *X. campestris* (Flaherty et al., 2001). Cyanophages have also been tested as a means of controlling cyanobacterial blooms, though greater success has been achieved given phage application prior to rather than following bloom formation (Desjardins and Olson, 1983). Phages have even been suggested as a means of controlling the biofouling of thermal power plant condenser tubes (Sakaguchi et al., 1989). Under much more controlled circumstances, phages have been employed in the laboratory as a means of reducing mixed-culture densities of specific bacteria including the removal of *Rhizobium trifolii* from protoplast cultures of clover. Phages also may be used to enrich cultures for comparatively rare species of soil bacteria or fungi (e.g., McKenna et al., 2002) or to kill donor strains of bacteria during *in situ* horizontal gene transfer experiments (e.g., Richaume et al., 1992).

Bacteriophage bioprocessing

Phage therapy may be described as bacteriophage biocontrol as practiced in a clinical setting or, at least, as administered to animals. *Bacteriophage bioprocessing*, in turn, is bacteriophage biocontrol as practiced in (or on the way to) the factory, particularly as a means of reducing or changing food bacterial loads. It is particularly in the production of minimally processed foods, usually for the sake of avoiding cooking-associated flavor or texture, that food contamination with undesirable bacteria often occurs. Thus, the preponderance of bacteriophage bioprocessing takes place under the guise of reduction in food loads of pathogenic bacteria, and predominantly under circumstances where cooking or other antibacterial treatments prior to consumer purchase is undesirable.

Oddly, regulatory concerns over phage applications to croplands do not seem to correlate with statements made by the Food and Drug Administration concerning phage application to food: "...for the safe use of a mixture of bacteriophages as an antimicrobial agent on foods, including fresh meat, meat products, fresh poultry, and poultry products... neither an environmental assessment nor an environmental impact statement is required" (Intralytix Inc., 2002). Perhaps this view is in response to the fact that phages are already ubiquitously present in unprocessed foods (Ackermann and DuBow, 1987), because of an expectation that the various animal products indicated in this quotation will be cooked prior to consumption, or because the overriding goal of pathogen reduction trumps hypothetical safety concerns over phage addition to foods. The Environmental Protection Agency, however, remains cautious over food contamination by applied phages (Stone, 2002).

Control of pathogens found on fruits and vegetables is a major concern since it is unlikely that these foods will undergo any further processing (i.e., cooking) that would kill any pathogens present. Ground meat (potentially containing *E. coli* O157:H7), eggs (potentially *Salmonella* contaminated), and cheeses (*Listeria*) represent animal products that similarly could benefit from reduction of bacterial pathogens. Therefore, there has been a great deal of research in the area of non-thermal processing of minimally processed foods. Phage-based control of pathogens is a non-thermal intervention, and has been demonstrated to control the growth of *Campylobacter* and *Salmonella* on chicken skin (Goode et al., 2003), *Salmonella enteritidis* in cheese (Modi et al., 2001), *Listeria monocytogenes* on meat (Dykes and Moorhead, 2002) and on fresh-cut fruit (Leverentz et al., 2003), and *Salmonella* also on fresh-cut fruit (Leverentz et al., 2001). The results of these studies are promising, with significant reductions of pathogenic bacteria observed.

Phage application has also been studied as a method to control the presence of biofilms in the food processing environment. Such a treatment is potentially useful in the control of *L. monocytogenes*, which is very effective at forming biofilms in drainage systems, making decontamination difficult (Hibma et al., 1997). The application of phages to control biofilms is also relevant to the medical community, where biofilms often form on the surfaces of embedded medical devices such as catheters. In addition, bacteriophage bioprocessing has been attempted as a means of extending animal-product shelf life (Greer and Dilts, 2002), and even to lyse lactic acid bacteria to contribute to the ripening of Camembert and Cheddar cheese (Crow et al., 1995).

Replacing antibiotics on the farm

Human phage therapy is fraught with regulatory concerns due to a combination of phage complexity and novelty to Western medicine. The employment of phages for the biocontrol of plant pathogens faces a different hurdle, and different regulatory agency, since cropland application occurs out of doors. Proposals to apply phages directly to foods have resulted in less regulatory concern, perhaps because phages are already known to be abundant in the human environment or because of an expectation that treated foods will be cooked prior to consumption. In comparison, farm animals often live within relatively enclosed environments, are already subject to a great deal of antimicrobial chemotherapy, typically are cooked prior to human consumption, and carry a plethora of bacterial pathogens affecting humans. We believe, therefore, that phage therapy of farm animals may lie within a regulatory sweet spot where application can do some good, i.e., by reducing food pathogen loads and overall antibiotic usage, and very likely would do no harm.

Because animals are subject to bacterial infection and can act as reservoirs of human bacterial pathogens, potential targets for animal phage therapy are abundant including: pustule disease in abalone; *Aeromonas hydrophila* and *Edwardsiella tarda* in eels; *Campylobacter jejuni* in broilers; *E. coli* in calves (diarrhea control); *E. coli* in poultry (respiratory-infection control); *Lactococcus garvieae* in yellowtail fish; *Pseudomonas plecoglossicida* in ayu fish; *Salmonella enterica* in chickens; *Salmonella typhimurium* in pigs and chickens; and *Vibrio anguillarum* in milkfish.[6] In addition, there exists an active research program at The Evergreen State College in Olympia, Washington, aimed at using phages to eliminate *E. coli* O157:H7 from the livestock reservoir (see www.evergreen.edu/phage/cev.htm). It is our expectation that if phage therapy is to be successful in the U.S., minimally it is going to be as a means of bacterial control in animal husbandry.

Phage-based bacterial detection

For successful biocontrol, effective bacterial diagnosis is pertinent. In contrast to phage typing, which has become more of an epidemiological than identification tool (Sharp, 2001), there has been much recent interest in the use of phages for the direct detection and identification of foodborne and clinical bacterial pathogens. In this section we provide a summary of various phage-based bacterial diagnostic techniques. Lysis of bacterial cells by specific phages results in release of intracellular materials that may then be assayed using a variety of techniques. The method that has received the greatest attention is the use of the firefly luciferase/luciferin enzyme system to detect intracellular ATP release (Entis et al., 2001). The concentration of ATP in the assay is directly proportional to the quantity of light produced by the luciferase/luciferin reaction. Other intracellular constituents also may be used to monitor phage-mediated cell lysis, including release of β-galactosidase (Neufeld et al., 2002).

Bacteria may be identified by their release of virion particles upon cell lysis. The resulting increase in phage particles forms the basis for so-called “phage amplification assays,” which have been applied for the rapid detection and identification of specific pathogenic bacteria including *Salmonella*, *E. coli* O157:H7 and *Mycobacterium tuberculosis* (Favrin et al., 2003, Mole and Maskell, 2001). Only if the target organism is metabolically active will addition of the phage result in the production of phage progeny, which can then be detected by plaque assay or other endpoint methods.

Another phage-based approach to bacterial detection is to tag the infecting phage itself. Phage nucleic acid, for example, may be labelled with a fluorescent stain. The resulting phages can then specifically tag bacterial cells, in a manner similar to antibodies, and may then be detected by several methods including epifluorescence microscopy, flow cytometry, confocal microscopy, and fluorescent plate readers. So far this method has been developed for analysis of marine bacteria (Hennes and Suttle, 1995) and for detection of *E. coli* O157:H7 (Goodridge et al., 1999a, Goodridge et al., 1999b).

Phage DNA encoding a reporter gene provides an additional highly sensitive method for both bacterial detection and determination of sensitivity to antimicrobial agents (Goodridge and Griffiths, 2002). Given the metabolic inertness of the virion particle, only once introduced into metabolically active target bacteria will these reporter genes be expressed. So far the reporter genes employed in this system are the prokaryotic and eukaryotic luciferase (*lux* and *luc*) genes and the bacterial ice nucleation (*inaW*) gene. To date, reporter phages have been developed that can detect *E. coli, Mycobacteria*,

Salmonella spp., *Staphylococcus aureus*, and *Listeria monocytogenes* (Entis et al., 2001).

Conclusion

Phages are the most numerous organisms on Earth, play key roles in bacterial gene exchange and bacterial pathogenesis, and continue to provide important insights into the basic molecular workings of life. Through a combination of their antagonistic but metabolically intimate relationship with their bacterial hosts, lytic phages possess ideal properties to serve as agents of both anti-bacterial biocontrol and bacterial identification. These two technologies, in turn, promise a profound and as-yet untapped synergy whereby phages are employed to rapidly identify bacterial infections and then, perhaps using the very same phages, to combat and destroy the infecting bacteria. We look forward to a time when phages, as both bacteria identifiers and biocontrol agents, are as ubiquitous in the clinic, on the farm, and even in the factory as Felix d'Herrele, over 85 years ago, so confidently hoped that one day they might be.

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Footnotes

1 For an English translation of this seminal report, go to *www.phage.org/bgnws001.htm#submissions*.

2 See *www.evergreen.edu/phage* for sampling of "Phage therapy in the news."

3 See *www.phage.org/beg*_links.htm#phage_companies for a list of companies doing phage-based research.

4 See *www.asm.org/division/m/Out.html* for phage taxonomy and structure links.

5 For a sense of what a number like 10^{31} means, see *www.phage.org/bgnws007.htm#submissions.*

6 For references to these other articles addressing bacteriophage biocontrol and bioprocessing see *www.phage.org/bib_pt.htm*.

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About the 2004 SIM election process

The first step in the election process is the identification of the Nominating Committee (NC) of at least three SIM members. The committee members are approved by the Board and serve only for the current year. Committee members can not be reappointed within a three-year period. The NC proposes a slate of candidates (at least two candidates for each position). The candidates must be Society members with a demonstrated interest and involvement in SIM. Upon acceptance of the nomination, the NC informs the candidates of the duties and responsibilities required by each position. In addition to the NC, candidates can be identified via the Article 5, section 4 in the SIM constitution using a petition process. The final slate of candidates is due to the President by August 1. Candidates must submit a biography and photograph by October 1 for publication in the SIM News and, starting with the 2004 election, for posting on the website for the online voting process.

In 2003, a website-voting module was activated. It will continue to be available in 2004. Individuals who do not have access to the internet should contact the SIM office for a paper ballot. Student members are not eligible to vote. After the voting ends, the Election Committee (consisting of a minimum of two SIM members) compiles the mail-in ballots, receives the tallies from online voting and delivers the results to the President and Secretary.

The election process and ballots are available for inspection for at least 30 days following the Annual Meeting. Ballots and records are destroyed six months after the election (unless otherwise directed by the Board) and final tabulations of the votes are preserved.