Bacteriophages as tools in drug discovery programs

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Screening of microbial natural products continues to represent an important route to the discovery of novel bioactive compounds for the development of new therapeutic or other important industrial agents[1,2]. However, a continuous supply of diverse compounds is needed to meet the needs of industry. Such a supply can only be derived through systematic screening of bioactive compound-producing microorganisms from natural sources [1-3].

Molecular ecology has undoubtedly revealed the fascinating diversity of microorganisms, which cannot be cultured. Regardless of the advances in the field, the ability to detect and isolate microorganisms effectively from their ecological niches is still lacking. Selective isolation of previously undetected bioactive microorganisms is thus one of the major targets of industrial microbiologists in the search for novel compounds for bioindustry [4-6].

Actinomycetes have been the most effective source of bioactive compounds, providing two-thirds of the currently used antibiotics [1]. However, while many representatives of actinomycetes have been isolated, it remains unclear which component of the isolated genera represents major functional groups in many environments [4,5]. As a result, approaches to the isolation of potentially valuable microorganisms for industry still remain largely empirical and restricted to the sampling of a tiny fraction of the microbial community found in diverse environments [4,5]. Therefore, detection of bioactive actinomycete taxa without understanding their true diversity and eco-physiology is a random approach rather than a target-directed one.

Actinomycetes are estimated to be present in soil at frequencies ranging from 10^6 to 10^7 cfu/g of soil, indicating that the top 10 cm of the earth's surface is compatible with actinomycete growth, which contains 10^{25} to 10^{26} actinomycetes [7]. These values, in turn, indicate that only a miniscule fraction of the soil on earth has been sampled for antibiotic-producing actinomycetes [7]. Furthermore, most cultured actinomycete species belong to common actinomycete taxa such as the genera *Streptomyces and Micromonospora* [1,2]. However, there are several clinically important antibiotics such as vancomycin, erythromycin, tobramycin, apramycin, and spinosyns, which are produced by less common or rare actinomycetes. Examples reported include vancomycin producer *Amycolatopsis* sp. or spinosyn producer *Saccharopolyspora* sp., which are 4% and 3% abundant as streptomycetes respectively [7].

It has already been demonstrated that the isolation of bioactive rare actinomycete taxa requires highly specialised isolation techniques [6,8]. Techniques employed range from the use of antibiotics to chemotaxis chambers, and excessive heat treatments [5,6]. In this context, bacteriophages have also proved to be useful tools in different applications [6,11]. They have so far been:

1. exploited as naturally present indicators of under-represented or rare actinobacterial taxa in environmental samples
2. used for deselecting unwanted taxa on the isolation plates in the process of target specific search for rare actinomycete taxa
3. used for the typing of novel isolates
4. used as indicators of antiviral activity to be screened further in bioassays.

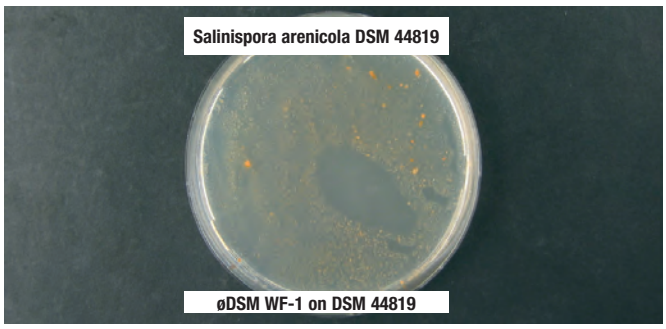
The following section will provide examples on the above mentioned applications of phages.

1. Phages as naturally present indicators of under-represented or rare actinobacterial taxa in environmental samples

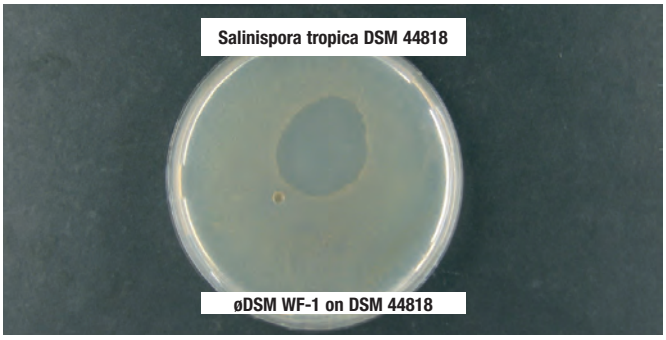
The existence of bacteriophages in natural habitats is dependent on the actively growing susceptible hosts. Consequently, if phages specific to rare actinomycete genera are present in the test sample, this can be a confirmation of the presence of the members of such genera in that sample [6,9].

Genus- and family-specific phage polyvalency can also be exploited to detect bioactive clusters of suborders such as the members of *Corynebacterineae, Micromonosporaceae and Pseudonocardineae* [9]. As an example, phages were isolated by the author from samples collected from the banks of a local creek in Wolfenbüttel, Germany, towards bioactive *Salinispora* species [10] indicating their presence in non-marine environments (Figures 1A and 1B).

Figure 1. Phage indicating presence of close representatives of bioactive *Salinispora* type species at the local creek banks of Wolfenbüttel, Germany.



(A) Salinispora arenicola



(B) Salinispora tropica

These findings might again confirm that bioactive taxa or their close relatives may be discovered in environments other than their first reported detection site if effective and highly selective isolation techniques are used. Phage battery can also be used to support biogeographical studies and provide evidence for the global distribution of species.

2. Use of phage for selective isolation of rare actinomycetes

Phages can be used to deselect unwanted taxa on isolation plates to be able to isolate the targeted ones [6]. With the exploitation of phage susceptibility of common bacteria, which impedes the growth of rare actinomycetes on isolation plates,

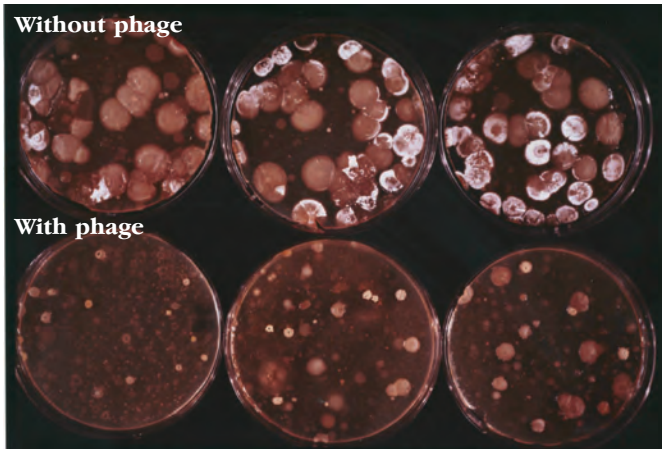
Table 1. Activity spectra of the thermophilic *Streptomyces* and *Thermoactinomyces* phages.

Type strain	ϕMP-1	ϕMP-2	ϕMP-3	ϕMP-4	ϕMP-5	ϕMP-6	ϕMP-7	ϕMP-8
Streptomyces thermoalcalitolerans (DSM 41741)	PH		+					
Streptomyces thermocarboxydovorans (DSM 44296)		PH		+	+			
Streptomyces thermocarboxydus (DSM 44293)	+		PH					
Streptomyces thermogriseus (DSM 41756)		+		PH				
Streptomyces thermolineatus (DSM 41451)					PH			
Streptomyces thermospinosisporus (DSM 41779)	+		+					
Streptomyces thermovulgaris (DSM 40444)			+	+				
Thermoactinomyces vulgaris (ATCC 43649)						PH		+
Thermoactinomyces intermedius (ATCC 33205)							PH	
Thermoactinomyces sacchari (ATCC 43356)						+		PH

many novel and rare actinomycete taxa can be isolated from different environments (e.g. termite guts, marine sponges) for pharmaceutical screens [6]. Polyvalent phages can reduce the numbers of colony forming units of unwanted bacteria on isolation plates; hence, increasing the probability of detecting rare or novel genera [6].

Thermophilic actinomycete phages (Table 1) have been used to detect rare thermophilic actinomycete taxa in overheated material (e.g. bagasse, hay, compost) [6]. Once the removal of large colonies is achieved, previously under-represented taxa can easily be isolated (Figure 2).

Figure 2. Use of phage battery to reduce the numbers of large colony forming and faster growing actinomycetes in overheated material to isolate rare actinomycetes.

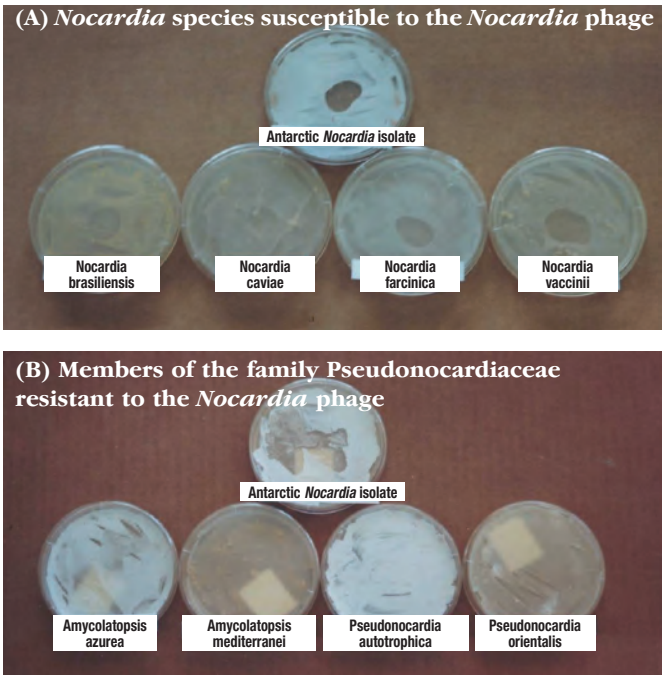


3. Phage as taxonomic tools

Phages can be species-, genus- or family-specific as well as defining suborder boundaries by recognising families in those suborders. Although rarely detected, species-specific phage presents significant value superior to genus- and family-specific ones in that it exhibits an extremely narrow spectrum, which renders it a very powerful tool for taxonomy. These narrow spectrum phages can also be a powerful tool for selectively removing common species of well-defined and investigated taxa such as streptomycetes (e.g. *Streptomyces albus)* on the isolation plates to isolate previously unknown bioactive members of the genus.

Activity spectra of a phage isolated against a *Nocardia* species from the same Antarctic soil sample [11] recognises its continental counterparts *(N. brasiliensis, N. caviae, N. farcinica and N. vaccinii)* but does not infect species outside the genus *(Amycolatopsis azurea, A. mediterranei, A. orientalis and Pseudonocardia autotrophica)* (Figures 3A and 3B). Phage displays cross-infectivity within the genus but does not cross the genus boundaries. These findings support the transfer of *Amycolata* and *Amycolatopsis* into the family of Pseudonocardiaceae from the family Nocardiaceae. Phage activity in this instance is again in full correspondence with the polyphasic taxonomical findings [12,13].

Figure 3. Activity spectra of *Nocardia* phage isolated from an Antarctic soil sample.



4. Phage as tools in antiviral research

The success of antiviral chemotherapy has been limited to a few viral diseases. One of the difficulties encountered has been the lack of effective screening systems. *In vitro* antiviral screening systems offer advantages over *in vivo* methodologies (e.g. large numbers of compounds may be screened quickly and economically) [14]. One such effective antiviral screening system is the use of anti-phage assays. Osada *et al.* (1990) reported the discovery of two novel antiviral compounds from actinomycetes discovered via the use of anti-phage assay [14,15]. Anti-phage assays can, therefore, be a rapid and effective way of screening for antiviral activity and by using such systems large numbers of compounds may be screened quickly and economically. Examples include the use of *Streptomyces* phage-host systems by the author to test anti-phage activity of actinomycetes deriving from different suborder members within the order *Actinomycetales* (Table 2).

Future directions

Bacteriophages can be effective tools in drug discovery programs indicating the presence of targeted bioactive taxa in natural substrates, which can aid in the isolation, preliminary identification and antiviral activity testing assays that are to be further confirmed in true viral assays.

To be able to exploit phages effectively for all these targets, in-depth information on host-phage interactions is needed. This requires the generation of substantial information at taxonomic level indicating taxon specificity of the host-phage interactions as well as the indication that results are in full correspondence with the data obtained from numerical, chemical and molecular biological studies. All these once again stress the need for a

Table 2. Anti-phage activity within family members of the order *Actinomycetales*.

SUBORDER (9 tested out of current total 13 suborders in the order *Actinomycetales*)	FAMILY	NUMBER OF GENERA WITH ANTIPHAGE ACTIVITY/FAMILY
Corynebacterineae	7	Marine: 2/7; Termite gut: 3/7; Tropical rain forest: 3/7; Activated sludge: 7/7; Desert sand: 0/7
Frankineae	7	Marine: 0/7; Termite gut: 0/7; Tropical rain forest: 2/7; Activated sludge: 0/7; Desert sand: 2/7
Kineosporiineae	1	Marine: 0/1; Termite gut: 1/1; Tropical rain forest: 1/1; Activated sludge: 0/1; Desert sand: 1/1
Micrococcineae	16	Marine: 0/16; Termite gut: 5/16; Tropical rain forest: 10/16; Activated sludge: 0/16; Desert sand: 5/16
Micromonosporineae	1	Marine: 1/1; Termite gut: 1/1; Tropical rain forest: 1/1; Activated sludge: 0/1; Desert sand: 1/1
Propionibacterineae	2	Marine: 1/2; Termite gut: 1/2; Tropical rain forest: 1/2; Activated sludge: 0/2; Desert sand: 1/2
Pseudonocardineae	2	Marine: 0/2; Termite gut: 2/2; Tropical rain forest: 2/2; Activated sludge: 0/2; Desert sand: 2/2
Streptomycineae	1	Marine: 1/1; Termite gut: 1/1; Tropical rain forest: 1/1; Activated sludge: 1/1; Desert sand: 1/1
Streptosporangineae	4	Marine: 0/4; Termite gut: 4/4; Tropical rain forest: 4/4; Activated sludge: 0/4; Desert sand: 4/4

sound understanding in microbial ecology, systematics, eco-physiology and functional diversity of microorganisms to utilize this information towards effective recovery of microorganisms for industrial applications.

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