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# Comparison of Bacterial and Phage Display Peptide Libraries in Search of Target-Binding Motif

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## Abstract

Genetic engineering allows modification of bacterial and bacteriophage genes, which code for surface proteins, enabling display of random peptides on the surface of these microbial vectors. Biologic peptide libraries thus formed are used for high-throughput screening of clones bearing peptides with high affinity for target proteins. There are reports of many successful affinity selections performed with phage display libraries and substantially fewer cases describing the use of bacterial display systems. In theory, bacterial display has some advantages over phage display, but the two systems have never been experimentally compared. We tested both techniques in selecting streptavidin-binding peptides from two commercially available libraries. Under similar conditions, selection of phage-displayed peptides to model protein streptavidin proved convincingly better.

**Index Entries:** Phage display library; bacterial display library; affinity selection; ligand; peptide; streptavidin.

## Introduction

Display of random peptides on the surface of bacteriophages and bacteria, combined with in vitro selection technologies, was introduced

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some 15 yr ago as a tool for discovering new ligands to virtually any chosen target *(1,2)*. Phage- or bacterial-displayed peptide library is a mixed population of an enormous number (up to ~$10^{12}$) of clones, each clone expressing multiple copies of a unique peptide sequence on its surface. Affinity selection of peptides from a biologic library is performed by incubating library vectors over the immobilized target. Clones displaying peptides with high affinity to target molecule bind to it, while others are washed away. Bound clones are subsequently eluted, multiplied, and used for further rounds of selection. Finally, individual clones are isolated, and their nucleotide sequence coding the displayed peptide is determined *(3)*. Receptor agonists and antagonists *(4,5)* as well as enzyme inhibitors *(6–10)* have been obtained with this technique.

The two similar techniques, phage display and bacterial display, differ in some important aspects. Bacteria are easier to cultivate with a selection marker (e.g., β-lactamase) that helps to prevent library contamination, whereas cultivation of phages requires a proper amount of host organism and is fairly susceptible to contamination with wild-type bacteriophages. In addition, bacterial concentration can readily be determined turbidimetrically. Phage concentration, on the other hand, is determined by time-consuming microbiological titration, in which dilutions of phage suspension are used to infect plated bacterial hosts. Another advantage of bacterial library utilizing flagella to display random peptides is that specifically bound clones can be eluted from the immobilized target by mechanical shearing of the flagella *(2)* without interrupting the possibly strong peptide-target interaction. Elution of phages can only be achieved by breaking the peptide-target interaction. In general, interaction can be overcome specifically with competitive ligand to the target of interest or nonspecifically by changing the target conformation. Nonspecific elution is most often done with 0.1 *M* glycine-HCl buffer (pH 2.2) *(11–15)*. Some clones with very high affinity to the target are not eluted by this method, even with two repeated elutions *(16)*.

There are reports of many successful uses of phage display libraries and considerably fewer cases describing bacterial display systems *(2,17–23)*. These two techniques have never been compared experimentally. In the present study, we compared two frequently used commercially available peptide libraries, the phage-displayed random cyclic heptapeptide library Ph.D.-C7C (New England Biolabs, Beverly, MA) and the bacterial-displayed random cyclic dodecapeptide library FliTrx (Invitrogen, Carlsbad, CA), for their ability to select a consensus peptide motif that binds to streptavidin. Apart from the general differences between bacterial and phage display, these two libraries differ in the length of displayed peptides (7 and 12 amino acids, respectively). Bacterial library containing all possible dodecapeptides should therefore include $4.1 \times 10^{15}$ independent clones. In fact, its actual size is only $1.77 \times 10^{8}$ independent clones, because the electroporation of FliTrx plasmids, coding diverse fusion library proteins, into *Escherichia coli* bacterial cells is a bottleneck of library construction.

Nevertheless, the size of the library is still large enough to encompass at least all theoretically possible hexapeptides located within displayed dodecapeptides. The size of phage-displayed heptapeptide library Ph.D.-C7C is $1.2 \times 10^9$ independent clones, which means that it should theoretically encompass nearly all possible heptapeptides. Thus, both libraries contain approximately the same number ($\sim 1 \times 10^9$) of different heptapeptides and are in this respect highly comparable.

Streptavidin was chosen as the target protein and biotin as a selective eluent molecule. Molecular interaction of biotin and streptavidin is one of the strongest noncovalent interactions in nature; therefore, biotin provides a very strong competitor against any peptide bound to streptavidin. Additionally, peptide ligands affinity selected to streptavidin's biotin-binding site are known to contain the tripeptide sequence HPQ *(24)*.

## Materials and Methods

### *Immobilization of Target Molecule*

Lyophilized streptavidin (Ph.D.-C7C kit; New England Biolabs) was dissolved in 50 m*M* $NaHCO_3$, pH 8.5, to a final concentration of 150 µg/mL. Maxisorp surface microtiter plate (Nalge Nunc, Roskilde, Denmark) wells were filled with 200 µL of target protein solution and incubated overnight at 5°C with gentle agitation. Additionally, microtiter plates precoated with streptavidin and preblocked by the manufacturer (Boehringer Mannheim, Mannheim, Germany) were used in some experiments. In-lab coated microtiter plates were blocked using 250 µL of 2% bovine serum albumin or 2% powdered skimmed milk in phosphate-buffered saline (PBS) buffer (135 m*M* NaCl, 3 m*M* KCl, 10 m*M* $Na_2HPO_4$, 2 m*M* $KH_2PO_4$; pH 7.4) for 1 h at room temperature and rinsed four times with PBS containing 0.1% Tween-20 (PBST).

### *Affinity Selection of Phage Display Library*

Selection of peptides from Ph.D.-C7C random cyclic heptapeptide phage-displayed library (New England Biolabs) was carried out according to the manufacturer's instructions. An aliquot of $2 \times 10^{11}$ phages was diluted to 100 µL with PBST and incubated in coated wells for 1 h at room temperature with gentle agitation. Nonbinding phages were discarded by washing the wells 10 times with PBST. Phages bound to streptavidin were eluted with 100 µL of 0.1 m*M* biotin in PBS for 1 h and amplified by infecting *E. coli* ER2738 host cells. After 5 h of growth at 37°C, bacteria were removed by centrifugation, and phages in the supernatant were precipitated by adding 1/6 vol of polyethylene glycol (PEG)/NaCl solution (20% PEG-8000, 2.5 *M* NaCl) and incubating overnight at 4°C. The precipitate was resuspended in a small volume of PBS, and amplified eluates were titered to determine phage concentration. This selection procedure was repeated three more times, while increasing the Tween-20 concentration to 0.5% in washing steps. Finally, eluates from the last round of selection were used

to infect plated bacterial host cells and resulting plaques were selected. Individual phage clones were then grown and purified for further analysis.

### *Affinity Selection of Bacterial Display Library*

Selection of peptides from the FliTrx random cyclic dodecapeptide bacterial-displayed library (Invitrogen) was carried out according to the manufacturer's instructions. The genotype of bacterial strain was $F^-$, $lacI^q$, ampC::$P_{trp}$ cI, ΔfliC, ΔmotB, eda::Tn10 *(2)*. A 1-mL aliquot of FliTrx library was cultivated overnight at 25°C in 50 mL of IMC medium (1X M9 salts, 0.2% casamino acids, 0.5% glucose, 1 m*M* $MgCl_2$, 0.1 g/L of ampicillin) on an orbital shaker (250 rpm). A total of $1 \times 10^{10}$ cells of overnight culture was transferred to 50 mL of fresh IMC medium with 100 mg/mL of tryptophan and cultivated an additional 6 h to induce the formation of flagella. Ten milliliters of induced culture was added to 0.1 g of nonfat dry milk, 0.3 mL of 5 *M* NaCl in 0.5 mL of 20% α-methylmannoside. A total of 0.2 mL of the prepared bacterial suspension was added to each of 16 microtiter plate wells, gently agitated for 1 min (50 rpm), and incubated for 1 h at room temperature. Unbound bacteria were decanted, and the microtiter wells were washed five times with 0.25 mL of IMC medium with 1% α-methylmannoside (wash solution) for 5 min at 50 rpm. The bound bacteria were eluted in a minimal volume (about 50 µL) of wash solution by vigorous vortexing for 30 s or, alternatively, by 1-h elution with wash solution containing 0.1 m*M* biotin with gentle agitation. Eluted bacteria were cultivated in IMC medium overnight, subsequently induced with tryptophan as just described, and used for four additional rounds of selection. Finally, the eluate from the last round of selection was plated on solid medium, and the resulting colonies selected for DNA isolation and sequencing.

### *DNA Sequencing*

Single-stranded DNA (ssDNA) from amplified selected phage clones was isolated by denaturing coat proteins with iodide buffer (10 m*M* Tris-HCl, pH 8.0); 1 m*M* EDTA; 4 *M* NaI) and precipitating with ethanol. Plasmid DNA was isolated from bacteria using a Wizard Plus Minipreps DNA Purification System (Promega, Madison, WI). Purified DNA (analyzed by agarose gel electrophoresis) was sent to MWG Sequencing Service (MWG Biotech, Munich, Germany) for sequencing.

## Results and Discussion

Selection of streptavidin-binding peptide sequences by phage display yielded a series of closely similar sequences (Table 1). The conserved motif was Gly-Ser/Thr-Phe/Tyr-Xaa-His-Pro-Gln, (G[S/T][F/Y]XHPQ) and corresponded to the streptavidin-binding motif (HPQ) previously published by Giebel et al. *(24)*. Selection was repeated independently three times, once with in-lab-coated microtiter plates and twice with plates precoated with streptavidin by the manufacturer. The peptide sequences

Table 1
Amino Acid Sequences of Peptides Selected
by Three Independent Phage Display
and Two Independent Bacterial Display Selections
on Streptavidin Bound to Microtiter Plates
in Our Laboratory or by Manufacturer

| Target | Selection | Amino acid sequence |
|---|---|---|
| Precoated streptavidin plates | Phage display no. 1 | G S Y W **H P Q** |
| | | G T F I **H P Q** |
| | | G T F I **H P Q** |
| | | G S Y W **H P Q** |
| | | G T F I **H P Q** |
| | Phage display no. 2 | G T F I **H P Q** |
| | | G T F I **H P Q** |
| | | G T F I **H P Q** |
| | | G T F A **H P Q** |
| | | G T F I **H P Q** |
| In-lab-coated streptavidin plates | Phage display no. 3 | G T F A **H P Q** |
| | | G T F D **H P Q** |
| | | G T F D **H P Q** |
| | | G T F A **H P Q** |
| | | G T F D **H P Q** |
| | | G T F A **H P Q** |
| | | G T F A **H P Q** |
| | | G T F A **H P Q** |
| Precoated streptavidin plates | Bacterial display no. 1 | STOP codon contained in all sequences |
| In-lab-coated streptavidin plates | Bacterial display no. 2 | A G F E G |
| | | S M W A H S S R D D A V |
| | | A K S S A K G K A S G V |
| | | STOP codon contained in 8 sequences |

Streptavidin binding motif (HPQ) is shown in boldface.

selected were nearly identical. All 18 samples of phage ssDNA that were sent to a commercial sequencing service were successfully sequenced.

A total of 38 clones was selected by five cycles of biopanning of the bacterial display library on streptavidin-precoated or in-lab-coated plates (6 and 32 clones, respectively). Bound clones were eluted by displacing with excess biotin or mechanical shearing of flagella. Samples of purified plasmid DNA were sequenced.

The first observation was that plasmid DNA sequencing was much less successful than phage ssDNA sequencing. Only 13 of 38 sequencing reactions were successful (Table 1), and 10 contained a stop codon in the region coding for the displayed random peptide. Given that the bacterial strain has no ability to suppress any stop codon, stop codon in this region is expected to stop the translation of functional flagellin. The construct

(random peptide together with trxA region, which enables the exposure of random peptide loop at the surface of the protein) is inserted in the middle of flagellin molecule, and, therefore, the partially translated flagellin is not expected to form functional flagella. Bacterial clones lacking flagella are not expected to bind to the target molecule and should be lost in the process of selection. The presence of stop codons in affinity-selected bacterial clones can be explained by nonspecific binding. According to a published procedure of library construction *(2)*, we calculated that the bacterial library FliTrx contains as much as 24% clones with at least one stop codon in the inserted randomized nucleotide region. The calculation was based on the fact that the ratio G:A:C:T on the first nucleotide of the triplet in the randomized region was 7:7:7:3. As a consequence, there is a 2.3% chance that an individual triplet is a stop codon. If the ratio were 1:1:1:1, the chance of stop codon would be 4.7%. We have observed higher frequency of stop codons in affinity-selected clones after five rounds of panning procedure.

One of the bacterial clones selected expressed a pentapeptide instead of the expected dodecapeptide (Table 1), most probably the result of an error in the process of library construction or a later mutation. As mentioned in the Introduction, the aliquot of bacterial library that was applied to selection did not fully represent all the theoretically possible different dodecapeptides. The bacterial clones with HPQ sequence on each position of the dodecapeptide are represented in $5.1 \times 10^{11}$ clones of fully represented library and in $10^6$ clones of the library aliquot (the same is true for any other sequence of three amino acids). The shorter sequences were therefore well represented also in bacterial library, and this should not be the reason for the failure in selecting target-binding clones.

Although bacterial display technology has in theory some advantages over phage display, in practice phage display turns out to be more successful. Many cases of successful use of phage display are described in the scientific literature and patents, and only a few cases of bacterial display *(2,17–23)*. On the basis of our experiments described herein and data in the literature, we conclude that the phage display technique, although technically more demanding, in general leads to convincingly better consensus sequence of binding peptides being selected, whereas the bacterial display technique in most cases fails to discover specific target-binding motif.

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