Production of polyclonal phages harbouring antibody fragment genes against Xanthomonas citri subsp. citri using phage display technology

H. Raeisi1, M. R. Saifarnejad2, S. M. Alavi3, S. A. Elahinia4 and N. Farokhi4

1 and 4. PhD student and Professor, Department of Plant Protection, College of Agricultural Sciences, Guilan University, Rasht; 2- Associate Professor, Department of Plant Viruses, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran; 3- Assistant Professor, Department of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran; 5- Associate Professor, Department of Cell & Molecular Biology, Faculty of Life Sciences & Biotechnology, Shahid Beheshti University, Tehran, Iran

Abstract
Citrus bacterial canker, caused by Xanthomonas citri subsp. citri (Xcc), is amongst the important diseases of lime orchards in southern parts of Iran. Phage display has been used to produce specific antibodies for detection of pathogen-infected plants as well as development of resistant varieties. An effector, namely phA and a pilus protein, HrpE, the major critical components of type III secretion (T3S) system with roles in pathogenesis, were chosen as antigens. Recombinant forms of the proteins (phA and HrpE) were expressed in a bacterial host and purified via affinity chromatography. Tomlinson phage display libraries including single chain variable fragments were used for isolation of the specific antibodies. Biopanning, 3 rounds against phA and HrpE proteins, allowed enriching antigen-specific phages. The specificity of phages was tested using ELISA. Moreover, the phages were able to detect the plants infected with citrus bacterial canker.

Key words: Biopanning, citrus bacterial canker, effector protein phA, HrpE protein, phage display.
Fujikawa et al., 2006; Swarup et al., 1992

in M13, Swarup et al. (2002) reported that overexpression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.

Swarup et al. (2002) showed that the expression of the M13 replication origin in a bacterial vector results in the formation of large, circular DNA molecules. These molecules are thought to be the result of the rolling circle mechanism of DNA replication, which is a process that occurs in bacteriophages belonging to the family that includes M13. The replication origin is a specific sequence of DNA that serves as a binding site for the origin recognition complex (ORC) and other proteins that are involved in the initiation of replication. The ORC is a heterotetramer composed of six proteins, each of which has a distinct function in the replication process. The recognition of the replication origin by the ORC is thought to be the first step in the initiation of replication, and the subsequent steps involve the recruitment of other replication factors and the unwinding of the DNA helix. The replication of M13 is thought to occur in a semiconservative manner, with each strand being replicated independently. This process is thought to be facilitated by the presence of a hairpin structure in the DNA molecule, which provides a site for the initiation of replication. The replication of M13 is thought to be a complex process that involves the coordinated action of multiple proteins, and further studies are needed to fully understand the molecular mechanisms that underlie this process.
میکروگرم در مول میکروگرم

در تحقیقات حاضر، جهت تکثیر کتابخانه فازی، Escherichia coli\textsuperscript{1} به سیستم TGI\textsuperscript{2} تقدیر شد و در این تحقیق به منظور بروز نژاد-بندی، سازه pha\textsuperscript{3} به کمک از تحقیقات قبلی تهیه سازه بود.

PHA\textsuperscript{4} قابلیت سنتز نژاد-بندی اکتور پروتئین‌های اکسپرسیون است. که دو فاز 108 جفت باز بوده و برای بیماری زایی

پاتوین بیماری متغیر با pET28a-HpRE

در این استفاده شد. این سازه حاوی زن کد نکاتی‌فلسی می‌باشد که در انتقال مستقیم افکت‌دهنده بیمار به دلایل سلول میزبان نقض دارد. پاسخگوی

ترکیب نواحی شناسایی هیستودین با سریون رژین نیکل سازه شرایط تحقیقات را بررسی نشان می‌نماید. سازه روی تولید پروتئین‌های تورتکوربی E. coli\textsuperscript{5} باکتری Rosetta

استفاده شد.

*Tomlinson I&J*, 2013\textsuperscript{a}, 1995)

*Escherichia pET28a-pthA*}

لیبل میکروگرم در مول

تولید پروتئین‌های تورتکوربی به منظور تولید بروتپی

روش تولید، کلینیک‌های تورتکوربی سویه به سیستم E. coli\textsuperscript{6} و pET28a-pthA

پیش‌گویی مثبت از تولیدی کانیکس 500 میکروگرم در مول

لایه LB میکروگرم در مول

کشت داده شد. پس از انجام کشت

شبانه، تجدید کشت به سبب 1:100 در

500 میلی لیتر میکروگرم

کشت ماک، حاوی آنتی‌بیوتیک کانیکس 500 میکروگرم در

میکروگرم در مول

در تاریخ ۱۴ ماه سیستم گردید. پس از ورود فاز

شردی باکتری به مرحله لگاریتمی (میزان جذب تنازی میکروگرم

در تاریخ ۶۰ ثانیه به 0/5 بردس) تحریک سلول‌های

باکتری جهت تولید پروتئین‌های تورتکوربی با انواع

فقط نهایی یک میلی‌مترول جریان و pET28a-pthA

به مدت 15 دقیقه سانتریفیوژ و به مدت 50 میلی‌لیتر از

سپس بی‌داه‌ای و خالص سازی پروتئین تورتکوربی با روش

کرومومگرافی و با استفاده از سترون زرین نیکل سیف‌نات

ماشین (۱۹) تاپید بیان و خالص سازی

SDS-PAGE (انجام شد (۱۹۹۵)).

تعیین افزایش گرفت. باکتری مورد استفاده در

تقریباً در تغییرات پایه تهیه شده بود pET28a-pthA

منتشر شود. (Mokhtari* et al.*, 2015)

PHA\textsuperscript{4} حاوی سازه

می‌باشد که با توجه به میل

نیکل سازه شرایط تحقیقات را بررسی نشان می‌نماید. سازه روی تولید پروتئین‌های تورتکوربی E. coli\textsuperscript{5} باکتری Rosetta

کشت انجام شد (۱۹۹۵).

نتایج کتابخانه فاز 1 و میکرو ۱۰۰ میکروگرم در مول

میکرو‌گرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگرم در مول

میکروگر
20 mls of E. coli grown in PBS buffer containing 1.5mM KH$_2$PO$_4$ and 2.5M NaCl.

PBS buffer was prepared by dissolving 137mM NaCl, 2.7mM KH$_2$PO$_4$, 0.18mM KCl, 0.0025mM CaCl$_2$, 0.025mM MgCl$_2$ in distilled water.

The pH of the buffer was adjusted to 7.4 with NaOH or HCl.

The cells were pelleted by centrifugation at 5,000 rpm for 10 minutes, and the supernatant was discarded.

The pellets were resuspended in PBS buffer containing 0.1% Triton X-100 and 0.5% sodium deoxycholate.

The suspension was sonicated for 15 seconds at 150W.

The resulting lysate was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was collected.

The concentration of the lysate was determined by measuring the optical density at 600 nm (OD$_{600}$) with a spectrophotometer.

The protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

20% polyethylene glycol 6000, (PEG), NaCl, and/or CaCl$_2$ were added to the lysate to precipitate the protein.

The precipitate was collected by centrifugation at 10,000 rpm for 10 minutes and washed with PBS buffer.

The protein content of the precipitate was determined by measuring the optical density at 280 nm with a spectrophotometer.

The results were expressed as mg of protein per ml of lysate.

The protein content of the precipitate was then compared with the control sample without any additions.
نتیجه و بحث


اهرام و پیامدهای گیاهی: جلد 28، شماره 2، اسفند 1396

روتانی فازهای نوترکیب در رشد گیاهان آلوده به پیامد شانکر می‌باشد: فازهای جداسازی شده از مراحل مختلف غربالگری کتابخانه فازی (برپنگ‌ها) جهت سنجش واکنش‌های سالم و آلوده در آزمون افزایش غیر مستقیم استفاده شدند. به این صورت که این داده‌ها فازهایی با عصاره گیاه سالم و آلوده XCC از Xanthomonas citri subsp. citri (نهایت) شده به پروهانگ می‌باشد. این تحقیق تکنیک و HrpE از پروتئین‌های نوترکیب خاص دارای خاصیت جدی‌گیری به عنوان دستگاهی فازی‌های اختصاصی ارزیابی داده شد.

واکنش‌های فازهای غربالگری کتابخانه فازی: کتابخانه‌های فازی حاوی فلکت و همگامی به عنوان تیپ‌بندی در صورت متناظرهجیپ و تاوازه استفاده می‌شود. در این کتابخانه، فاز نوترکیب حاوی تیپ‌بندی و از پروتئین پیش‌بندی شده از سطح فاز ظاهر می‌شود (شکل 1). E. coli تست کتابخانه فازی از سویه ABTS تاکریکی TGL باکتری گرفت (جدول 1).
**Fig. 1.** recombinant phage contains recombinant antibody fragment fused to the N-terminus of coat protein III; (Kierny et al., 2012)

<table>
<thead>
<tr>
<th>Output phage</th>
<th>Input phage</th>
<th>Panning rounds</th>
<th>Phage display library (Tomlinson I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4*10^8</td>
<td>10^11</td>
<td>1</td>
<td>phA</td>
</tr>
<tr>
<td>3.8*10^4</td>
<td>10^11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7.4*10^3</td>
<td>10^11</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2.6*10^6</td>
<td>10^11</td>
<td>1</td>
<td>HrpE</td>
</tr>
<tr>
<td>4.0*10^4</td>
<td>10^11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7.9*10^3</td>
<td>10^11</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Comparison of optical density in 405 nm from phages obtained in biopanning processes of Tomlinson I against HrpE. x axis: phages from the 1st, 2nd and 3rd rounds of biopanning. BSA and phA protein used as a negative control. Different letters indicate significant differences between samples (P < 0.05).

Fig. 3. Comparison of optical density in 405 nm from phages obtained in biopanning processes of Tomlinson I against phaA. The horizontal axis contains the phages from the 1st, 2nd and 3rd round of biopanning. BSA and HrpE protein used as a negative control. Different letters indicate significant differences between samples (P < 0.05).
References


COLETTA-FILO, H. D., M. A. TAKITA, A. A. SOUZA,


Engineering of a single-chain variable-fragment (scfv) antibody specific for the stolbur phytoplasma (mollicute) and its expression in Escherichia coli and tobacco plants. Applied and environmental microbiology, 64: 4566–72.


