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(54) Title: A METHOD OF PRODUCING BACTERIOPHAGES

(57) Abstract: The proposed method permits the single-stage and effective purification of phage preparations of therapeutic significance, makes it possible to retain the antibacterial activities of bacteriophages, both in the case of a strategy based on the displacement of bound bacteriophages from the gel as well as on proteolytic release. Modification of phage capsid proteins with appropriate binding motifs makes it possible to purify therapeutic strains of bacteriophages using affinity chromatography.

A method of producing bacteriophages

The present invention relates to a method of producing purified bacteriophage preparations. The resulting bacteriophage preparations are useful in many applications, particularly in the manufacturing of products requiring a high degree of purity, such as medicinal products.

During the lytic cycle during therapy, bacteria are destroyed by the bacteriophages multiplying in them. Bacteriophage progeny, are released in amplified numbers into the environment, and subsequently lyse the following bacterial populations.

Regarding the production of bacteriophage lysates for technical purposes, not only the number of progeny phage molecules is significant, but the bacterial structural elements released into the environment as well, such as nucleic acids, proteins and cell wall components. A significant portion, as much as 70%, of the wall of Gram-negative bacteria is composed (as much as 70%), of lipopolysaccharides (termed pyrogens or endotoxins), peptidoglycans and proteins.

The effective removal of pyrogens from bacterial lysates is a key requirement in the production of bacteriophage preparations for dedicated bacterial infection therapies. Endotoxins are strong stimulants of the immune system by inducing the production of interleukins, TNF, NO, etc.

Endotoxin isolation or removal procedures are based on organic solvent extraction, such as an aqueous phenol solution Westphal O., Lueritz O., Bister F. Uber die Extraktion von Bakterien mit Phenol/wasser. Z. Naturforsch. 7: 148-155, 1952), mixtures of acids and aliphatic amines (Patent application Publicatiom US 2007/0020292A1), or extractive and chromatographic methods (Patent Application publication US 2007/0031447 Al). Purification of biological preparations of endotoxins is performed using metal ion-protein interactions (patent US 6,942,802 B2 Sep. 13, 2005; W002083710A1; W004003215A1), via the alcohol precipitation of endotoxins and with bivalent counterions (US 5039610). The use of bivalent ions in combination with alcohols, gels and detergents is the subject of many patents, such as EPO 407037B1. Crab haemolymph proteins have been used for endotoxin removal (US 5760177). Many column chromatography methods have been descrigel. They make use of lipopolysaccharide affinity for alkaline haptens such as polymyxine (Petsch D, Beeskow TC, Anspach FB, Deckwer WD, (1997) Membrane adsorbers for selective removal of bacterial endotoxin. J. Chromatogr B Biomed Sci Appl. 693(1):79-91),

złoże calcium silicate (Hang JP, Wang Q, Smith TR, Hurst WE, Sulpizio T, (2005) Endotoxin removal using a synthetic adsorbent of crystalline calcium silicate hydrate. *Biotechnol Prago* 21(4):1220-5), synthetic polymers (Hirayama Ch, Sakata M, (2002) Chromatographic removal of endotoxin from protein solutions by polymer particles. *Journal oj Chramatography B, 781:419-432)* or polyanionic gels (Boratyński J, Syper D, Weber-Dabrowska B, Łusiak-Szelachowska M, Poźniak G, Górski A. Preparation of endotoxin-free bacteriophages Celi Mol Biol Lett. 2004; 9(2):253-9).

There is still a need for methods of purifying bacteriophage lysates, particularly of endotoxins, which could be used for the industrial production of bacteriophage preparations meant for use in the treatment of bacterial infections which occur in humans. Despite the multiple aforementioned methods of purifying bacteriophage lysates, they fail to sufficiently fulfill the requirements for industrial production methods of preparations for the above mentioned uses.

Unexpectedly, a method fulfilling the above mentioned requirements has been proposed within the present invention.

The subject the present invention is a method of producing bacteriophages characterised in that: a) a bacterial host culture is maintained on an appropriate medium, the culture is inoculated and a bacteriophage lysate is produced,

b) the bacteriophage lysate is purified using affinity chromatography, d) the purified bacteriophage preparation is made from the the resulting eluate,

wherein in stage a) the bacterial host strain consists of bacterial cells containing a sequence encoding a fusion protein tat is a part of the capsid of the bacteriophage present in the produced lysate capsid, which also has an affinity for the chromatography gel used in stage b) as well as a polypeptide from a phage structural protein.

Preferably, a polypeptide with an affinity for the chromatography gel is selected from among a group encompassing a HisTag and GST. An example polypeptide from a phage structural protein is encoded by a sequence encoding the HOC protein shown in Figure 1. Preferably, during stage a), the bacterial host culture is maintained in a culture broth with a pH of about 7.2 which contains a meat extract, an enzymatic lysate of casein, yeast hydrolysate, peptone and NaCl.

Preferably, during stage a), the bacterial host strain used is a bacterial strain sensitive to the phage being amplified.

Preferably, during stage a), the culture is inoculated with a bacteriophage strain lacking the gene encoding the structural phage protein which is a part of the fusion protein encoded by the sequence contained in the bacteriophage host.

Preferably, during stage a), the resulting phage lysate is filter-sterilised through a sterilising filter with 0.22 µm pores.

Affinity chromatography is an established, highly efficient purification strategy for individual proteins of various origins. Unexpectedly, the use of such a method in the method according to the present invention, for the isolation of complete bacteriophage capsids, constituting complex and extensive spatial protein structures made it possible to retain the antibacterial properties of the isolated bacteriophages, despite the fact that their capsids have been extensively modified to enable the use of affinity chromatography. In the example embodiment, we made use of the phage display technique for introducing binding motifs into the phage capsid, and then we based on binding such modified bacteriophages to the affinity gel appropriate for the selected binding motif.

Example 1

The procedure bases on the preparation and use of a parental bacteriophage strain, in which one of the non-essential structural genes (genes encoding facultative capsid proteins, whose absence does not inhibit phage activity) is deleted or damaged. In bacteriophage T4 this may be a defective *hoc* or *soc* gene. As the host for the defective T4 bacteriophage we used expressive strains of *Escherichia coli* transformed with expressive plasmids containing a proper *hoc* gene fused with a sequence encoding the selected binding motif. In such a culture, a bacteriophage defective in the selected capsid protein is able to supplement this absence with the Hoc protein with binding motif simultaneously expressed in the bacterial cell from the expression plasmid. This results in stable capsid structures containing the recombinant protein, and thus containing motifs with a strong affinity for the binding gels and expressing them on the capsid surface.

Two alternate means may be used to release the bacteriophages from the gel: (i) competitive elution, i.e. flushing by compounds capable of interacting with the binding motifs of the capsids and/or the binding gel (glutathione, imidazole), or (ii) proteolytic elution with the aid of a protease that recognises rare amino-acid motifs. The second strategy requires the introduction of sequences recognised by an appropriate pro-

tease at the design stage of the expression plasmid construct meant for the production of the recombinant protein in the cell. In the case of proteolytic elution, the bacteriophage capsid devoided of binding motifs.
A detailed example of an embodiment of the method according to the present invention is as follows.

Bacterial host cells were obtained using expressive strains of *Escherichia coli* which were transformed with expression plasmids containing the correct *hoc* gene fused to a sequence encoding a selected binding motif. In the example embodiment we made use of the plasmid pDEST15 (Invitrogen), whih contained an expresion cassette that made it possible to obtain a protein containing Hoc fused with GST (Figure 2) or an expression cassette encoding a protein containing Hoc fused with Histag (Figure 3).
The efficacy of the transformation of *E.Coli Rosetta* cells was evaluated by observing the expression of the Hoc protein fused with the GST or Histag tags (Figure 4).
Recombined host cells were cultured at a temperature of 37°C to an OD_{600} of 0.7 LB medium (LB-Broth, high salt) containing: enzymatic casein hydrolysate 10g/l, yeast extract 5g/l, sodium chloride lOg/l, pH 7.5. Next the cels were transferred into fresh LB (at a ratio of 1:100 in relation to the LB) with an addition of 0.0025 M IPTG and 1:100 in relation to the lysate of the HAP1 phage (~3x109 pfu/mi). The induction of the expression and infection were thus simultaneous. The infected cells were cultured at 37°C at 160 RPM for 8 hours.
In the example embodiment of the present invention being described, the recombined *Escherichia coli* bacteria were infected with defective bacteriophage T4 (strain HAP1 deposited in the PCM repository as F/00028 and disclosed previously in the patent application PL355355, for which patent PL 195 815 has been granted), at the same time inducing the production of the Hoc protein. The phage lysis was performed concurrently, along with the induction of the expression of the recombined protein.
The phage lysate was filtered, whereafter it was incubated with an appropriate agarose gel: glutathione sepharose or agarose with metallic ions that form complexes with the imidazole residues of the histidines (i.e. agar NiNTA). The lysates were incubated with the appropriate gel overnight at 4℃ with gentle rocking. After removing the unbound fraction, the gels were rinsed with 3 l of the following buffer: 50 mM Na_2HP04, 300 mM NaCl, pH 7.5.

Next, we used a typical purification procedure for affinity chromatography, the gel was rinsed, and the bacteriophages were eluted from the gel proteolytically.

For GST, we used two elution methods:

competitive elution with 40mM reduced glutathione;

- elution buffer: 40mM reduced glutathione, 50mM Tris, pH 8.0. Before the collection of eachfraction, the gel was incubated in the buffer for 20 minutes;

proteolytic elution was performed using the AcTEV protease;

- protease buffer: 50mM Tris, 0.5mM EDTA, 1mM DTT, pH 8.0. The enzyme was added at 1µl per 1ml gel (activity 10U/µl). Proteolysis was performed over 7 days.

From NiNTA gels, the phages were flushed out with imidazole in a 100-500mM gradient

-elution buffer: 100-500mM imidazole (depending on the fraction), 50 mM Na2HP04,300 mM NaCl, pH 7.5 The rinsing and elution were performed at room temperature.

Methods of analyzing the results

The specificity of bacteriophages modified in relation to the gel was estimated on the basis of a comparison of the elution profiles of modified T4 phages, unmodified T4 phages and T4 phages modified with a tag incompatible with the gel used. The elution profile was determined by evaluating the phage titer in individual fractions. The effectiveness of the purification was evaluated by determining the endotoxin level in the eluted (or released) fractions.

Results

Te results obtained are shown in the attached Figures and Tables.

The graphs shown in the Figures contain comparisons of individual elution profiles. In the case o comparative experiments meant to show the specificity of the phages for a gel, the initial titres of the control preparation and potentially specific preparation are the same. The same volumes of lysates were incubated with the same amount of gel, rinsed and eluted in identical conditions.

Fig. 5 shows a comparison of the elution profiles of phages modified with the GST tag and unmodified ones. The affinity of the modified preparation for the gel is almost 1000-fold that of the unmodified phage, which shows its specificity towards GST.

Fig. 6 shows a comparison an elution profile of phages modified with a GST tag as well as of phages modified with a Histag tag. The affinity of the modified preparation

for the gel is nearly 1000-fold that of the preparation modified with the incompatible tag, which shows its specificity towards GST.
Fig. 7 shows a proteolytic release profile of modified T4 phages from a GST column.
Fig. 8 shows a comparison an elution profile of phages modified with a Histag tag as well as unmodified ones. The affinity of the modified preparation for the gel is almost 100000-fold that of the unmodified phage, which shows its specificity towards NiNTA.
Fig. 9 shows a comparison of the proteolytic release profile of phages modified with a GST tag as well as of phages modified with a Histag tag from a GST column. The affinity of the modified preparation for the gel i nearly 100-fold that of the preparation modified with the incompatible tag, which shows its specificity for GST.
Tab.1. The table shows the resulting endotoxin values of purified phage preparations and their corresponding titres.

Phage preparation	Titre (pfu/mi)	Endotoxin level (EU/ml)
T4 modified with GST	1,3 x 10	186
T4 modified with NiNTA	$4,7 \times 10^8$	253
T4 modified with GST-	$4,7 \times 10^H$	363
Proteolysis		
T4 modified with GST-	1 x 10	11
Proteolysis *		
T4 modified with HisTaq*	1 x 10'	16
T4 modified with GST*	7×10^8	13

Conclusions
The proposed method facilitates the single-stage and effective purification of phage preparations, makes it possible to retain the antibacterial activity of bacteriophages, both in the case of the strategy based on the displacement of bound bacteriophages from the gel, as well as proteolytic elution. It does not require additional steps for removing proteinaceous and non-proteinaceous contaminants (including LPS). The modification of phage capsid proteins with appropriate binding motifs can also facilitate the purification of other bacteriophage strains of using the affinity chromatography technique.

SEQUENCE LISTING

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<120> A method of producing bacteriophages

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Claims

1. A method of producing bacteriophages, characterised in that:

a) a bacterial host strain is cultured in a medium appropriate to it, the culture is inoculated with bacteriophages and a bacteriophage lysate is obtained,

b) the bacteriophage lysate is purified using affinity chromatography,

c) the purified bacteriophage preparation is obtained from the resulting eluate,

wherein in stage a) the host cell used is a bacterial cell containing a sequence encoding a fusion protein that is a part of the bacteriophage capsid present in the obtained lysate, which contains a polypeptide with an affinity for a chromatography gel used in stage b) as well as a polypeptide from a phage structural protein.

2. A method according to Claim 1, characterised in that the polypeptide with an affinity for the chromatography gel is selected from a group encompassing HisTag as well as GST.

3. A method according to Claim 1, characterised in that during stage a), the bacterial host cell culture is conducted in a culture broth with a pH of about 7.2 containing a meat extract, enzymatic casein hydrolysate, yeast hydrolysate, peptone and NaCl.

4. A method according to Claim 1, characterised in that during stage a) the bacterial host strain used is a bacterial strain sensitive to the lytic activity of the amplified bacteriophage.

5. A method according to Claim 1, characterised in that during stage a) the culture is inoculated with a bacteriophage strain lacking the gene of the phage structural protein being a component of the fusion protein encoded by the sequence contained in the bacterial host strain.

6. A method according to Claim 1, characterised in that during stage a) the resulting phage lysate is sterilised through filtration via a 0.22 µm sterilising filter.

HOC gene sequence:

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GTTGATGTCCAAGAATCGCGTAATGGATACATCATTCATAAAACTGCTTTAGAAACTGGTATCATCTATACCTAT
CCATAA

Fig. 1

Expression casette – Hoc protein fused with GST – expected HocGST product mass: 66 kDa

T7 Promotor	RBS (Riboso Binding Side)	GST	digestion site for AcTEV	3xSer	HOC	T7 Terminato

Bold print highlights sequences from the vector pDEST15 (Invitrogen), underlining the restriction sites characteristic of the vector pDEST15

Normal print describes the remaining operatorelements, additionally shown with the following markings: single underline: T7 promoter, double underline: RBS, dashed underline: GST, zigzag underline: T7 terminator;

Italics denote a construct that contains the HOC gene, encompassing cleavage sites for AcTev (single underline), 3xSer (double underline), HOC gene (dashed underline)

TAATACGACTCACTATAGGG**AGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAA**GAAGGA**GATA**

TACATATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGGAATATC

TTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGG

GTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTT

ATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGG

TTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTA

GCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAA

CCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCC

CAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATA

TAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAA**TCGGATCTGGTTCCGC**

GTCCATGGTCGAATCAAACAAGTTTGTACAAAAAAGCAGGCT

GAAAACCTGTATTTTCAGGGCTCATCATCAATGACTTTTACAGTTGATATAACTCCTAAAACACCTACAGGGGTT

ATTGATGAAACTAAGCAGTTTACTGCTACACCCAGTGGTCAAACTGGAGGCGGAACTATTACATATGCTTGGAGC

GTAGATAATGTTCCACAAGATGGAGCTGAAGCAACTTTTAGTTATGTACTAAAAGGACCTGCCGGTCAAAAGACT

ATTAAAGTAGTTGCAACAAATACACTTTCTGAAGGAGGCCCGGAAACGGCTGAAGCGACAACAACTATCACAGTT

AAAAATAAGACACAGACGACTACCTTAGCCGTAACTCCTGCTAGTCCTGCGGCTGGAGTGATTGGAACCCCAGTT

CAATTTACTGCTGCCTTAGCTTCTCAACCTGATGGAGCATCTGCTACGTATCAGTGGTATGTAGATGATTCACAA

GTTGGTGGAGAAACTAACTCTACATTTAGCTATACTCCAACTACAAGTGGAGTAAAAAGAATTAAATGCGTAGCC

CAAGTAACCGCGACAGATTATGATGCACTAAGCGTTACTTCTAATGAAGTATCATTAACGGTTAATAAGAAGACA

ATGAATCCACAGGTTACATTGACTCCTCCTTCTATTAATGTTCAGCAAGATGCTTCGGCTACATTTACGGCTAAT

GTTACGGGTGCTCCAGAAGAAGCACAAATTACTTACTCATGGAAGAAAGATTCTTCTCCTGTAGAAGGGTCAACT

AACGTATATACTGTCGATACCTCATCTGTTGGAAGTCAAACTATTGAAGTTACTGCAACTGTTACTGCTGCAGAT

TATAACCCTGTAACCGTTACCAAAACTGGTAATGTAACAGTCACGGCTAAAGTTGCTCCAGAACCAGAAGGTGAA

TTACCTTATGTTCATCCTCTTCCACACCGTAGCTCAGCTTACATCTGGTGCGGTTGGTGGGTTATGGATGAAATC

CAAAAAATGACCGAAGAAGGTAAAGATTGGAAAACTGACGACCCAGATAGTAAATATTACCTGCATCGTTACACT

CTCCAGAAGATGATGAAAGACTATCCAGAAGTTGATGTCCAAGAATCGCGTAATGGATACATCATTCATAAAACT

GCTTTAGAAACTGGTATCATCTATACCTATCCATAA

ACCCAGCTTTCTTGTACAAAGTGGTTTGATTCGACCCGGGATCCGGCTGCTAACAAAGCCCGAAAGGAATAGCAT

AACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

Fig. 2

Expression casette – Hoc protein fused with Histag – expected HocHistag

T7 Promoter	RBS (Ribosom Binding Side)	His tag	digestion site for protease	3xSer	HOC	Tenninator T7

product mass: 40 kDa

Bold print highlights sequences from the vector pDEST15 (Invitrogen), underlining the restriction sites characteristic of the vector pDEST15
Normal print describes the remaining operatorelements, additionally shown with the following markings: single underline: T7 promoter, double underline: RBS, dashed underline: GST, zigzag underline: T7 terminator;
Italics denote a construct that contains the HOC gene, encompassing cleavage sites for AcTev (single underline), 3xSer (double underline), HOC gene (dashed underline)

TAATACGACTCACTATAGGG**AGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAA**GAAGGA**GATA**
TACATATGTCGTACTACCATCACCATCACCATCAC**CTCGAATCAACAAGTTTGTACAAAAAAGCAGGCT**
GAAAACCTGTATTTTCAGGGCTCATCATCAATGACTTTTACAGTTGATATAACTCCTAAAACACCTACAGGGGTT
ATTGATGAAACTAAGCAGTTTACTGCTACACCCAGTGGTCAAACTGGAGGCGGAACTATTACATATGCTTGGAGC
GTAGATAATGTTCCACAAGATGGAGCTGAAGCAACTTTTAGTTATGTACTAAAAGGACCTGCCGGTCAAAAGACT
ATTAAAGTAGTTGCAACAAATACACTTTCTGAAGGAGGCCCGGAAACGGCTGAAGCGACAACAACTATCACAGTT
AAAAATAAGACACAGACGACTACCTTAGCCGTAACTCCTGCTAGTCCTGCGGCTGGAGTGATTGGAACCCCAGTT
CAATTTACTGCTGCCTTAGCTTCTCAACCTGATGGAGCATCTGCTACGTATCAGTGGTATGTAGATGATTCACAA
GTTGGTGGAGAAACTAACTCTACATTTAGCTATACTCCAACTACAAGTGGAGTAAAAAGAATTAAATGCGTAGCC
CAAGTAACCGCGACAGATTATGATGCACTAAGCGTTACTTCTAATGAAGTATCATTAACGGTTAATAAGAAGACA
ATGAATCCACAGGTTACATTGACTCCTCCTTCTATTAATGTTCAGCAAGATGCTTCGGCTACATTTACGGCTAAT
GTTACGGGTGCTCCAGAAGAAGCACAAATTACTTACTCATGGAAGAAAGATTCTTCTCCTGTAGAAGGGTCAACT
AACGTATATACTGTCGATACCTCATCTGTTGGAAGTCAAACTATTGAAGTTACTGCAACTGTTACTGCTGCAGAT
TATAACCCTGTAACCGTTACCAAAACTGGTAATGTAACAGTCACGGCTAAAGTTGCTCCAGAACCAGAAGGTGAA
TTACCTTATGTTCATCCTCTTCCACACCGTAGCTCAGCTTACATCTGGTGCGGTTGGTGGGTTATGGATGAAATC
CAAAAAATGACCGAAGAAGGTAAAGATTGGAAAACTGACGACCCAGATAGTAAATATTACCTGCATCGTTACACT
CTCCAGAAGATGATGAAAGACTATCCAGAAGTTGATGTCCAAGAATCGCGTAATGGATACATCATTCATAAAACT
GCTTTAGAAACTGGTATCATCTATACCTATCCATAA
ACCCAGCTTTCTTGTACAAAGTGGTTGATTCGAGGCTGCTAACAAAGCCCGAAAGGAAGTAGCATAACCCCTTGG
GGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

Fig. 3

Expression of the Hoc protein fused with GST and Histag tags in *E. coli* Rosetta cells.

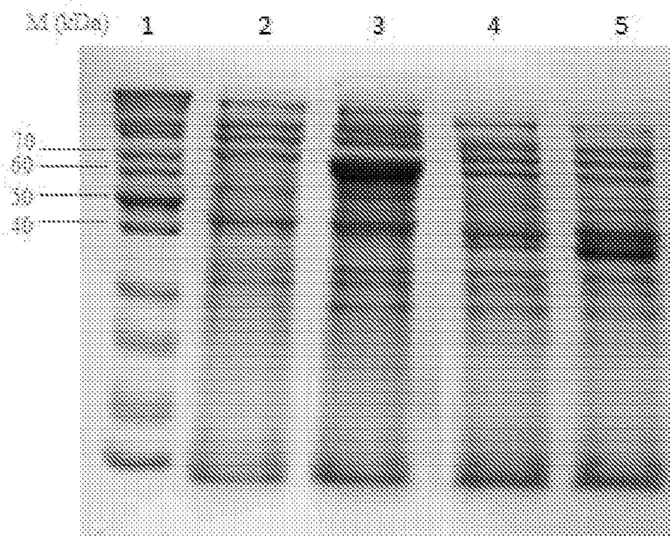


Image of electrophoresis gel- supernatant following lysis of cells expressing **the Hoc protein fused with GST and Histag** :1- Marker, 2- HocGST prior to IPTG induction, 3-HocGST 3h after IPTG induction, 4 HocHistag before induction, 5- HocHistag 3h after IPTG induction

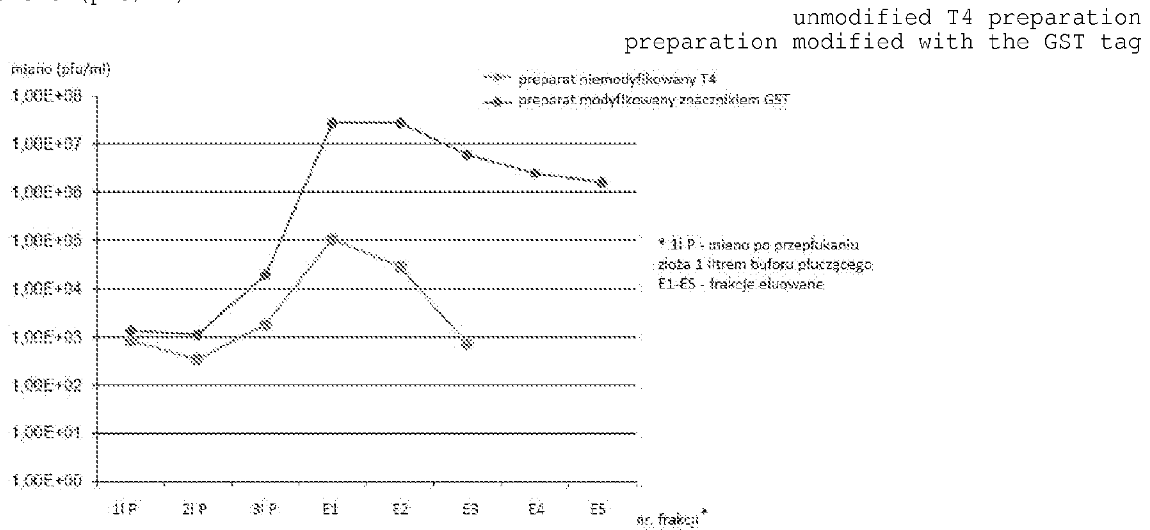
Fig. 4

Rinsing and elution profile of phages modified with the GST tag and unmodified phages

titre (pfu/ml)

unmodified T4 preparation

preparation modified with the GST tag



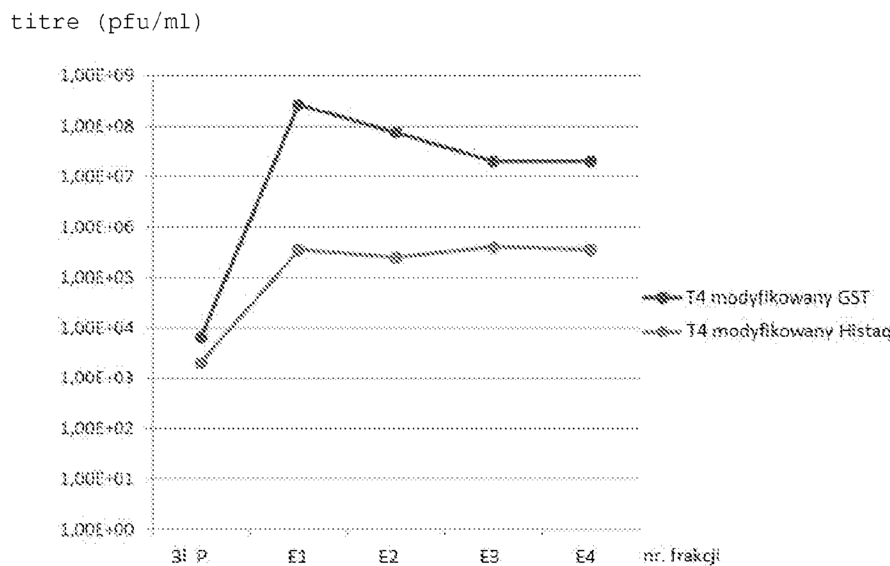
* 11 P - titre following rinsing the gel with 1 lire of rinsing buffer E1-ES - eluted fractions

* fraction No.

Fig. 5

Rinsing and elution profile phages modified with the GST tag and phages modified with the Histag tag from a GST gel

titre (pfu/ml)



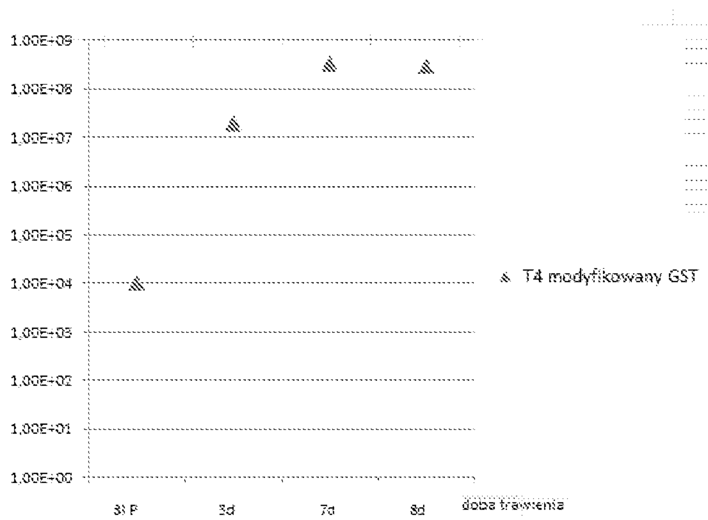
T4 modfied with GST

T4 modified with Histag

fraction no.

Fig. 6

Proteolytic release profile of modified phages from a GST gel



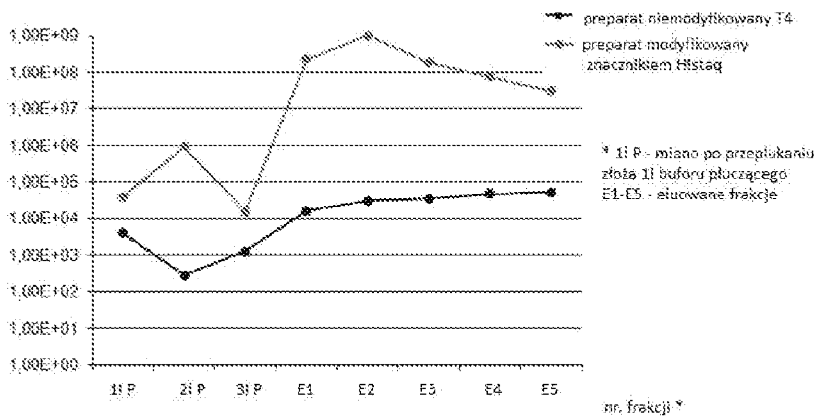
T4 modified with GST

day of digestion

Fig. 7

Rinsing and elution profile of phages modified with the Histag tag as well as unmodified phages

titre (pfu/ml)

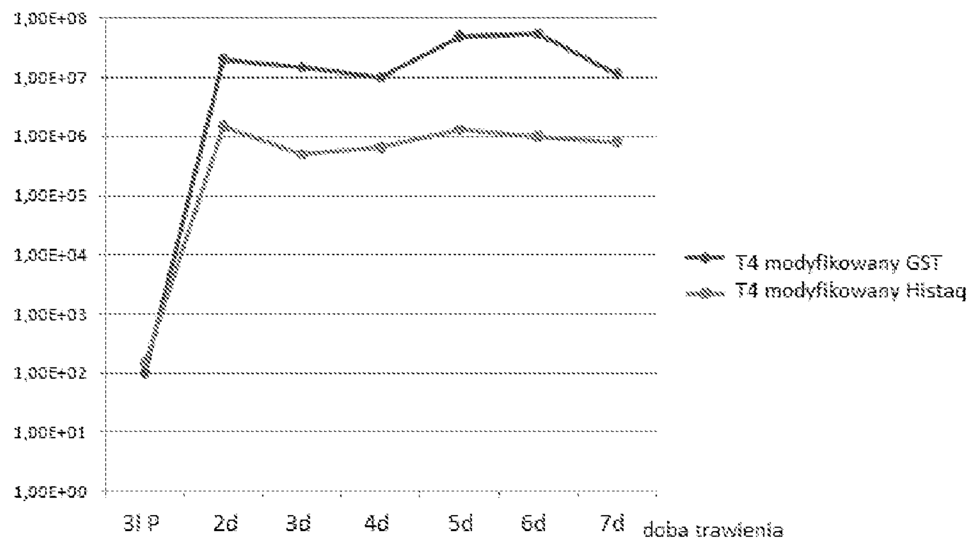


unmodified T4 preparation
preparation modified with the Histag tag
1l P - titre following rinsing the gel with 1 lire of rinsing buffer E1-E5 - eluted fractions
fraction No.*
*ALTERED CONDITIONS OF GEL RINSING

Fig. 8

Proteolytic release profile of phages modified with GST and Histag from a GST gel

titre (pfu/ml)



T4 modified with GST

T4 modified with Histag

day of digestion

Fig. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2011/050026

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/02 C07K14/01
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIANG J ET AL: "Display of a porA peptide from neisseria meningitidis on the bacteriophage T4 capsid surface", INFECTION AND IMMUNITY, vol. 65, no. 11, 1 January 1997 (1997-01-01), pages 4770-4777, XP002903999, ISSN: 0019-9567 left-hand column, paragraph 9 page 4771, right-hand column, paragraph 3 - paragraph 4 figure 1 ----- -/--	1,3,4,6

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2011	31/10/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mandl, Birgit

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	ANNA OSLIZLO ET AL: "Purification of phage display-modified bacteriophage T4 by affinity chromatography", BMC BIOTECHNOLOGY, vol. 11, no. 1, 1 January 2011 (2011-01-01), pages 59-59, XP55010029, ISSN: 1472-6750, DOI: 10.1186/1472-6750-11-59 the whole document -----	1-6
A	BORATYNSKI JANUSZ ET AL: "Preparation of endotoxin-free bacteriophages", CELLULAR AND MOLECULAR BIOLOGY LETTERS, vol. 9, no. 2, 1 January 2004 (2004-01-01), pages 253-259, XP002485107, ISSN: 1425-8153 cited in the application the whole document -----	1-6
A	US 2005/226892 A1 (RAO VENIGALLA B [US]) 13 October 2005 (2005-10-13) figures 2,3 paragraphs [0008] - [0011] paragraph [0058] -----	1-6
A	US 2005/158712 A1 (LEBOULCH PHILIPPE [US] ET AL) 21 July 2005 (2005-07-21) examples 1-4 -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/PL2011/050026

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005226892 A1	13-10-2005	NONE	
US 2005158712 A1	21-07-2005	NONE	