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INTERACTION BETWEEN PHAGES AND BACTERIA AS A TOOL FOR THE OBTAINING OF IMAGES

The obtaining of images by lytic action of bacteriophage T4 on the Escherichia coli bacterial lawn is considered. Methodical aspects of the approach are discussed, namely, use of different stencil types, total and partial staining of obtained image by different dyes. The perspectives of the practical use are proposed namely restriction of the action of microorganisms in out-of-the-way places etc.

Key words: bacteriophage T4, Escherichia coli, lytic action, lytic zone.

Introduction. Creation of images by controllable culturing of microorganisms in certain patterns (microbial art) now became a very special branch of skill at the interface between science and art [2]. In this case agar plates can be considered as a background, while pigmented or fluorescent bacteria or yeasts represent the paint. In the first case it can be performed by the application of the microorganisms with the intensively colored colonies. Another approach based on the using transgenic bacteria expressing fluorescent protein genes [3]. In this case images can be visible in ultraviolet light.

Images as well can be obtained by the growth of microscopic green alga on the nutrient medium [4]. In this case exposing different areas of the algal lawn to light over varying intervals of time changes their colors along the green spectrum.

In all these approaches the stiff nutrient medium (agar) is used as a background, where the image can be formed as the result of the growth of the bacterial colonies as well as by the change of the color of the medium or by the combination of these factors. In both cases bacteria appear as a tool.

However the authors did not found any scientific publication, where bacterial lawn itself was used as the as a background and the image was formed by the lytic action of the virus (bacteriophage). Whereas the mentioned approach could be used not only with artistic aim but for the practical use.

The aim of this work was to demonstrate a possibility to obtain the image on the bacterial lawn by the lytic action of the bacteriophage on the example of the bacteriophage T4 and *Escherichia coli*.

Materials and methods. For the obtaining of the bacterial lawn by the standard metod [1] the 1,5% agar with the nutrient medium was disposed to the Petri dishes. After the congelation of the solid medium its' surface was coated by the 0,7% agar containing *Escherichia coli* culture (concentration about 10^9 cells per ml).

For the introduction of the virus on the bacterial lawn the use of the micropipette and the application of the preliminarily autoclaved stencil from printing or filter paper were combined.

After the chilling of the substrat the stencils were applied and the preparation of the bacteriophage T4 (concentration 10^8 PFU (plaque-forming unit) per ml was introduced at the stencils by micropipette. Samples obtained by such a way were incubated during the twenty-four hours at +37°C.

The scheme of the experiment is shown on the Fig.1.

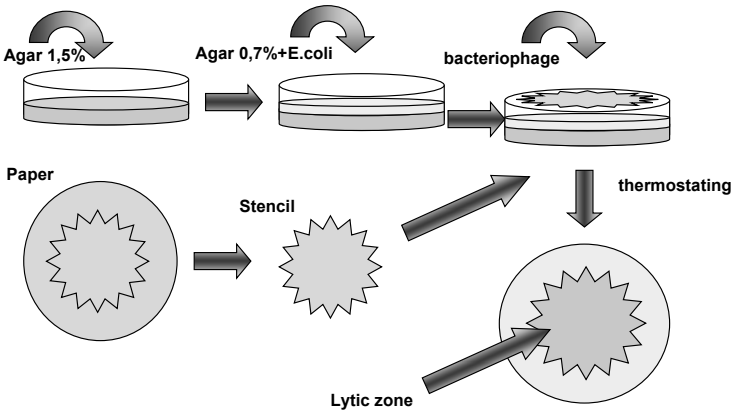

Fig.1.The scheme of the experiment

After the incubation the stencils were removed and the samples were stained by *Coomassie blue R-250 (Coomassie blue 100mg, methanol 50 ml, acetic acid 10 ml, H_2O 40ml or fuchsine (1 ml of concentrated solution – 9g of fuchsine in 100 ml of ethanol – diluted in 10 ml of H_2O) with further fixation by the 7% acetic acid.*

Results and discussion. For the obtaining of the image by the lytic action of the bacteriophage several approaches were applied. At the first series of the experiment stencils made from printing paper and filter paper were compared. It was demonstrated, that generally the use of filter paper stensil (Fig.2a) allows one to obtain more accurate and controllable image, than the use of the printing paper stensil (Fig.2b). This result can be explained by the porous structure of the filter paper which provides more regular and controllable penetration of the bacteriophage to the stencil. As the result more uniform distribution of the bacteriophage to the bacterial lawn can be achieved in

comparison with the print paper stencil. The last one can not absorb virus containing liquid effectively and therefore can not then distribute it regularly to the bacterial lawn causing the forming of wide area of lytic zones. Also It was shown, that the wetting of the whole stencil in the phage preparation instead of the using the micropipette for the application of the virus containing liquid leads to the total lysis of the bacterial lawn (data not shown).

a b

Fig.2. Unstained images, obtained by the lytic action of the bacteriophage T4 on the *Escherichia coli:*
a – using filter paper stencil, b – using printing paper stencil

In the second series of the experiment the possibility of the use of the reversed stencil (where the image is formed not by the lytic zone but by the zone of bacterial growth) was demonstrated (Fig.3) However this approach is more restricted because of the fact, that in this case the zone of lysis is more wide and thereof less controllable. Besides, this approach is more labor-consuming.

Fig.3. Image obtained by the use of the reversed stencil (stained by fuscine)

Also the possibility of the partial staining of the obtained image (namely, only zone of lysis) was explored (Fig.4). It gives an opportunity to obtain polychrome images using available colorants (for example, *Coomassie blue, fuchsine)*.

Fig.4. Partially stained by *Coomassie blue* image

Summarizing the above it should be noted, that it was the firs time when the graphical image was obtained by the lytic action of the virus on bacteria. This approach could be used not only for the artistic aims but as well for the practical use, for example, for the restriction of the action of microorganisms in out-of-the-way places.

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ВЗАЄМОЗВ'ЯЗОК ФАГІВ ТА БАКТЕРІЙ ЯК ІНСТРУМЕНТ ОТРИМАННЯ ЗОБРАЖЕНЬ

Розглянуто отримання зображень шляхом літичної дії бактеріофага T4 на бактеріальний газон Escherichia coii. Обговорюються методичні аспекти підходу, зокрема, використання шаблонів різних типів, повне та часткове фарбування отриманого зображення різними барвниками. Запропоновано можливі перспективи використання, зокрема, обмеження дії мікроорганізмів у важкодоступних місцях.

Ключові слова: бактеріофаг, Escherichia coii, літична дія, літична зона.

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ВЗАИМОСВЯЗЬ ФАГОВ И БАКТЕРИЙ КАК ИНСТРУМЕНТ ПОЛУЧЕНИЯ ИЗОБРАЖЕНИЙ

Рассмотрено получение изображений путем литического действия бактериофага T4 на бактериальный газон Escherichia coii. Обсуждаются методические аспекты похода, в частности, использование шаблонов разных типов, полное и частичное окрашивание полученного изображения различными красителями. Предложены возможные перспективы использования, в частности, ограничение действия микроорганизмов в труднодоступних местах.

Ключевые слова: бактериофаг, Escherichia coii, литическое действие, литическая зона.

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TEST SYSTEM BASED ON ROOT EXUDATES FOR HIGH-YIELDING COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH.) FORM SCREENING

A new effective non-invasive method of screening of highly productive forms of buckwheat sowing (Fagopyrum esculentum Moench.) based on rapid testing of buckwheat seedling intensity exudation of organic acids root system in the laboratory is offered. Buckwheat seeds were germinated on agar gel layer which contains in its composition acid-base indicator followed by visual assessment of the indicator color changes around primary root and plants with the largest area of color change were selected. The effectiveness of the method was confirmed in the field conditions by phenotyping of plants and significant differences in determining the structure and yield performance of selected plants were found. Statistical analysis of indicators grain number and grain weight showed that these indicators in selected plants were over 6 times higher than in the control variant with the degree of reliability of 99%.

Keywords: screening, buckwheat, phenotyping, seedlings, root exudates.

Introduction. Buckwheat is a well known valuable agricultural cereal culture used traditionally in the food industry of Ukraine. However, due to the peculiarities of the secondary metabolism this culture can be widely used in the pharmaceutical and medical industries as a source of a significant amount of bioactive substances [1]. But buckwheat may not have the performance as cereal crops because of their biological characteristics. At the same time the potential of buckwheat are not always used sufficiently. Development of new methods of selection can greatly affect the disclosure of genetic potential of the crop and expand its use. Taking into account the substantial value of buckwheat in the food industry the search for effective and rapid methods for the selection of high-performance forms of culture do not stop. Development of new methods requires complex analysis and improvement and modification of previous methods of selection.

Most known methods of selection of high-performance forms of buckwheat is selection at the stage of budding or flowering plants [2, 3, 4]. They are held on the last phenological phases of plant development and aimed at creating productive large inflorescences. A method of selecting plants for buckwheat complex features [5] is that plants are selected for buckwheat sign of breeding and structure characteristics at the crop ripening phase. Plants selected are propagated *via* meristem culture *in vitro*, encouraging re-blooming. Seeds from regenerative plants are got by directed re-pollination with known genotypes. The essence