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The kinetics of *Escherichia coli* B growth and bacteriophage T4 multiplication in SM-1 novel minimal culture medium

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The aim of this study was to develop a minimal medium for the cultivation of *Escherichia coli* B, which could be especially suitable for the industrial propagation of bacteriophage T4. The new defined, minimal SM-1 culture medium, contains free amino acids as the only nitrogen source and enables the bacteria generation time to be prolonged and satisfactory phage titers to be achieved. The presence of organic ingredients, such as meat extracts, yeast hydrolysates, enzymatic protein hydrolysates, in a culture medium may cause problems in the case of bacteria or phage cultures for therapeutic purposes. In the present study, we introduce a new medium, together with some procedures and applications for its usage. We also present new kinetics of *E. coli* B growth. Some traits such as the lack of high molecular proteins, a bacterial growth comparable to that in a rich medium, and the cost effectiveness of the medium, makes it highly competitive with currently used microbiological media. The surprisingly high titers of bacteriophage T4 obtained in our experiments suggest that SM-1 medium has the potential to find a broad application in medicine, especially in infectious disease therapy, pharmacy and biotechnology.

Key Words: bacterial growth; bacteriophage multiplication; culture medium

Introduction

Different species of microorganisms are characterized by various requirements of culture medium composition and environmental conditions that affect their dynamics and growth rate (Willey et al., 2007). By various, orderly transformations of substances in the cell, absorbed from the environment, microorganisms obtain the energy necessary for life and the synthesis of cellular components. Moreover, different mechanisms of growth rate regulation have evolved in different bacteria (Jin et al., 2012). The processes that occur inside the cell are specific enzymatic reactions forming specific metabolic pathways (Kim and Gadd, 2008). Medium composition and the availability of nutrients strongly determine the growth rate, cell size and division in gram-negative and gram-positive bacteria, such as *Salmonella typhimurium*, *Bacillus subtilis* or *Escherichia coli*. In single-celled organisms, the nutrient-dependent control of growth and cell size influence quick adaptations to rapid changes in environmental conditions (Chien et al., 2012; Yao et al., 2012).

Thus far, several types of microbial culture medium with different compositions have been proposed and employed

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in the field of microbiology (Neidhardt et al., 1974; Schneebeli and Egli, 2013; Wegkamp et al., 2010). The basic requirement to be fulfilled by any medium is the presence of water and a mixture of chemicals providing digestible substrates of all the elements involved in the formation of cell mass. Glucose or glycerol are often used as carbon sources. Whereas nitrogen and amino acid sources are different extracts such as beef or yeast extract, caseine hydrolysates, ammonium salts or nitrates (Durnin et al., 2009; Gonçalves et al., 2002; Kim and Gadd, 2008). The presence or absence of various bacterial growths is dependent on the occurrence of mineral salts, or specific elements in the culture medium, such as Zn, Cu or Se, that regulate the activity of several, metabolic enzymes (Blencowe and Morby, 2003; Ding et al., 2010). In addition, the culture medium must have the appropriate pH, a parameter to which the bacteria are very sensitive. Culture media can be divided according to state, derivation and standard of ingredients. The basic media used for bacterial culture are minimal media containing only the components necessary to maintain vital functions, and simple ones that are specific only for the least demanding microorganisms (Lech and Brent, 2001; Madigan et al., 2009). In steady-state culture conditions with no growth barriers, such as limited resources, bacterial growth is stable and proceeds according to an exponential growth model. The logistic growth model shows growth, as it approaches the maximum capacity the environment can sustain. As the population approaches the limit, the rate of increase decreases (Vandermeer, 2010).

Nowadays, microbiological culture media are used in many biotechnological processes for the preparation of biomass and specific metabolic products of microorganisms (Thomsen, 2005). One such process is the culture of appropriate bacterial strains to multiply therapeutic bacteriophages. Bacteriophages are used in medicine for the treatment of chronic bacterial infections, like *Staphylococcus*, *Enterococcus*, *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Shigella*, *Serratia*, *Salmonella*, *Proteus*, *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter* and *Bulkholderia* (Gorski et al., 2009). It is worth mentioning that the only center of phage therapy in Europe is the highly specialized Phage Therapy Unit (PTU) at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, Poland (Miedzybrodzki et al., 2012). The presence of high molecular proteins (meat extracts, yeast hydrolysates, enzymatic protein hydrolysates) in a culture medium may cause problems in the case of bacteria or phage cultures for therapeutic purposes. Situations requiring the removal of these components during purification may increase the time, or stop the registration process, of the potential medical preparations. In addition, yeast extract, as a component of a medium, seems to show an inhibitory effect relative to the rate of phage multiplication (Hadas et al., 1997).

The aim of this study was to develop a simple culture medium for bacteria such as *Escherichia coli* B, which are used in the replication of bacteriophage T4, and to eliminate high molecular proteins that are particularly undesirable in the preparation of phages for therapeutic purposes. The main goal of SM-1 was to achieve as high as possible multiplication of bacteriophage T4. Moreover, *E. coli* B growth kinetics in the simple medium SM-1 and the generation time of bacteria in the logarithmic growth phase were determined. These results enabled us to obtain sufficiently high titers of bacteria cells and especially bacteriophage T4 multiplication.

Materials and Methods

Organisms. *E. coli* B strain was obtained from the Polish Collection of Microorganisms at the Institute of Immunology and Experimental Therapy (IIET), Polish Academy of Sciences. The strain was maintained on MacConkey agar (BTL, Poland) at 4°C and subcultured on MacConkey agar monthly during the course of experimentation.

Bacteriophage T4 was obtained from the American Type Culture Collection (Rockville, Maryland, USA). The strain was stored in Polish Collection of Microorganisms at IIET, the Polish Academy of Sciences. The bacteriophage was cultured with *E. coli* B obtained from IIET. The bacterial lysate with bacteriophage T4 was purified by filtration through a syringe filter (0.2–0.45 μm Millex-HPF, Merck Millipore) and maintained at 4°C. The phage concentrations were measured by the double-layer method as described previously (Adams, 2005).

Media. SM-1 (pH 7.2 ± 0.2) the main, new medium used in the experiments was the minimal medium designed by our team (M. Sochocka et al., 6 October 2014, Polish Patent Office, Patent application 20 July 2012). It contained (per liter) 10 g of casein acid hydrolysate from bovine milk (Hy-Case®Amino, Sigma Aldrich, Poland), 3.5 g of K_2HPO_4 (POCH, Poland), 5 g Na_2SO_4 (POCH, Poland) and 1% glucose (5% glucose solution, Baxter, Poland). Three prime components were dissolved in miliQ water (Laboratory of Buffers, IIET) and sterilized in a bioreactor (BioFlo 415, New Brunswick) according to standard sterilization procedures. Sterile glucose was added to the basal medium after the sterilization process.

Peptone water (pH 7.2 ± 0.2), used as a control medium, was obtained from the Laboratory of General Chemistry IIET and consisted (per liter) of 10 g enzymatic protein hydrolysate (BTL, Poland), 5 g NaCl (POCH, Poland), 9 g Na_2HPO_4 (POCH, Poland), 1.5 g KH_2PO_4 (POCH, Poland) and 1% glucose (5% glucose solution, Baxter, Poland). Four prime components were dissolved in distilled water and sterilized in a bioreactor. Sterile glucose was added to the basal medium after the sterilization process.

Nutrient agar plates (pH 7.4 ± 0.2) were obtained from the Laboratory of General Chemistry IIET and consisted (per liter) of 5 g peptic digest of animal tissue, 5 g NaCl, 1.5 g beef extract, 1.5 g yeast extract and 15 g agar. All ingredients were dissolved in distilled water and sterilized by autoclaving.

Fermentor. BioFlo 415 (New Brunswick), a versatile sterilizable-in-place (SIP) fermentor that provides a fully equipped system in one compact package, was used for the experiments. The fermentor was employed for batch culture with process control of pH, dissolved oxygen (DO), agitation, temperature, pump feed, antifoam, level and addition analog/digital inputs and outputs. A computer

connected to the fermentor was used to remotely control all the fermentation parameters. The fermentation process was carried out at 100% O_2 enrichment, 100% DO and minimal agitation speed (50 rpm). During the experiments, the temperature was maintained at 37°C, gas flow was kept at the minimal level of 1.0 SLPM (standard liters per minute) and an optimal pH value was kept constant at 7.0 by the addition of 20% NaOH through the pump.

Culture conditions. SM-1 and peptone water with 1% glucose (control medium), the media used in the experiments, were prepared and sterilized in a bioreactor according to standard sterilization procedures. Sterile glucose was added to the basal medium after the sterilization process. Prior to each experiment, the bacterial strain was grown on MacConkey agar at 37°C for 24 h and then suspended in phosphate-buffered saline (PBS). Each medium (8 liters) in the bioreactor was inoculated with 500 ml of *E. coli* suspension. The inoculum was prepared by suspending the required amount of bacteria from the Petri plate culture. The optical density (OD) of the bacterial inoculum, estimated with a densitometer DEN-1 McFarland (OD_{565}) (BIOSAN, Latvia), was approximately OD = 3.0. During the 6-h fermentation process, the bacterial growth was monitored by measuring the OD and samples for determining the bacterial amount in the culture were collected every hour. The total amount of bacteria per ml of the liquid culture was determined by using dilution and a spread plate technique. The experiment (fermentations) was carried out eight times.

Dilution and spread plate technique. The Petri plates with nutrient agar were applied with 0.1 ml of selected, serial dilutions of bacterial samples. The material was spread on the surface with sterile palp and left to dry. The plates were incubated at 37°C for 24 h. After incubation, the colonies on each Petri plate were counted and the amount of bacteria per ml was calculated.

Statistical analysis.

Determination of the growth model: To determine the growth curve for the bacterial cultivation in SM-1 and in the control medium, the experimental data were fitted to a model based on a four-parameter logistic curve, described by the equation:

$$y_t' = f(t) = a + \frac{b-a}{1+e^{\frac{c-t}{d}}} \quad (1)$$

where,

y_t': number of bacterial cells estimated with the model;

a, b, c, d: model parameters (a: minimum asymptote; b: hill slope, steepness of the curve; c: inflection point, the point on the curve where the curvature changes direction or signs; d: maximum asymptote);

t: time of incubation;

e: base of natural logarithms.

Determination of the biological parameters of the growth curve: On the basis of the models, for the two growth curves, the following parameters were determined:

1. The beginning of the logarithmic growth phase (log phase);
2. The time point of the maximum growth rate of bacterial culture;
3. The end of the logarithmic growth phase;
4. The generation time.

Based on the bacterial growth in the log phase, the growth rate μ was estimated using the following equation:

$$N_t = N_0 e^{\mu(t-t_0)} \quad (2)$$

where,

N_t: number of bacterial cells at time t;

N_0: number of bacterial cells at the start of the log phase;

μ: specific growth rate;

t: culture time point at which the number of cells is N_t;

t_0: culture time point at which the population starts a log phase.

Based on the resulting specific growth rate the generation time, (the time between two cell divisions), was determined from the equation:

$$g = \frac{1}{\mu}. \quad (3)$$

The time point at which the population starts and ends of the log phase was determined by equating the third derivative of the logistic function, given by Eq. (1), to zero (Buchanan and Cygnarowicz, 1990). The time point when the bacterial culture has the fastest growth rate was determined by equating the second derivative of the function (1) to zero (Zwietering et al., 1990).

Hypothesis H0: *there is no difference between two curves* (i.e. SM-1 growth compared to the control medium) was tested with a bootstrapped *paired-t* statistic

$$T^* = \frac{\sum_{t=1}^{6} \log_{10} N_{t,SM-1} - \log_{10} N_{t,control}}{SE^*},$$

where t is the time of growth, $\log_{10} N_{t,SM-1}$ is the difference between $\log_{10}$ number of cells at time t and $\log_{10}$ of cells at time $t = 0$ for the SM-1 culture, SE is the standard error estimated in $b = 1000$ bootstrapped samples. Distribution of T^* was estimated based on $B = 10{,}000$ samples.

Results

SM-1 preparation

The basis for the development of a new culture medium, was to produce a different standard media to those used in microbiology, such as nutrient broth (meat extract 3.0 g; peptone 10.0 g; NaCl 5.0 g; glucose 10.0 g; distilled water 1000 ml) and peptone water (enzymatic casein hydrolysate 10.0 g; NaCl 5.0 g; $Na_2HPO_4 \cdot 12H_2O$ 9.0 g; KH_2PO_4 1.5 g; distilled water 1000 ml).

Modifications made in the composition of the new culture medium removed high molecular proteins (meat extracts, yeast hydrolysates, enzymatic protein hydrolysates), and determined the most limited mineral composition in order to allow bacteria culture. The presence of high molecular proteins in standard culture media is a major ob-

Table 1. Means and standard deviations of $\log_{10}$ number of cells obtained at time t for SM-1 and the control medium.

Time [h]	n	SM-1			Control		
		Mean $\log_{10} N$	SD	SE	Mean $\log_{10} N$	SD	SE
0	2	6.477	0.351	0.248	6.389	0.125	0.088
1	2	6.675	0.345	0.244	6.906	0.132	0.094
2	3	7.236	0.063	0.036	7.197	0.327	0.189
3	3	7.738	0.334	0.193	7.182	0.253	0.146
3.5	3	7.917	0.216	0.125	7.903	0.345	0.199
4	3	8.075	0.073	0.042	8.705	0.117	0.068
4.5	3	8.171	0.151	0.087	8.712	0.127	0.074
5	3	8.130	0.098	0.057	8.463	0.431	0.249
5.5	3	8.137	0.122	0.071	9.199	0.241	0.139
6	4	8.039	0.054	0.027	8.957	0.244	0.122

Difference between the two curves: $T^* = -3.51$; $p < 0.0001$.
SD: standard deviation.
SE: standard error.
T^*: bootstrapped *paired-t* statistic; distribution of t estimated numerically (B = 10000).

Table 2. Growth model coefficients for the SM-1 and the control medium.

Medium	a	b	c	d
SM-1	6.5114	8.1783	2.2588	0.6755
Control	6.7577	8.9546	3.4451	0.4379

a: minimum asymptote; b: hill slope, steepness of the curve; c: inflection point, the point on the curve where the curvature changes direction or signs; d: maximum asymptote.

stacle in the pharmaceutical industry. Potential medical preparations obtained using such media must be further purified and tested for biological purity. SM-1 was developed with the aim of possible application in the pharmaceutical industry, particularly for bacteriophage multiplication. Thus, SM-1 consists of the amino acid hydrolysate of casein *Amino Hy-Case*® (not the enzymatic protein hydrolysate), as the only nitrogen source, in the least amount beneficial to bacterial growth. Casein proteins were obtained by the hydrolysis of bovine milk under acidic conditions, without the digestive enzymes. In addition, the hydrolysate contains 30% NaCl, which enabled the elimination of this salt from the final composition of SM-1. The quantity of casein (*Amino Hy-Case*®) in SM-1 was tested and it was found that the smallest amount of casein required for bacterial growth, comparable to the growth in the control medium, was 10 g/l, which corresponds to the amount of the hydrolysate used in the control medium. In order to verify if *Amino Hy-Case*® was an optimal source of amino acids, the amino acid hydrolysate of casein (Acid Caseine, Calcium Caseine, Extruded Caseine and Sodium Caseine) from different sources were also tested. However, a supplement of these hydrolysates did not improve bacterial growth compared to *Amino Hy-Case*®. Therefore, in further studies, *Amino Hy-Case*® casein was used. Acid hydrolysates of different proteins were not tested due to the fact that this study was part of a project for developing a simple and unique medium for bacteriophage multiplication.

One of the essential requirements of bacterial metabolism is the presence of organic compounds, as a carbon source, in the medium. In our investigations, 1% glucose was used as the carbon source. Studies with an alternative, cheaper source of carbon, glycerol, did not provide satisfactory growth and titers of bacteria, compared with the control medium.

Mineral composition with the use of different inorganic acid salts was also tested. The medium was supplemented with various mineral salts and single micronutrients. However, no improvement in bacterial growth was observed. In addition, precipitation of some of the compounds from the solution (for example, Mg) had restricted their use. This would require a change in the medium preparation procedure, i.e. a time extension. We determined the SM-1 composition only for the two, optimal inorganic compounds, K_2HPO_4 and Na_2SO_4. Finally, the accepted composition of the medium enabled the pH to be maintained in the range of 7 to 7.4.

Kinetics of E. coli B growth in the new-defined SM-1 medium in fermentor BioFlo 415 conditions

The first studies were examined for small volumes of media, and the final results presented here are based on bacterial culture in a fermentor BioFlo 415. Peptone water with 1% glucose was used as the control medium. After the sterilization process, the SM-1 medium or the control medium were supplemented with glucose. Eight liters of each medium were then inoculated with 500 ml of bacterial inoculum in PBS with an OD = 3.0. Inoculum was prepared by suspending the required amount of bacteria from the Petri plate culture. The culture conditions in the bioreactor were set as follows: stable pH 7.0 (20% NaOH), stable temperature 37°C, minimal agitation speed (50 rpm) and minimal gas flow (1.0 SLPM). The samples of bacterial culture were collected hourly and the OD of the bacterial culture was determined. Next, the samples were placed on nutrient agar according to dilution and a spread plate technique and incubated for 24 h at 37°C. Based on the number of colonies on the plate, the amount of bacteria per ml was calculated. The experiments were conducted for 6 h in order to determine the growth curves.

The growth curves for the bacterial culture in SM-1 and the control medium were determined using Eq. (1). Table 1 contains means and standard deviations of $\log_{10}$ number of cells obtained at time t for SM-1 and the control medium. The two growth curves are significantly different ($p < 0.0001$). Table 2 contains the coefficients obtained from the SM-1 model and the control medium model.

Figure 1 presents the growth curves in SM-1 and the control medium, respectively. The graphs show the actual experimental results.

Figure 2 shows the growth curves for *E. coli* B culture in SM-1 and the control medium. The dashed line shows the growth in the control medium, and the continuous line the growth in SM-1. The logarithmic growth phase (log phase) is highlighted in bold. The start and the end of the log phase is also marked (black dots on the curve). The abscissa indicates the cultivation time in hours and the ordinate indicates the log of the ratio of the number of bacteria at time t, designated as N, to the initial number of bacteria, designated as N_0. Table 3 presents the growth

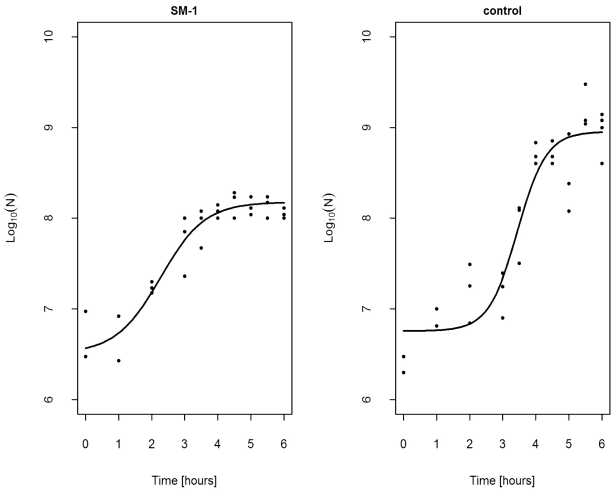

Fig. 1. *E. coli* B growth curve in the new-designed culture medium SM-1 and in a control medium.

The graph shows the observed and estimated number of bacterial cells in time based on a fitted model.

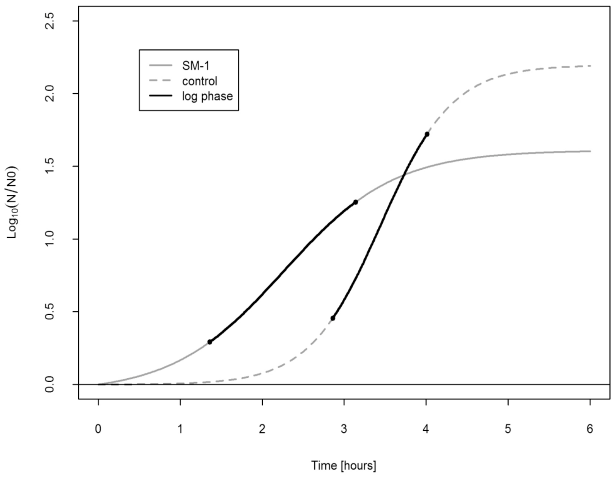

Fig. 2. Estimated bacterial growth curves in a control medium (dashed line) and SM-1 (solid line).

The logarithmic growth phase (log phase) is highlighted in bold. The start and the end of the log phase is marked with black dots on the curve. N: number of bacterial cells at a certain time, N_0: initial number of cells.

curves characteristics for SM-1 and the control medium.

From Table 3, it can be interpreted that the bacteria growth in SM-1 started the log phase after 1 hour and 22 minutes of culture. Bacteria growth in the control medium initiated the log phase after 2 hours and 52 minutes of culture (also see Fig. 1). The maximum growth rate in the SM-1 occurred 2 hours and 15 minutes from the start of a culture while, in the control medium, this was after 3 hours and 26 minutes. The log phase in SM-1 ended after 3 hours and 8 minutes, which suggests that the log phase was obtained at 1 hour and 46 minutes. The log phase in the control medium ended after 4 hours from the start of the culture and thus took 1 hour and 8 minutes.

Based on the bacterial growth in the log phase, the specific growth rate μ was estimated using Eq. (2). Then, the resulting μ was used to determine the generation time g using Eq. (3). For the *E. coli* B culture in SM-1, the generation time was g = 45 minutes and for the culture in the control medium it was g = 22 minutes (see Table 3).

Table 3. The characteristics of bacterial growth curves in the control medium and SM-1.

Culture characteristics	SM-1	Control medium
Start of log phase	1 h 22 min	2 h 52 min
Max growth rate	2 h 15 min	3 h 26 min
End of log phase	3 h 8 min	4 h
Time of log phase	1 h 46 min	1 h 8 min
Generation time (g)	45 min	22 min

The times given in hours and minutes represent the time point of culture.

Max growth rate-time moment when the fastest growth of bacteria was observed.

Bacteriophage T4 multiplication

After determining the *E. coli* B growth kinetics in SM-1, the multiplication of bacteriophage T4 was carried out. For this purpose, 8 l of SM-1 was inoculated with 500 ml of inoculum of *E. coli* B (OD ~ 3.0). Culture conditions were established as follows: stable pH 7 (20% NaOH), poor mixing and poor aeration of bacterial culture. Then, *E. coli* B culture at the beginning of the log phase was inoculated with a titer of bacteriophage T4 (4×10^9 pfu/ml). The culture was incubated in the bioreactor for 24 hours at 37°C. The resulting lysate was filtered with 0.22 micron filters. Phage titer was determined by two-layered plates. A phage titer of 1.2×10^{16} pfu/ml (mean ± SD; 16.38 ± 1.33) was obtained, which confirms that both the SM-1 medium and the conditions for *E. coli* B growth are favorable to obtain high titers of bacteriophage T4. The phage titer of 1×10^{12} (mean ± SD; 11.86 ± 0.79) was obtained when fermentations were carried out with the control medium. The data presented are logarithmic. The experiment was carried out eight times.

Discussion

Currently, many different media for bacterial culture are commercially available in microbiology and biotechnology. The composition of the medium influences the rate and kinetics of bacterial growth or enzymatic activity of bacterial cells, which often determines the industrial or scientific utility of the medium. One of the most popular and widely used media in bacteriology is the Luria-Bertani Broth (LB Broth). It is a rich medium, containing tryptone, yeast extract and NaCl, which permits fast growth and good growth yields for many species. This medium has been, and still is, being used in a number of studies on the physiology of *E. coli* with cultures in steady-state growth (Sezonov et al., 2007). New or modified simple media have, however, reduced or changed the amount of different nutrients, sometimes enriched with a single ingredient like Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} or Se ions (Ding et al., 2010; Paliy and Gunasekera, 2007). These media are developed with the aim to achieve the growth of microorganisms as well as a rich medium, or to enhance the activities of sev-

eral enzymes. Interestingly, Jia and Zhong (2011) have reported that the addition of Mg^{2+} into a culture medium increases the production of anticancer ansamitocin P-3 (AP-3) by submerged cultures of *Actinosynnema pretiosum*. New-defined media can also be used to produce high-level recombinant protein using *E. coli* expression systems (Fu, 2010; Li et al., 2011). Synthetic minimal medium MCLMAN for *Campylobacter jejuni* strain NCTC 11168 is useful in physiological assays and responses to exogenous agents. In addition, it is based only on inorganic salts and necessary amino acids and vitamins (Alazzam et al., 2011).

SM-1 is a simple, cost-effective medium and contains free amino acids as the only nitrogen source. Bacteria growing in SM-1 do not require the synthesis of as many enzymes necessary for the metabolism of several nutrients as compared with the control medium (peptone water with 1% glucose). Population growing in SM-1 has thus a shorter resting phase (lag phase) and the logarithmic phase (log phase) starts sooner. These are very important features of the new medium, which allows for potential use in industry, or in the field of medicine, especially for the multiplication and development of bacteriophages. The presence of high molecular proteins in microbiological media causes a problem for many industries; for example, during the culture of probiotic bacteria for applications in strict vegetarian food products. Pathak and Martirosyan (2012) modified de Man Rogosa Sharpe culture medium (MRS) for the selective cultivation of the probiotic strain *Lactobacillus lactis*. In the tested medium TM2, animal extract was replaced by plant (lentil) seed powder. The authors demonstrated better bacterial growth in TM2 than in an MRS culture medium. The results provide an alternative and efficient vegetal culture medium. They declared that the modified culture medium could provide a pure vegetarian way to achieve pure vegetarian products.

E. coli growing in classical media (such as LB Broth) doubles every 15–20 minutes (Sezonov et al., 2007; Todar, 2013). In our studies, bacterial growth in the control medium showed that the culture was carried out properly. The estimated generation time, for *E. coli* was 22 minutes, which was the expected result. SM-1, however, is limited in nutrients so it doesn't allow for such fast cell divisions as classic media. The generation time was extended to 45 minutes and the curve of the culture growth runs smoother. The achieved results could be profitable and have a direct application in the culturing of bacteriophage T4. The bacteriophage T4 lytic cycle takes about 25–35 minutes (sometimes up to 45 minutes) (Doermann, 1947; Ramanculov and Young, 2001), which correspond with the generation time of *E. coli* B growth in SM-1. This provides for continuous and efficient phage multiplication, and furthermore, the virus takes complete advantage of the host. It allowed us to obtain high titers of phage (1.2×10^{16} pfu/ml), which indicated that the new medium is optimal for T4 multiplication. Hadas et al. (1997) investigated supplementation of different carbon and nitrogen sources to an M9 minimal medium and its impact on the rate of T4 phage synthesis in *E. coli* B/r. Surprisingly, the highest titer of phage multiplication was obtained not using an LB medium with glucose, but an M9 minimal medium supplemented with casein hydrolysate and glucose. Thus, it was concluded that the bacteriophage replication cycle is dependent on the host cultured in an appropriate medium. Obtaining a satisfactory rate of phage multiplication requires the establishment of balance between a high cellular content of protein synthesizing-system (PSS) and delayed cell lysis. Inomata et al. (2012) compared the infection cycle of lytic RNA bacteriophage Qβ in *E. coli* cultured in rich and minimal media. They observed differences in the rate of phage release. Moreover, they noticed that the addition of Mg to a minimal medium affected the Qβ infection cycle.

It is also important in phage culture to choose the appropriate viral titer, bacteria titer and time of inoculation of the bacterial culture. Bacterial culturing in SM-1 allows for a much more precise selection of an appropriate phage T4 titer as compared with the control medium. In addition, as we demonstrated in the model of *E. coli* B growth, the log phase lasts longer in the case of SM-1, which makes it easier to "fit in" with phage inoculation in the "log window" than in the case of the control medium. It is noteworthy that in the six-hour culture (360 minutes), bacteria growing in SM-1 were in log phase for 107 minutes, i.e. 30% of the time of culture. In the case of the control medium, where the log phase lasts 68 minutes, it was only 19% of the time of culture. Moreover, *E. coli*, growing in SM-1, needed 188 minutes from the start to the end of the culture log phase. During this time, bacteria grew for 107 minutes in the log phase, which was 57% of this time. Thus, if we decide to stop the bacteria culture at the end of the log phase, the total culture time will be about 52 minutes shorter (188 vs. 240 minutes) in the case of SM-1. In addition, 57% of this time, bacteria culture was in the log phase. In the case of the control medium, *E. coli* B culture took 240 minutes before the end of the log phase. At that time, bacteria were in the log phase for only 68 minutes, which was about 28% of the time of culture.

The final composition of SM-1, the results of *E. coli* B culture, and the achieved titers of bacteriophage T4, testify to the potential use of the medium in medicine, pharmacy and biotechnology. The possibilities of using bacteriophage T4 and its mutant HAP1 in cancer therapy were presented by Dabrowska et al. (2004). They reported that treatment with purified preparations of bacteriophage T4 resulted in a significant reduction of tumor size. They also showed that HAP1 was more effective than T4. Szczaurska-Nowak et al. (2009) observed antitumor and antimetastatic effects of bacteriophage T4 in the transplantable B16 mouse melanoma model *in vivo*. Moreover, phage preparations did not negatively interfere with the application of any popular cytostatic agents (CY, CPt, 5-FU). The authors, thus, suggest the possibility of a safe combination of bacteriophage preparations with popular antitumor drugs.

The SM-1 medium was developed for its possible application in medicine and the pharmaceutical industry. The new medium is characterized by a minimal and simple composition. It has also been deprived of high molecular proteins. SM-1 consists of the amino acid hydrolysate of casein, not enzymatic protein hydrolysate, which makes

it more cost effective than most of the microbiological culture media found on the market. The kinetics of *E. coli* B growth in SM-1 is different in comparison to a classical medium. New growth kinetics allows for a steady and more controlled bacterial culture. It also enables the accurate propagation of bacteriophage T4 and the obtaining of high virus titers.

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