

Efficiency of *Salmonella enterica* serovar Enteritidis bacteriophage amplification the CellMaker disposable bioreactor system.

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Introduction

Nowadays, when the use of antibiotics in veterinary medicine has been restricted, bacteriophages are gaining popularity in the prevention and treatment of bacterial diseases. Polish company Proteon Pharmaceuticals, which is directed towards the production of pioneering and innovative treatments, medicines and vaccines in the veterinary industry, is working on a bacteriophage cocktail for prevention of *Salmonella* infections in farm animals.

Cellexus CellMaker 8L is an easy scalable, single-use bioreactor suitable for bacterial cultivation. Thus Proteon have evaluated this system for bacteriophage production on *Salmonella enterica* serovar Enteritidis culture.

Experimental conditions

The CellexusBag was filled with 5L of LB cultivation media and pre-warmed to 37°C. Then media was inoculated with overnight culture of *S. enterica* Enteritidis strain. The bioreactor was supplied continuously by 5 liters of air per minute and the temperature was stable at 37°C. Optical density of growing bacteria was measured every half an hour. When density reached OD₆₀₀ 0.4-0.5 (2h after bacterial inoculation) the inoculum with bacteriophages, specific for *Salmonella*, in amount of 5x10¹⁰ PFU (Plaque Forming Units) was added. Then the culture was sustained for another 3h to let the bacteriophages to amplify. Samples were taken to measure titre of bacteriophages (in PFU/ml) and optical density of bacteria. Another process in the same conditions but without bacteriophages was conducted as a control to compare the growth of bacteria. The results are shown in Table below.

Time	<i>S. Enteritidis</i> growth control [OD ₆₀₀]	<i>S. Enteritidis</i> growth with bacteriophages [OD ₆₀₀]	Titre of bacteriophages [PFU/ml]
0h	0,101	0,020	
30'	0,103	0,046	
1h	0,197	0,072	
1h30'	0,456	0,276	
2h	0,937	0,480	5,0 x 10 ⁷
2h30'	1,960	0,933	
3h	3,020	1,290	6,8 x 10 ⁸
3h30'	3,910	0,580	
4h	4,500	0,332	8,5 x 10 ⁹
4h30'	4,620	0,356	
5h	4,700	0,440	1,0x 10 ¹⁰

The kinetics of optical density of bacteria growing alone and with bacteriophages was compared and is illustrated in Figure 1. Dashed line represents bacteria growing alone and continuous line shows the changing optical density when phages were added to the culture in time 2h. Orange line presents the growing number of bacteriophages.

Application Note:



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Experimental conditions (continued)

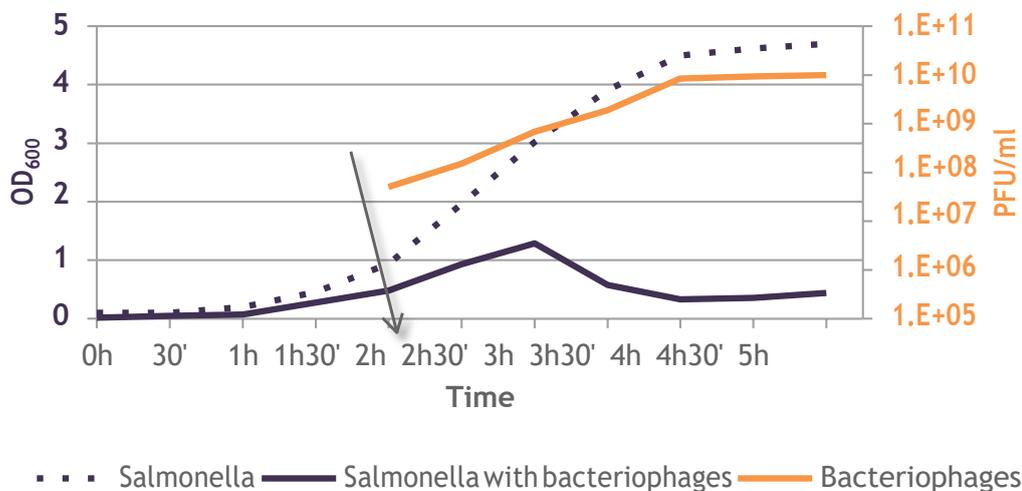


Figure 1. *Salmonella* Enteritidis growth and amplification of bacteriophages in bioreactor Cellexus CellMaker comparing to *S. Enteritidis* growing alone.

Conclusions

The Cellexus CellMaker is a user friendly ready to use system for production of bacteriophage, and the single-use CellexusBag, that does not need to be sterilized prior to cultivation of bacteria, is a great alternative to traditional bioreactors. The computer software included allows for continuous control over the conditions in the bioreactor and samples can be taken from the culture during cultivation without pausing the system. The results prove that the Cellexus CellMaker bioreactor system can be successfully used for cultivation of *Salmonella enterica* serovar *Enteritidis* and further high amplification efficiency of bacteriophages specific against these bacteria. Additional optimization may improve the yield of bacteriophages production.

Data was kindly provided by Justyna Klimczak and Arkadiusz Wojtasik (Proteon Pharmaceuticals SA).

[For further details, or to request a quotation, contact us now.](#)

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