



Application Note:

Efficiency of *Salmonella enterica* serovar Enteritidis bacteriophage amplification using the CellMaker single-use bioreactor system.

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.

The CellMaker is an easy to use, unique airlift 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 CellMaker Bioreactor 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 5x10⁷ PFU (Plaque

Forming Units) was added. Then the culture was sustained for another 3h to let the bacteriophages 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 the table below.

Time	S. Enteritidis growth control [OD600]	S. Enteritidis growth with bacteriophages [OD600]	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	5.00E+07
2h30'	1.960	0.933	
3h	3.020	1.290	6.80E+08
3h30'	3.910	0.580	
4h	4.500	0.332	8.5E+09
4h30'	4.620	0.356	
5h	4.700	0.440	1.00E+10

The kinetics of optical density of bacteria growing alone and with bacteriophages was compared and is illustrated in the graph below. The dashed line represents bacteria growing alone and the continuous line shows the

changing optical density when phages were added to the culture after 2 hours. The orange line presents the growing number of bacteriophages.

Efficiency of *Salmonella enterica* serovar Enteritidis bacteriophage amplification using the CellMaker single-use bioreactor system.

Experimental conditions (continued)

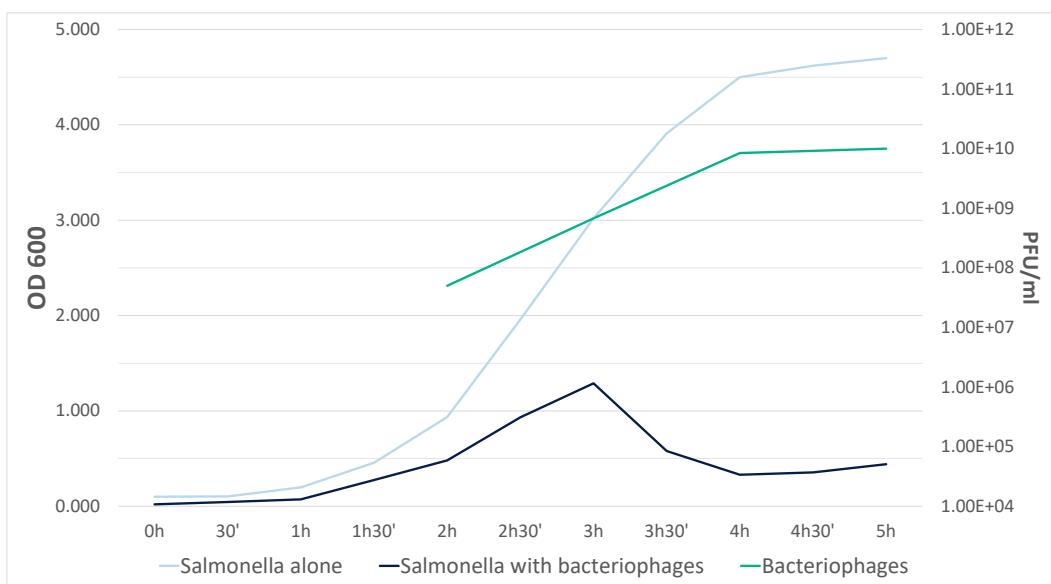


Figure 1. Salmonella Enteritidis growth and amplification of bacteriophages in the CellMaker bioreactor compared to S. Enteritidis growing alone.

Conclusions

The CellMaker is a user-friendly ready-to-use system for production of bacteriophage, and the single-use CellMaker bioreactors, that does not need to be sterilised prior to cultivation of bacteria, is a great alternative to traditional bioreactors. The integrated control software 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 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. Since, additional optimisation of the process has been carried out to improve the yield of bacteriophages and is now used as production process.

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

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