

Application Note:

Scale-up of *Staphylococcus aureus* bacteriophage production in the CellMaker Regular single-use bioreactor system.

QUALITY | RELIABILITY | SERVICE EXCELLENCE | PRODUCTIVITY

Introduction

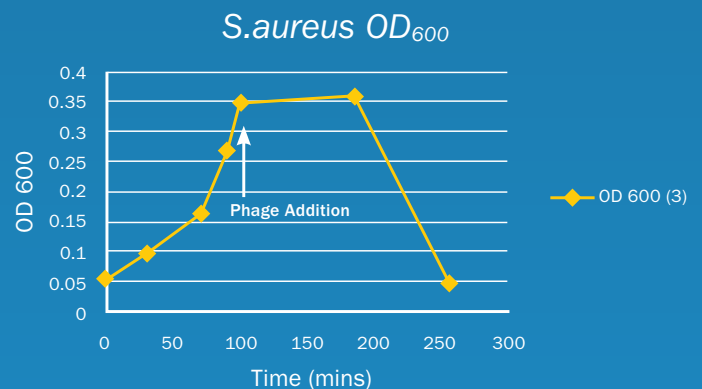
There is much commercial interest in exploiting the use of bacteriophages to control bacterial infections (phage therapy), with clear potential for control of wound infection and also general sterilization applications.

Novolytics, a company whose aim is to develop phage therapies to treat a number of problematic bacterial infections, is initially targeting Meticillin-Resistant *Staphylococcus aureus* (MRSA). Currently shaker flask cultures are used to progress these research programs, but clearly as progress is made this shaker flask scales will become limiting. Novolytics therefore evaluated the Cellexus CellMaker Regular (8L) to assess simple scale-up of *Staphylococcus aureus* bacteriophage production.

The protocol developed for this application dictates the need to seed the CellexusBag with *S.aureus*, then allow the bacteria to progress to early exponential growth phase before inoculating with bacteriophage. The phage-enriched media is then later harvested following successful phage infection and cell lysis.

Experimental

The CellexusBag was filled with 8L LB media (+ 2mls SE-15 antifoam) and pre-warmed in the CellMaker enclosure before inoculation with 0.5% (40mls) *S.aureus* (overnight culture). Upon attaining an OD600 of 0.3, bacteriophage was introduced into the CellexusBag at a multiplicity of infection (MOI) of 0.25. The phage-enriched media was later harvested following phage infection and cell lysis (associated with a drop in OD600). These experiments are run at relatively low ODs and therefore the oxygen demand of the culture is not high. Typical run parameters are shown in the table (below).



TIME (mins)	OD600	Set Temp	Process Temp	Gas Mix (Air/O ₂ /Headspace) (all L/min)	Pressure (mBar)	Comments
30	0.097	37	37	5/0.5/0.4	40	
70	0.162	37	37	5/0.5/0.4	40	
90	0.269	37	37	5/0.5/0.4	40	
95	-	37	37	5/0.5/0.4	40	1% phage (80ml) added
100	0.350	37	37	5/0.5/0.4	40	OD taken straight after phage addition
185	0.358	37	37	5/0.5/0.4	40	Sign of cell lysis
255	0.046	37	37	5/0.5/0.4	40	Culture cleared; harvest



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Three different *S.aureus* bacteriophage were evaluated in this way, and all three gave similar profiles illustrating reproducibility. The final phage titres obtained from the Cellexus CellMaker Regular were comparable to those recovered from standard shake flask experiments, see table (below). Further optimization of run parameters, OD at time of infection and MOI could increase phage yields.

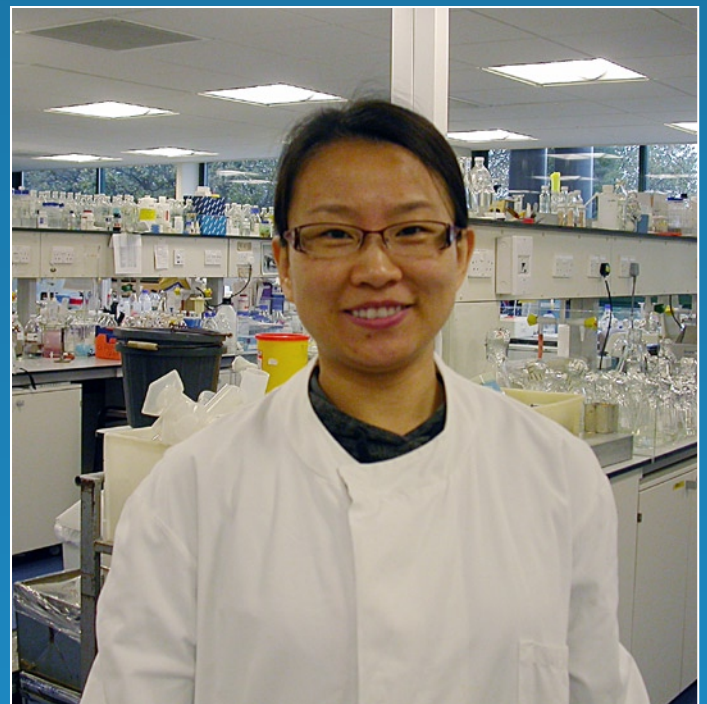
Phage	CellMaker titre (pfu/ml)	Shake flask titre (pfu/ml)
Phage#1	5×10^{10}	5×10^{10}
Phage#2	4.5×10^9	6.7×10^9
Phage#3	5.8×10^9	8.3×10^9

Conclusions:

This data demonstrates that bacteriophage production can be readily scaled-up from shaker flask to a single vessel 8L culture in the Cellexus CellMaker Regular. The performance and profile obtained was similar across a range of three distinct *S.aureus* bacteriophages. The Cellexus CellMaker Regular offers a convenient, user-friendly and consistent system for scale-up of bacteriophage production. Further scale up may be achieved using the Cellexus CellMaker Regular 50L system.

Data kindly provided by Y Jia (Novolytics, University of Warwick)

Please check the specification of the in-line 'vent' filter (used with the Cellexus CellMaker) for suitability to specific applications and identify if additional biohazard precautions are required.



"As a microbiologist I found the Cellexus CellMaker Regular exceptionally easy to use. I even completed my first large scale phage preparation the same day that the Cellexus system was delivered!"

Dr Ying Jia, Novolytics Ltd., Coventry, UK

For further details, or to request a quotation, contact us now.

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