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Process Development and Media Optimisation for a New Vaccine Producing Avian Cell Line

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Introduction

The production of vaccines in embryonated chicken eggs is associated with shortcomings like flock variance and complex supply logistics . To overcome these issues permanent suspension cell lines were developed. Among others, the muscovy duck retinal cell line Age1.CR was established. To enhance the virus production of the cell line the adenoviral pIX protein was stably transfected to yield the cell line AGE1.CR.pIX.

First, growth characterisation and determination of nutrient consumption rates for both cell lines were performed. In successive steps, optimisation of a chemically-defined media, DMF.CRI, has been accomplished to provide high cell densities and virus yield. Further attention focused on process development, especially on the effects of temperature and pH-value on growth rate and maximum cell density.

Methods

Cultivation

The standard cultivations of AGE1.CR and AGE1.CR.pIX cells were carried out in 250 mL baffled and non-baffled Erlenmeyer shake flasks (Corning Life Sciences). The cultivation medium was AEM (Invitrogen) for the CR and Ex-Cell GTM-3 (Sigma-Aldrich) for CR.pIX with 5 mM Glutamine. Conditions for standard cultivations:
37.0°C, 5% CO_2 , shaking revolution 185 rpm, orbital movement 2", working volume 150 mL.
For bioreactor cultivations three systems have been used: Triple Biostat® B-DCU, two Biostat® MD (each 2L) and the Cellferm pro® (1L). The working volumes were 1.2L/0.4L.
Initial conditions were 37.0°C, 40% DO and pH 7.2.

Modelling

For the determination of nutrient consumption rates a model-based approach using Berkeley Madonna™ was chosen.
The design of experiment and statistical evaluation were done using JMP®7.

Conclusions

- a chemically-defined medium, DMF.CRI, has been optimised to support growth of the AGE1.CR and AGE1.CR.pIX cells up to $1 \cdot 10^7$ cells/mL
- virus titers up to $5 \cdot 10^8$ pfu/mL could be obtained using DMF.CRI as proliferation medium with a specially formulated virus production medium
- without pH control the AGE1.CR cells displayed an unique intrinsic pH profile with lower specific nutrient consumption rates
- cell growth differed in Biostat B-DCU and Cellferm pro bioreactors. A possible explanation could be the different type of agitation with rushton turbines in the B-DCU system and a glassball stirrer in the Cellferm pro.
- the cells showed optimal growth at a temperature of 39.5°C and without controlled pH
- in a fed-batch cultivation with DMF.CRI medium and optimised growth conditions, the AGE1.CR.pIX cells have been grown to $1.3 \cdot 10^7$ cells/mL

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* for further information on media development see also the poster:
Northoff *et al.* "Development of a new chemically-defined medium for various animal cell lines"

Results

Media Optimisation

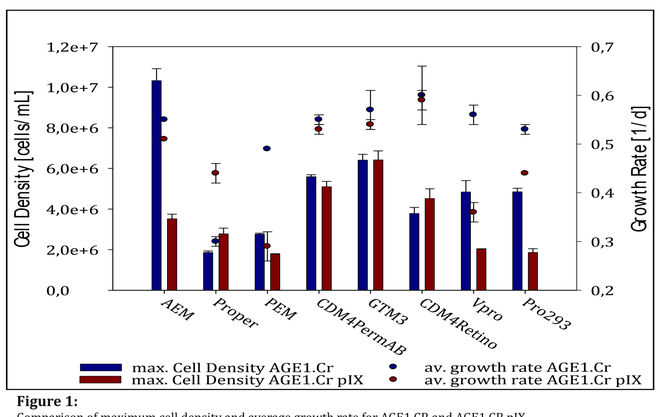

Figure 1:
Comparison of maximum cell density and average growth rate for AGE1.CR and AGE1.CR.pIX cells in different commercially availabe media

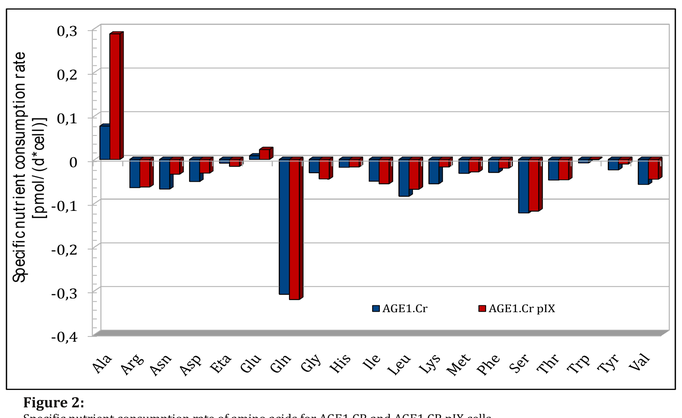

Figure 2:
Specific nutrient consumption rate of amino acids for AGE1.CR and AGE1.CR.pIX cells

The maximum cell densities and the average logarithmic phase growth rate of both cell lines differ considerably in the tested commercially available media (Fig 1). Additionally, some media contain components that are shown to be dentrimental for virus replication. To optimise a chemically-defined medium, the specific nutrient consumption rates for both cell lines have been determined (Fig 2). Simultaneously to the fortification of the nutrient concentrations potential inhibitors of virus replication have been investigated and optimal concentrations of growth factors were identified using design of experiment.

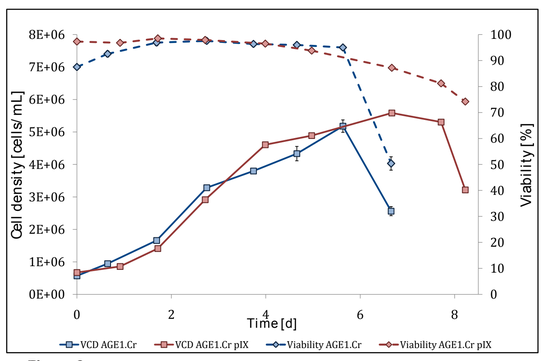

Figure 3:
Growth curves for AGE1.CR and AGE1.CR.pIX cells in DMF.CRI in culture flasks

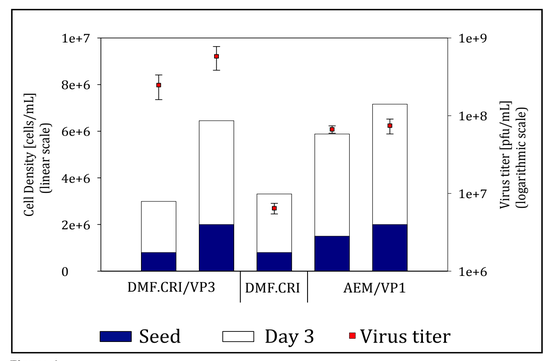

Figure 4:
Cell densities at inoculation (■), and prior dilution (□) to $2 \cdot 10^6$ cells/mL for infection with MVAATTC at 0.1 MOI in different production and proliferation media

Test cultivations of the optimised DMF.CRI media showed maximum cell densities of $5 \cdot 10^6$ cells/mL (Fig 3) for both cell lines, CR and CR.pIX, , thus comparable to commercially available chemically-defined media.
Virus infection with wild-type MVA showed poor titers for the DMF.CRI media. However, in combination with virus production media excellent virus titers up to $5 \cdot 10^8$ pfu/mL were obtained (Fig 4).

Process Development

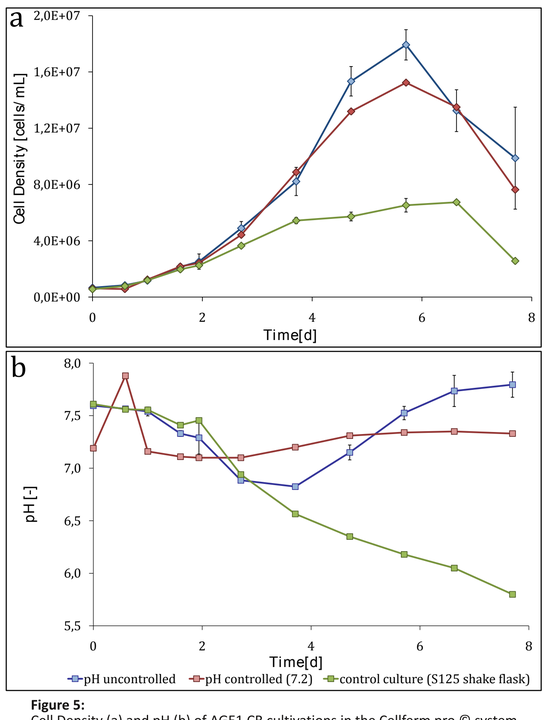

Figure 5:
Cell Density (a) and pH (b) of AGE1.CR cultivations in the Cellferm pro © system with/without pH control and of shaker flask control cultures

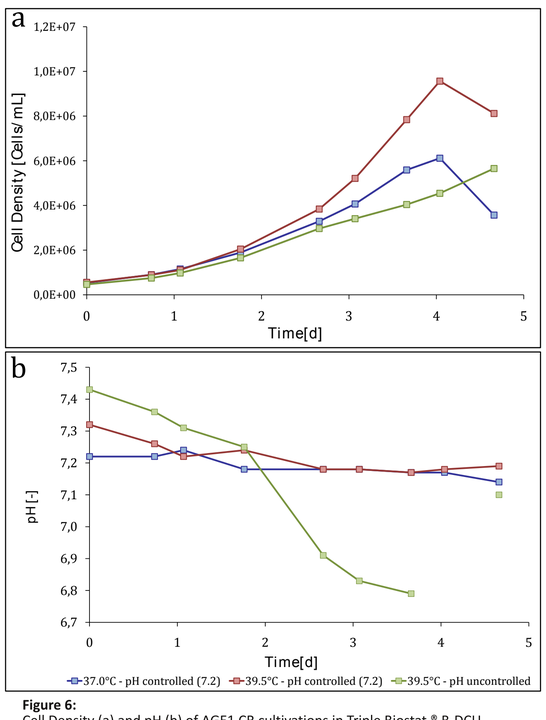

Figure 6:
Cell Density (a) and pH (b) of AGE1.CR cultivations in Triple Biostat ® B-DCU bioreactors at different temperatures and pH values

Parallel cultivations in a Biostat® B-DCU bioreactor at different temperatures indicated better growth of the avian cells at 39,5°C (Fig. 6a). Compared to the shake flask cultivations, considerably higher cell densities were observed. In bioreactor cultivations, a further increase in cell density could be achieved using no pH control, resulting in a unique pH profile (Fig 5). During this profile the decreased pH value after 2 days of cultivation affected the specific nutrient consumption rates and prelonged the logarithmic growth slightly (data not shown).

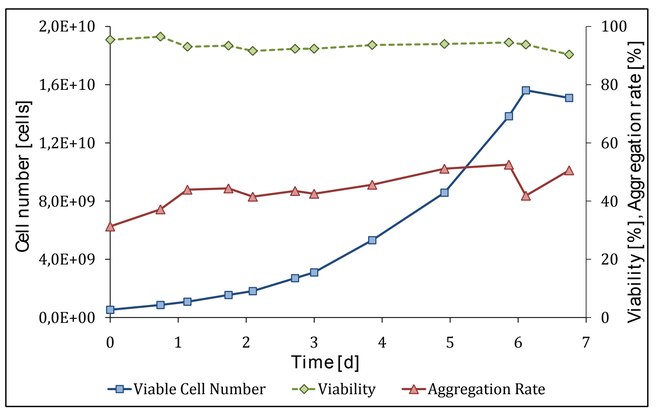

Figure 7:
Cell number, viability and aggregation rate for AGE1.CR.pIX cells in a fed-batch cultivation with the DMF.CRI medium and the optimised growth conditions

The poor growth of the control culture may be explained with the constant decrease in pH to a pH value of < 6.0.

Finally, a fed-batch cultivation of AGE1.CR.pIX in DMF.CRI medium and with optimised process parameters was carried out. A maximum cell density of $1.3 \cdot 10^7$ cells/mL and 6 days of logarithmic growth represent a major improvement of the process (Fig 7). Though, the superior pH profile, promising even higher cell densities, that were obtained in non pH-controlled cultivations could not be recreated.