Serum-free Influenza Vaccine Production with MDCK Cells in Wave-bioreactor and 5L-stirred Tank Bioreactor

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Abstract: A serum-free process for influenza virus vaccine production (equine and human) in roller bottles and microcarrier systems in 5L-stirred tank and 2L-wave bioreactor (Cytodex 1) is described. MDCK cells were adapted from growth in serum containing GMEM medium to serum-free Ex-Cell MDCK medium. Virus titers of 2.0-2.9 log HA units/ 100 µL were obtained. Omission of the medium exchange before infection has clearly simplified the process.

Key words: serum-free media, influenza vaccine, MDCK cells, wave bioreactor, microcarrier, stirred tank bioreactor, cell attachment, metabolites, bioprocess engineering, virus, roller bottles

1. INTRODUCTION

The switch from serum containing media to serum-free media in mammalian cell culture has become a major issue in the last years [1]. Especially for virus vaccine production processes such as influenza, where the infection phase has to be serum-free, even when cultivating the cells in serum containing media, a complete serum-free process has many advantages.

Here, we present the successful adaptation of an influenza vaccine production process from serum containing GMEM medium [2] to serum-free

R. Smith (ed.), Cell Technology for Cell Products, 563–565.

Ex-Cell MDCK medium for microcarrier systems (Cytodex 1). Cultivations in roller bottles, 2L-Wave and 5L-stirred tank bioreactor are compared.

2. MATERIALS AND METHODS

Madin-Darby canine kidney cells (MDCK) (ECACC No. 84121903) were cultivated in serum containing medium (SC) as described elsewhere [2]. For serum-free cultivation (SF) MDCK cells had been adapted to Ex-Cell MDCK medium (JRH Bioscience) supplemented with 2 mM glutamine (Sigma).

Cells were infected with either equine influenza A/Equi 2 (H3N8) Newmarket 1/93 or human influenza A/PR/38 (H1N1) (NIBSC) in GMEM medium without serum or in Ex-Cell MDCK medium both containing low levels of porcine trypsin (12.5 mg/L; Invitrogen) at 37°C. Virus seed was stored in aliquots of 1-10 mL (2.1-2.4 log HA units/100 µL) at -70 °C, thawed and added with moi of 0.1-0.025 based on plaque forming units of the virus seed. Cells were grown in roller bottles (250 mL wv) (Greiner) (start 1 x 10^5 cells/mL), 2L-Wave bioreactor (1 L wv) (Wave Biotech) or 5L-stirred tank bioreactor (STR) (start 2 x 10^5 cells/mL) (details see [2]) (B. Braun Biotech) on Cytodex 1 solid microcarriers (1.7-2 g/L) (GE Healthcare).

In all cultivation vessels the cells were grown to confluency after 4 days of cultivation. For serum containing cell growth the medium was removed and the remaining suspension was washed several times with PBS (without Ca^{2+}/Mg^{2+}) before virus medium addition and infection. In serum-free medium infection was directly without washing and medium exchange.

3. RESULTS AND DISCUSSION

After adaptation of MDCK cells to serum-free Ex-Cell medium (SF) roller bottle experiments were carried out to compare metabolite profiles and virus titers with results in serum containing medium (SC).

Further scale-up into a microcarrier process in a 5L-stirred tank bioreactor and a wave bioreactor was then tried. Here, the adhesion to the chosen Cytodex 1 carriers [2] was most problematic. Data on corresponding typical runs are summarized in Table 1.

Although SF medium contained higher starting glucose concentrations less glucose was consumed than in SC medium. Thus, no limiting concentrations even without washing steps and medium exchange before infection were obtained. Correspondingly, higher lactate concentrations were

reached in SC medium. In SC medium gln uptake after virus infection was about 0.7-1.0 mM compared to only 0.1 mM in SF medium. For the cell growth phase ammonia release correlated with the glutamine uptake. However, during virus infection the increase in ammonia did not correspond to the glutamine uptake. The glutamate starting concentration was clearly higher in SF than in SC medium. The glutamate profile for cell growth as seen in SC medium with a release phase and almost complete uptake after 50 h [2, 3] could not be observed in SF medium. Instead a steady increase of glutamate was found. After infection a stronger release of glutamate than during growth was observed directly after infection. Finally, virus titers and profiles were similar for all cultivations with 2.4-2.9 log HA units/100 µL. Only for the SF wave bioreactor low HA-values of 2.0 were found. This was probably due to difficulties in pH control, resulting in a final pH of 6.25. In SF medium for the overall process (growth + infection) lower glucose uptake and ammonia release was found in the wave bioreactor compared to the stirred tank bioreactor. This could result from additional viable cells (suspension) that were present in the supernatant of the stirred tank bioreactor (data not shown).

Table 1. Serum containing and serum-free influenza production in different cultivation vessels.

vessel	med.[a]	end[b]	moi	gln[c]	NH_3[c]	gluc[c]	lac[c]	glu[c]	HA[d]
RB	SC	n.a	0.1	-1.2/-0.7	+0.7/+1.1	-10.5/-6.4	+13.6/+12.5	-0.2/+0.5	2.6
RB	SF+	n.a	0.1	-1.1/-0.4	+1.0/+1.1	-5.3/-5.3	+10.6/+10.1	+0.2/+1.1	2.4
RB	SF	n.a	0.1	-1.1/-0.1	+1.1/+1.1	-3.5/-5.6	+10.3/+8.5	+0.2/+0.5	2.4
wave[e]	SC	2.8	0.05	-1.9/-1.0	+1.5/+1.7	-18.4/-13.3	+33.8/+24.8	-0.4/+0.8	2.6
wave[e]	SF	0.9	0.05	-1.5/+0.1	+1.7/+1.3	-13.0/-4.7	+20.3/+1.6	+0.5/+0.6	2.0
STR	SC	1.2	0.03	-1.6/-0.8	+1.6/+0.8	-23.2/-12.4	+38.3/+22.3	-0.3/+0.4	2.4
STR[f]	SF	1.3	0.05	-1.2/-0.1	+0.9/+0.8	-13.1/-11.8	+27.3/+20.8	+0.4/+0.3	2.9

amedium used; bend cell number on microcarriers (x 106 cells/mL); coverall consumed (-) or released (+) gln, NH3, gluc, lac & glu (mM) for cell growth phase/virus replication; dvirus titer in log HA units per 100 µL (4 days p.i.); ewave angle 7°, frequency 15 min-1, 2-5% CO2 mixed with air at 0.1 mL/min; fhere human influenza instead of equine; n.d.: not applicable; SF +: Ex-Cell cultivation with medium exchange.

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