Plant-derived peptones are a suitable replacement for Tryptone N1 (TN1) in recombinant protein expression using Human Embryonic Kidney (HEK293-6E) cells

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Introduction

Workflow

Cell density and viability during culture

Cell density and viability (Trypan-blue method) were measured before addition of peptone (Day 1 post transfection) and on day 4 and 5; an aliquot from each triplicate flask was taken out for cell density and viability measurement.

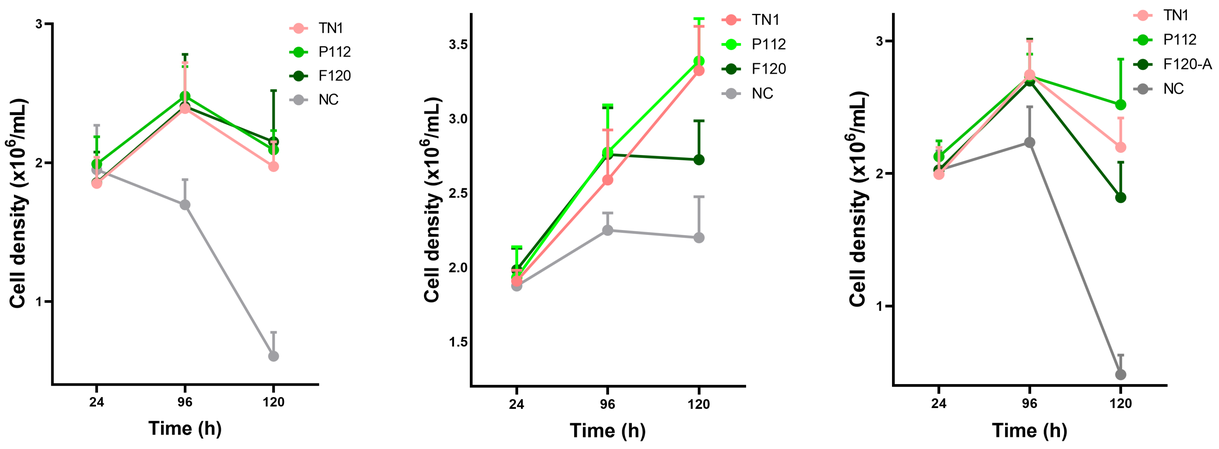

Fig. 2: Viable cell counts of HEK293-6E cells from 24 to 120 hours post transfection (hpt) in three independent experiments, each performed in triplicate, using the average of two cell counts for each sample at each time point.

Quantification of expression with Western blotting

Comparison of protein expression

Discussion and conclusion

Recombinant protein production depends on several culture and transfection parameters including cell density, DNA concentration, volume, temperature, harvesting time, serum addition or deprivation and nutrient supplementation. Supplementation with amino acids, glucose, or serum have been previously studied. Feeding of free amino acids has been shown to negatively affect the yield[1]. Although the serum supplementation has shown increased productivity, the high concentration of proteins in serum interferes with downstream purification of secreted proteins[2], making this a less suitable option. The addition of TN1 has been shown to increase the expressed protein and is considered a standard supplement. It has been suggested that peptones increase recombinant protein synthesis by stimulating gene expression mostly during the post-transfection phase, instead of perceived effect on increasing the efficiency of transfection. The effect of multiple feedings of TN1 has indicated that a single feed between 24-48 hpt is adequate, because additional feedings did not increase the expression of recombinant protein. Previously, it has been shown that TN1 does not increase growth, but increases the yield. This may be due the fact that synthetic and proliferative processes require different nutritional blocks[1] a similar phenomenon also observed by Franek and colleagues[3].

Here, we compared the effect of two novel plant-based peptones on expression of a tegumental protein from the eukaryotic parasite *Echinococcus granulosus* in HEK293-6E cells with the standard TN1 peptone. As opposed to common perception as a qualitative technique, Western Blotting can be utilised as a quantitative tool when a stringent workflow is followed[4]. In case of overloading the gels, densitometry is not directly proportional to increase in quantity[5]. Hence, we quantified the expression after determination of the linear dynamic range for protein loading by running a 2-fold serial dilution of pooled sample and using a broader linear range fluorescent antibody. After the necessary optimisation steps, we observed that the plant-derived peptones resulted in comparable yields of recombinant protein. We conclude that pea or broad bean-derived peptones, and possibly plant-derived peptones more broadly, are a suitable replacement for TN1 in HEK293 recombinant protein expression protocols.

References

1. Pham, P. L. et al. (2005): *Biotechnology and Bioengineering* 90 (3), pp. 332–344. DOI: 10.1002/bit.20428.
2. Butler, Michael (2005): *Appl Microbiol Biotechnol* 68 (3), pp. 283–291. DOI: 10.1007/s00253-005-1980-8.
3. Franek, F. et al. (2000): *Biotechnology Progress* 16 (5), pp. 688–692. DOI: 10.1021/bp0001011.
4. Pillai-Kastoori, L. et al (2020): *Analytical Biochemistry* 593, p. 113608. DOI: 10.1016/j.ab.2020.113608.
5. Taylor, S. C. et al. (2013): *Mol Biotechnol* 55 (3), pp. 217–226. DOI: 10.1007/s12033-013-9672-6.

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