OPTIMISATION OF PROTEIN PRODUCTION IN FREESTYLE 293-F CELLS WITH SIX PEPTONES

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ABSTRACT

The FreeStyle™ 293-F cells were utilized to optimize proDPPI expression using six different peptones, including plant-based peptones and animal-derived Tryptone N1.
The goal of the study was to evaluate the effectiveness of these peptones in producing the proDPPI-Twin-Strep-tag construct from FreeStyle™ 293-F cells, conducted in suspension flasks.
Results revealed that peptones derived from guar, soy, and pea proteins improved protein production by approximately eightfold compared to the control without added peptones. The plant-based peptones used were G115, S146B, and P112.

MATERIALS AND METHODS

EXPRESSION SYSTEM

- **Cells**: FreeStyle™293-F suspension cells
- **Vector**: pDSG-IBA104
- **Protein**: DPPI-Dipeptidyl-peptidase I

MEDIA COMPOSITION

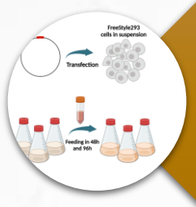
- FreeStyle 293 Expression Medium (12338026)
- 0.5% Peptones

C-CELL PEPTONES

- **Tryptone N1**(#19553) of animal origin)
- **C-CELL P112**, (#17112) and **P118**, (#17118) of pea origin
- **G115**, (#17115) of guar origin
- **S204**, (#17204) and **S146B** (#E0003) of soy origin

PRE-CULTURE

Before transfection, pre-culture of cells.



PROTEIN EXPRESSION

Transfection with pDSG-IBA104 plasmid.

Feeding with 0,5% peptones at 48h and 96 h.

Sample collection at 120h.

SDS-PAGE AND WB

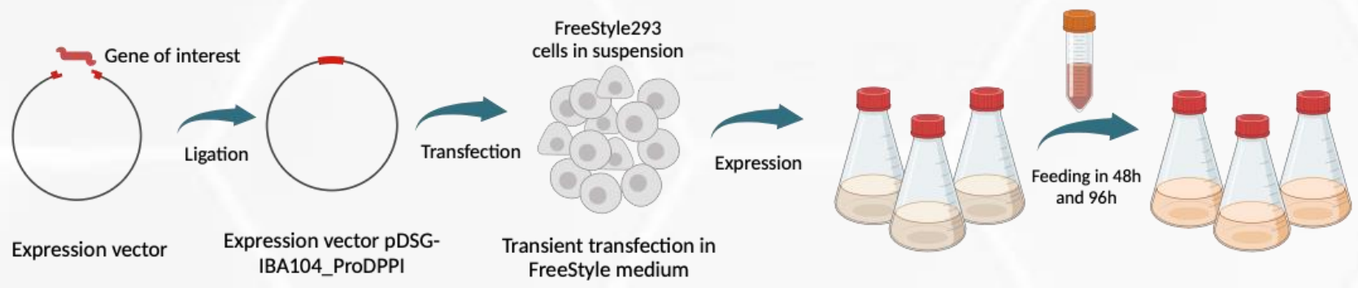
Coomassie staining;

Western blot, **HRP-streptavidin antibody.**

QUANTIFICATION

Quantification of pro DPPI in the conditioned medium from Western blot by using Bio-Rad Image lab 6.1

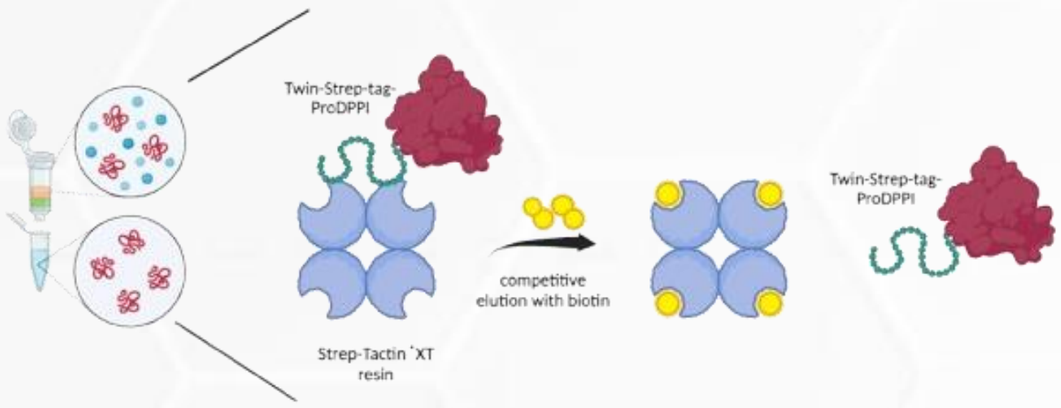
INTRODUCTION



Producing proteins in the right quantity and quality is a crucial need in modern times. The use of mammalian cells for protein production has notably increased due to their ability to ensure proper protein folding, post-translational modifications, and product assembly, all of which are vital for full biological activity. The ultimate goal of process development in animal cell culture is to increase product quality and yield while reducing cost.

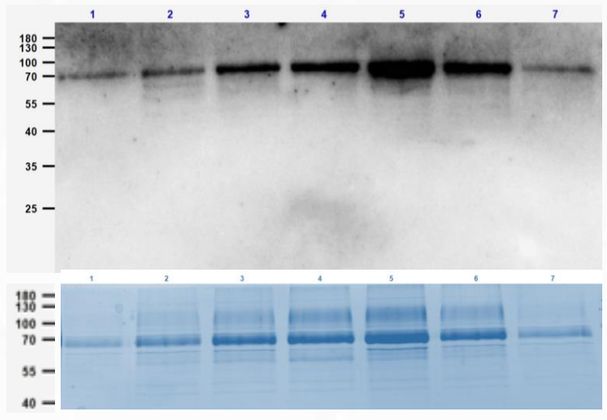
Serum is essential for cell growth but is costly, inconsistent, and poses contamination risks. To address this, plant-based hydrolysates, rich in amino acids, vitamins, and peptides, are gaining popularity as serum alternatives. They not only support cell growth but also improve protein quality and reduce production costs. Overall, hydrolysates offer a promising solution to replace animal serum in cell culture.

In our study, we expressed the proDPPI protein from FreeStyle™ 293-F cells five differet plant-based protein hydrolysates. To enhance protein solubility and purification efficiency, we employed the Twin-Strep-tag for this experiment.



RESULTS

Protein expression detection by SDS-PAGE and Western-blot analysis



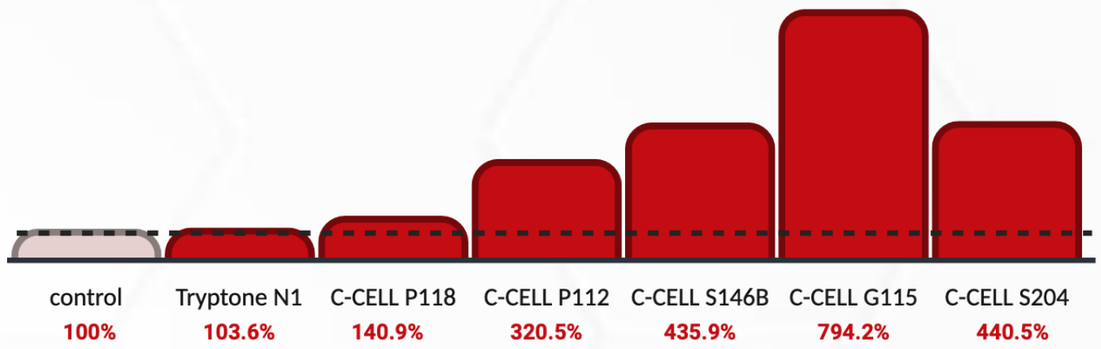
Western blot, HRP-streptavidin antibody

Coomassie staining

Line	Peptone 0,5%	Expression volume/ mL	Cell density/ cells/mL	A280 nm	Mass [µg/mL]*
1	Tryptone N1	25	1,25 x10 ^{6}	0,060	36
2	**C-CELL** P118	25	1,25 x10 ^{6}	0,140	83
3	**C-CELL** P112	25	1,25 x10 ^{6}	0,200	119
4	**C-CELL** S146B	25	1,3 x10 ^{6}	0,245	145
5	**C-CELL** G115	25	1,3 x10 ^{6}	0,250	148
6	**C-CELL** S204	25	1,1 x10 ^{6}	0,160	95
7	Control	25	1,25 x10 ^{6}	0,043	25

*The mass was calculated from the measured absorbance at 280 nm on NanoDrop and the amino acid sequence of the protein with the tags.

Quantification of pro-DPPI in the conditioned medium from Western-blot



DISCUSSION AND CONCLUSIONS

A proDPPI expression study was conducted to assess the ability of various peptones to enhance protein production yields. Different peptones were compared to standard expression without supplementation, with the protein secreted into the culture medium. The results indicated that plant-derived peptones significantly boosted proDPPI secretion compared to the control.

After purification via affinity chromatography, protein quantification was done using absorbance at 280 nm, and Western blot provided semi-quantitative analysis. Peptones enhanced the protein production by approximately eightfold compared to the control, particularly C-CELL G115, C-CELL S146B and P112 also showed more significant increase in protein production. All six peptones dissolved easily in a preheated expression media.

ACKNOWLEDGEMENTS

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