Schmidt A.[1*], Steffens T.[1], Schierbaum I.[1], Schilling M.[2], Heinrich C.[1]

1 Xell AG, Bielefeld, Germany | www.xell.ag
2 Evonik Nutrition & Care GmbH, Darmstadt Germany
* presenting author | Anica.Schmidt@xell.ag

Novel Dipeptides For Cell Culture Media and Their Cellular Response

In this work, effects of replacing more challenging amino acids by dipeptides on cell growth, productivity and cellular response are evaluated. High performance media for efficient biopharma processes need to be closely adjusted to cells' needs. Thereby, nutrients must not only be available in sufficient amounts but also stable and easy to use in media preparation, storage and cultivation. This is of special relevance for slightly soluble or instable amino acids. While glutamine dipeptides have been applied for decades, tyrosine or cysteine dipeptides are rarely available. Moreover, there may be more suitable carriers than in presently established dipeptides.

RESULTS

A. Impact of different dipeptides on cell growth & productivity

Tyrosine-containing Dipeptides

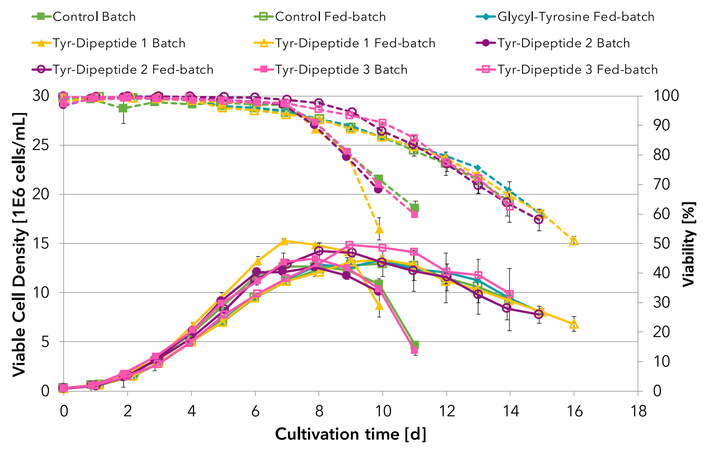

FIG. 1: Viable cell densities (solid lines) and viabilities (dotted lines) of control cultures containing only free tyrosine (green) and respective best perfoming fed-batch runs with different tyrosine-containing dipeptides either in the medium (batches, filled symbols) or the feeding supplements (fed-batches, blank symbols).

Cysteine-containing Dipeptides

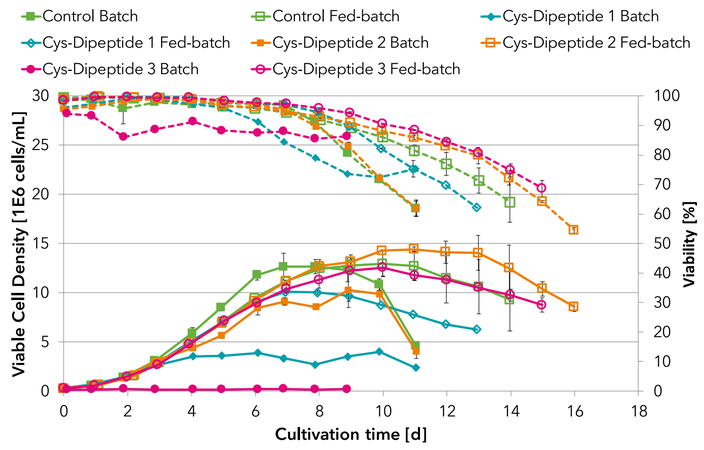

FIG. 2: Viable cell densities (solid lines) and viabilities (dotted lines) of control cultures containing only free cysteine (green) and respective best perfoming fed-batch runs with different cysteine-containing dipeptides either in the medium (batches, filled symbols) or the feeding supplements (fed-batches, blank symbols).

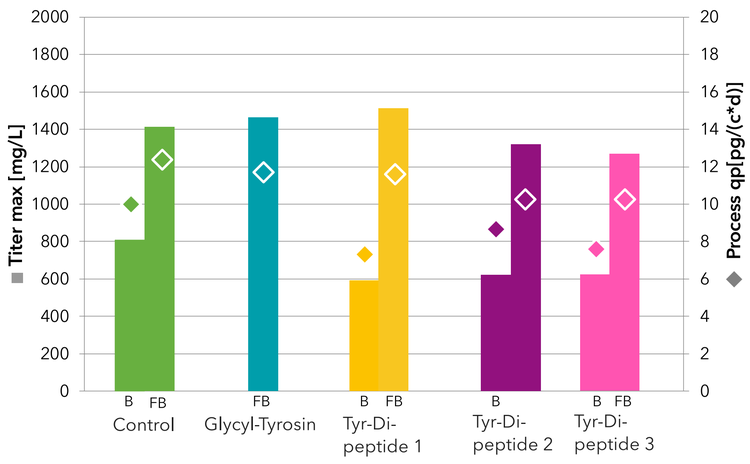

FIG. 3: Final mAb titers (bars) and process productivities (qP, rhombs) achieved in initial CHO GS batches and consecutive fed-batch cultivations with free tyrosine (control, green), newly evaluated tyrosine-containing dipeptides or the commercially available dipeptide glycyl-tyrosin. Final titers could be enhance by switching from batch to fed-batch mode as well as by replacing free tyrosine by a dipeptide.

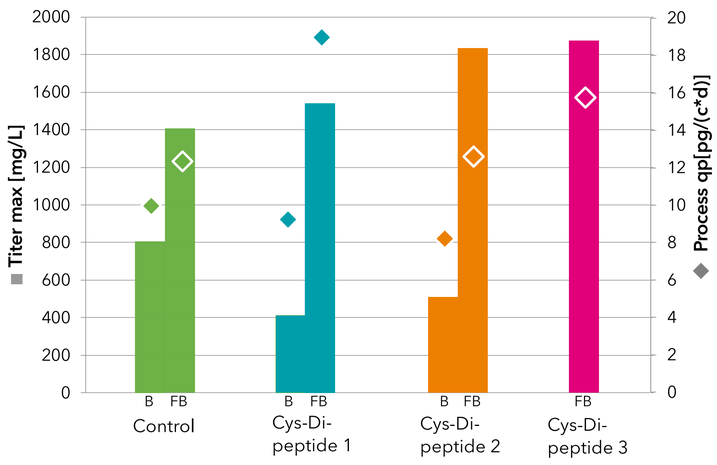

FIG. 4: Final mAb titers (bars) and process productivities (qP, rhombs) achieved in initial CHO GS batches and consecutive fed-batch cultivations with free cystein (control, green) and newly evaluated cysteine-containing dipeptides. Both, final titers and process qP, could be enhanced by replacing free cysteine through dipeptids in respective feeding supplements. Hereby, titers could be increased up to 1870 mg/L (Cys-Dipeptide 3) and process qP up to 18.9 pg/(cell*d) (Cys-Dipeptide 1).

B. Transcriptomic analyses after different dipeptide supplementations

EC	Name	Gen
3.4.13.9	Xaa-Pro dipeptidase	Pepd
3.4.13.19	membrane dipeptidase	Dpep1
3.4.15.5	peptidyl-dipeptidase A	Ace3
3.5.1.1	isoaspartyl dipeptidase	Asrgl1
3.4.13.20	carnosine dipeptidase I	Cndp1
3.4.13.18	carnosine dipeptidase II	Cndp2
3.4.17.21	glutamate carboxypeptidase II	LOC100769032
3.4.13.19	Carboxpeptidas Q	Cpq
3.4.11.9	Xaa-Pro aminopeptidase	Xpnpep1
3.4.13.19	membrane-bound dipeptidase-2	Dpep2
3.4.13.19	dipeptidase 3	Dpep3
3.4.11.2	aminopeptidase N	Anpep

FIG. 5: Overview of assorted potentially di- and tripeptidases, which might be differentially expressed under dipeptide feeding compared to the use of free amino acids. Enzymes were identified via literature and database search and chosen based on their general expression rates in CHO cells.

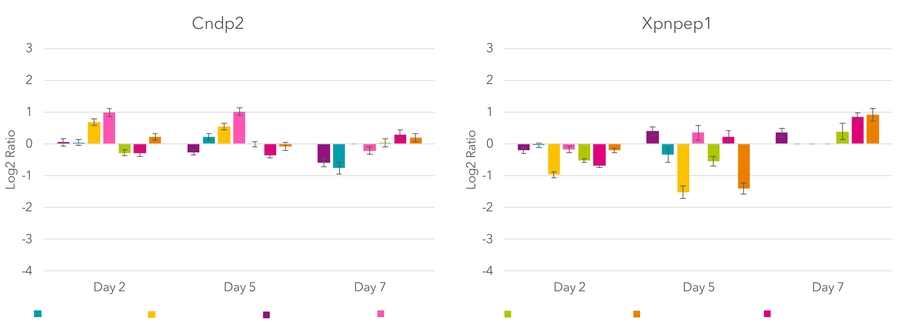

FIG. 6: Exemplary log 2 rations for the evaluated enzymes Carnosine dipeptidase II (Cndp2), a nonspecific cytosolic dipeptidase, and Xaa-Pro aminopeptidase (Xpnpep1). Although minor changes for the respective dipeptidases under dipeptide treatment as well as over time course of cultivation were detectable, transcriptomic effects need to be further analyzed. Therefore, RNA sequencing experiments will be carried out.

METHODS

Cultivation An antibody-producing CHO GS cell line was cultivated in plain shake flasks using standard conditions in a proprietary GS medium. Duplicate cultivations were carried our either in batch or fed-batch mode, where selected amino acids were replaced by different dipeptides at equimolar concentrations in proprietary feeding supplements. Appropriate volumes were added once per day from day 3 on.

Analytics Viable cell density and viability were measured daily using a Cedex automated cell counter. Antibody yield was measured by Protein A HPLC and amino acid profiles were determined via UPLC.

Transcriptomic analyses To test for relative expression levels of di- and tripeptidases, qRT-PCR analyses were performed. RNA from cell samples on cultivation days 2, 5 and 7 were extracted using Trizol (Invitrogen) and qRT-PCR was performed using the SensiMix SYBR (Bioline). Gapdh, Eif3i and Vezt were used as reference genes.

CONCLUSIONS

- Slightly soluble and instable amino acids could be replaced by their respective dipeptides and thus process duration and maximum VCD could be enhanced by improved nutrient concentrations.
- For some dipeptides, solubility could be increased by more than 100x compared to cystine (data not shown).
- Final titers and process qP of an antibody producing CHO GS cell line could be enhanced in fed-batch processes by replacing poorly soluble amino acids with newly developed dipeptides.
- Amino acid profiles of cultivations with tyrosine- and cysteine-containing dipeptides revealed a release of the respective amino acid from used dipeptides thus indicating the ability of CHO cells to metabolize these dipeptides (data not shown).
- Higher solubility of dipeptides can have additional benefits compared to their free amino acids. Process intensification can be achieved by more concentrated feeding solutions and formation of precipitates can be prevented resulting in increased process robustness.
- More than 900 potential enzymes with peptidase activity in *Cricetulus griseus* or CHO K1 could be identified via extensive data base search.
- Investigated di- and tripeptidases were chosen based on their comparatively high expression rates in CHO cells.
- Changes of dipeptidase transcriptional activities under dipeptide feeding were detectable, but cellular response need to be further investigated.
- For more in-depth investigation of differentially expressed di- and tripeptidases under dipeptide feeding, RNA sequencing experiments will be performed.

PARTNERS & ACKNOWLEDGEMENTS

We would like to thank the German Federal Ministry of Education & Research (BMBF # 031L0077D DipImpact) for funding.

SPONSORED BY THE
Federal Ministry of Education and Research
EVONIK POWER TO CREATE
10 Years Xcell