A MUTANT γ-GLUTAMYLTRANSFERASE WITH IMPROVED TRANSPEPTIDASE ACTIVITY

Michela Massone[1], Cinzia Calvio[2], Alessandra M. Albertini[2], Giovanna Speranza[1], Carlo F. Morelli[1]

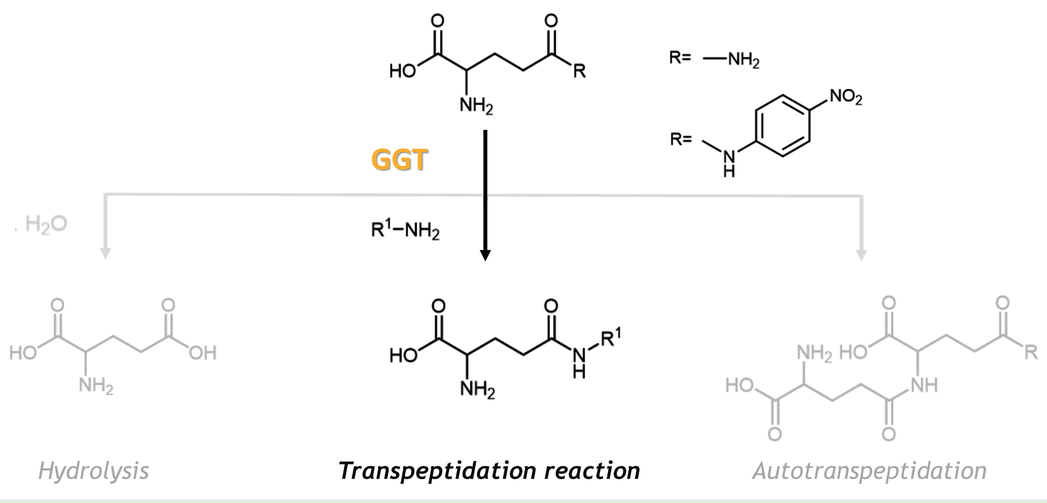
[1]Università degli Studi di Milano Dipartimento di Chimica, via Golgi, 19 – 20133 Milano

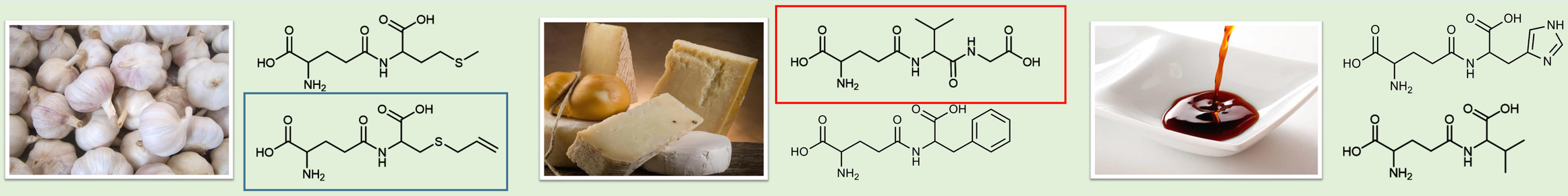
[2]Università degli Studi di Pavia Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", via Ferrata, 9 – 27100 Pavia

michela.massone@unimi.it

TAILGLUTRAN

Fondazione CARIPLO



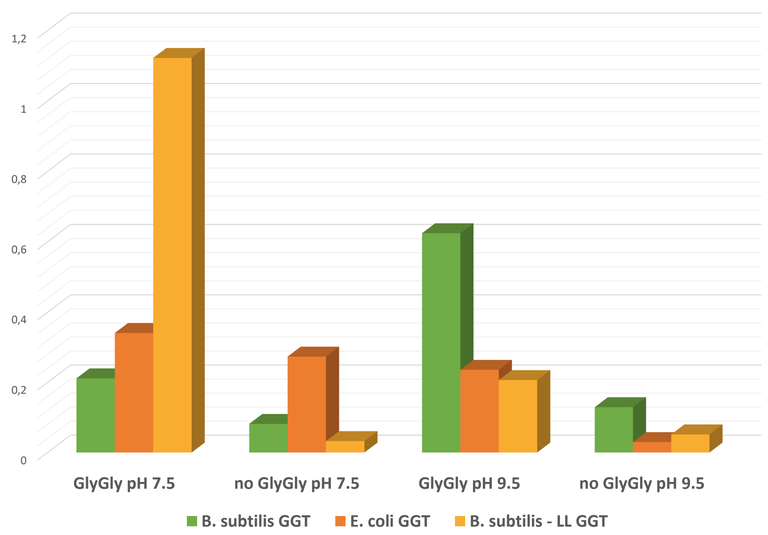


γ-Glutamyl derivatives of both unmodified and modified amino acids are naturally occurring flavor enhancers found in many foods such as cheese, garlic, mushrooms and fermented sauces, as soy sauce, used in cuisine worldwide[1]. Despite their potential applicative, they are commercially underexploited compounds due to the difficulties connected with their supply at a reasonable cost. Enzymatic approaches to their preparation based on the use of γ-glutamyltransferases (GGTs) as biocatalysts have been proposed[2] to circumvent both the low-yielding extractive procedures from natural sources and the uneconomical chemical synthesis. However, also the use of GGTs is not free from drawbacks because of the hydrolase activity towards both the donor substrate and the newly formed transpeptidation product, affording irreversibly glutamic acid[3].

AIM and RESULTS

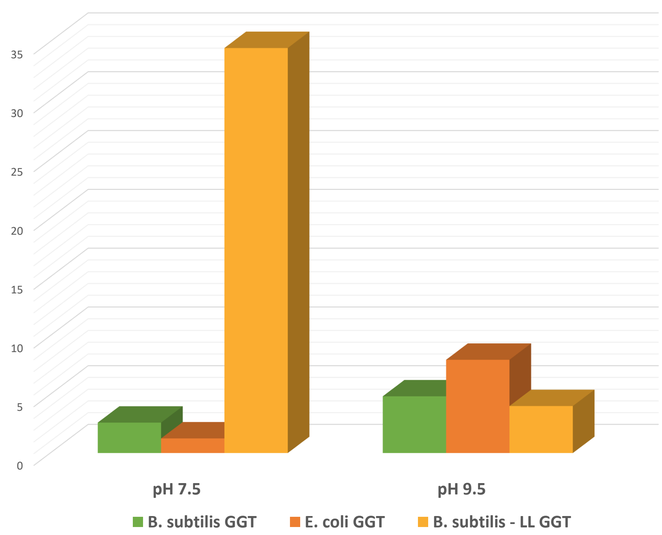
Development of mutant GGTs with improved transpeptidase activity, to be used as biocatalysts in the synthesis of γ-glutamyl derivatives of commercial interest.

Sequence alignment of *B. subtilis* – LL GGT, *B. subtilis* GGT and *E. coli* GGT, showing the insertion of the «Lid Loop» sequence which covers the *E. coli* GGT's binding site, into the structure of *B. subtilis* GGT, which lacks it, to give the **mutant *B. subtilis* – LL GGT**.[4,5]

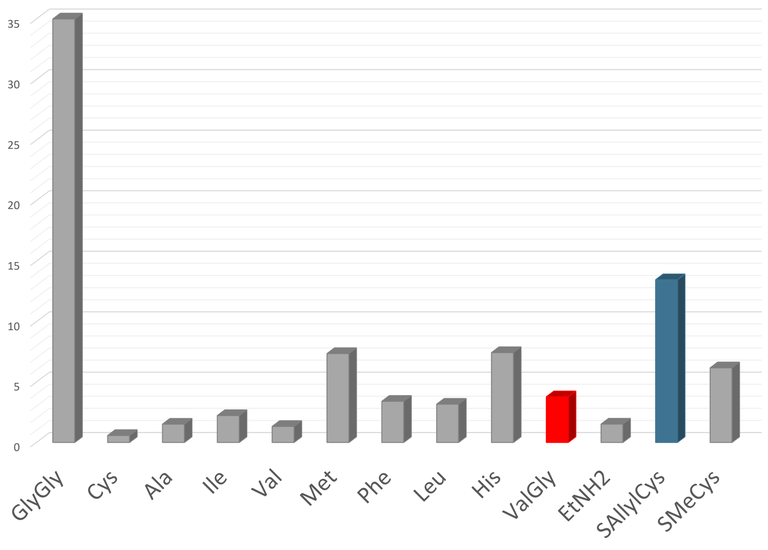


Comparison between activities (μmol s^{-1} ml^{-1}) of *B. subtilis* GGT, *E. coli* GGT and *B. subtilis* – LL GGT in the absence and presence of glycylglycine as acceptor, at two different pHs.

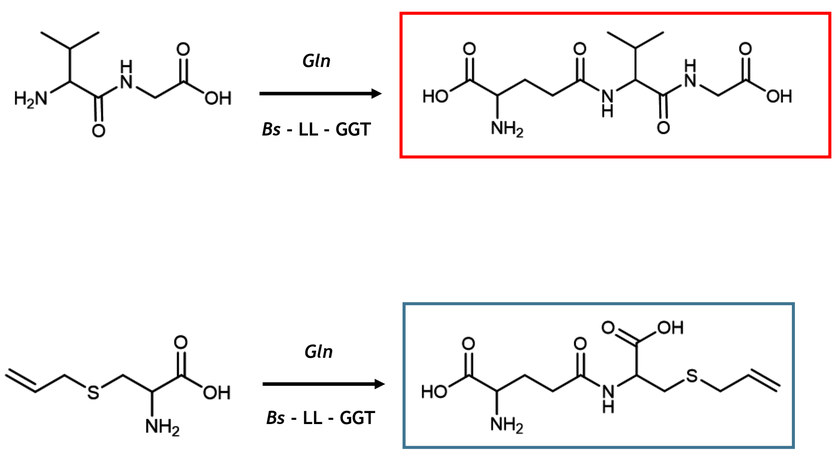
Mutant *B. subtilis* – LL GGT shows an improved transpeptidase activity at a pH close to neutrality.



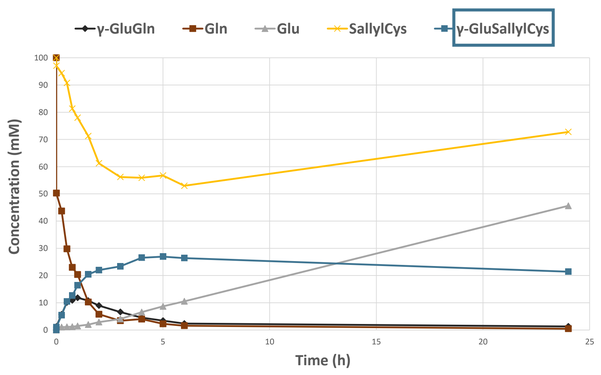
Ratio between transpeptidase and hydrolase activities of *B. subtilis* GGT, *E. coli* GGT and *B. subtilis* – LL GGT at two different pHs.



Substrate specificity of mutant *B. subtilis* – LL GGT towards different acceptors at pH 8.5.



32% not optimized yield.



[1] Zhao, C. J.; Schieber, A.; Ganzle, M.G. *Food Res. Int. 2016, 89, 39-47.*
[2] Speranza, G.; Morelli, C. F. *J. Mol. Catal. B: enzymatic*. 2012, *84*, 65-71.
[3] Morelli, C. F.; Calvio, C.; Biagiotti, M.; Speranza, G. *FEBS J.* 2014, *281*-232-245.
[4] Calvio, C.; Romagnuolo, F.; Vulcano, F.; Speranza, G.; Morelli, C.F. *Enzyme Microb. Technol. 2018, 114, 55-62.*
[5] The **TailGluTran Project** is founded by Fondazione Cariplo, Bando 2016 sulle Biotecnologie Industriali e la Bioeconomia, No 2016-0741.