Trends in Peptide and Protein Sciences
Volume 6 (2021): e5

DOI: http://dx.doi.org/10.22037/tpps.v6i0.35579

Original Article

Isolated Metallopeptidase from *Lactobacillus casei*: A Computational Study

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Article history:
Received: 24 July 2021
Accepted: 24 August 2021

Keywords:
3D structure
Molecular modeling
Lactobacillus casei
Metallopeptidase
RNA folding

HIGHLIGHTS

- Metallopeptidase is a stable protein with suitable RNA folding.
- It has proper half-life for production in prokaryotic and eukaryotic systems.
- The amino acids R217, G63, K64, F27 and G58 in active site participate in the reaction with the substrate.
- Predicted structure of bacterial metallopeptidase has 68% coverage with human protease.

ABSTRACT

One of the common studies on industrial proteins is to study the RNA Folding and stability, protein stability, physicochemical properties and conformational structure. In our previous proteomics study, Metallopeptidase (MP) was isolated from *Lactobacillus casei,* which in this study, it was evaluated with RNA fold, Protparam, I-TASSER and Phyre2 web servers. The results showed that it fairly has a suitable RNA folding in terms of minimum energy. In addition, Phyre2 could model the protein with 100% confidence and 68% coverage with human protease. Secondary structure of MP contains 45% alpha helix and 8% beta strand, 31% Disordered and 3% TM helix. The estimated TM-score for final predicted MP model was 0.38±0.13 and calculated RMSD was 15.9±3.2Aº. Phyre2 investigator showed that R217, G63, K64, F27 and G58 in active site participate in the reaction with the substrate. In conclusion, predicted structure of bacterial metallopeptidase in acidic conditions has 68% coverage with human protease.

Cite this article as: Dadfarma, N., Bandehpour, M., Nowroozi, J. and B. Kazemi, (2021). Isolated metallopeptidase from *Lactobacillus casei*: A computational study. *Trends Pept. Protein Sci.*, ***6***: e5.

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Introduction

Lactobacillus casei is one of the acid tolerant bacterium used as probiotic supplements (Wu et al., 2011). They were confronted with a strongly acidic environment in

the stomach during the transition into the intestine (Cotter et al., 2003). One of the *L. casei* critical metabolites is an ATP dependent metallopeptidase. We isolated this enzyme (FtsH) from acidic culture of *L.casei* through proteomics studies (Dadfarma et al., 2020). FtsH is a metalloprotease that is associate with ATP-dependent zinc for cytoplasmic and membrane proteins. A large number of earlier investigations studied this protein in *Escherichia coli*. They reported that this protein plays role in many biological processes, including quality control of proteins by degrading out-of-membrane proteins, like the subunit alpha of the F1F0 ATP synthase complex and the type 2 secretion system translocon protein SecY. It also involves in changing between lysis and lysogeny upon bacteriophage l infection, as well as, in LPS biosynthesis, and cell division (Kamal et al., 2019). FtsH is a homohexamer in the inner membrane. When a substrate degrades in the proteolytic chamber of the protease, FtsH uses its ATPase to unfold and translocate it. Therefore, FtsH separates misfolded and proper folded proteins due to quality control or regulatory reasons, respectively. FtsH is considered as a key protein of *E. coli* in quality control and other related processes (Biswas et al., 2019).

Metallopeptidase (MP) activity is the catalysis of the peptide bonds hydrolysis by a mechanism in which water acts as a nucleophile, one or two metal ions hold the water molecule in place, and charged amino acid side chains are the ligands for the metal ions. The reports have indicated that metallopeptidase produced from the acidic metabolism in *L. casei* is most likely involved in the probiotic effects described for this bacterium. Our previous study showed that endogenous recombinant MP under colon specific promoter could inhibit the proliferation of SW480 colorectal cancer cells by increasing the MAP2K1 and P53 activation (Dadfarma et al.,2021). Nevertheless, *L. casei* metallopeptidase under the same circumstances could not affect the growth rate and viability of MDA-MB231 breast cancer cells *in vitro*.

Following our previous studies about metallopeptidase of *L.casei,* as a probiotic bacterium, we specifically analyzed the RNA stability, 3D structure and molecular modeling of the protein in acidic condition, here.

Materials and Methods

Protein sequence retrieval

The metallopeptidase protein introduced from our previous study of *L. casei* proteomics analysis (Dadfarma et al., 2020), MP with significant MOWSE score in mass spectroscopy, was considered for the following analysis steps.

Metallopeptidase physicochemical properties

The ProtParam server (https://web.expasy.org/protparam/) (Gasteiger et al., 2005) was applied for the analysis of the protein physicochemical parameters. These parameters include molecular weight, instability index, aliphatic index, theoretical pI, amino acid composition, and *in vitro* and *in vivo* half-life.

Metallopeptidase RNA analysis

The RNAfold web server predicted the secondary structures of metallopeptidase single stranded RNA (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi?PAGE=3&ID=sZSyzLGXM2).

Metallopeptidase secondary structure

Secondary structure and homology prediction of the MP protein was performed using the I-TASSER server (Yang et al., 2015) during job id number S631621 at https://zhanglab.ccmb.med.umich.edu/I-TASSER/. For the target, the I-TASSER simulations introduce large similar 3D conformations, called decoys. To report the final models, the I-TASSER uses the SPICKER program to categorize all the decoys based on the pairwise structure similarity and clusters up to five models. The validity of each model is quantitatively measured by the C-score that is estimated based on the significance of threading template alignments and the convergence parameters of the similar assemble structures.

Metallopeptidase tertiary structure

The tertiary structure of the MP protein was predicted using the Phyre2 server (Kelley et al., 2015) (http://www.ebi.ac.uk/). The assessment of the 3D structure of the protein was also performed by using the I-TASSER server.

Metallopeptidase- substrate interaction study

For characterization of the best interaction force between refined MP and a random sequence peptide from Phyre2 investigator (described in results), the model quality assessment by ProQ221 and catalytic site detection from the CSA24 were carried out for Metallopeptidase protein sequence. This server was used for automatically estimating the direct protein substrate interaction.

Table 1. Physicochemical properties of the isolated metallopeptidase

Number of amino acids	Molecular weight Da	Theoretical pI	Extinction coefficients M^{-1} cm^{-1}	Estimated half-life	Instability index	Aliphatic index
706	76941.05	5.86	35870	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli,* in vivo)	35.20 stable	79.94

Da, Dalton; pI, pH Isoelectric

Results and Discussion

The isolated metallopeptidase protein produced in acidic condition from *Lactobacillus casei*, was analyzed by computational methods, as the prerequisite studies for its industrial production.

Metallopeptidase RNA folding

RNA folding was predicted using RNAfold 2.4.18. A minimum free energy of -966.40 kcal/mol was calculated for optimal secondary RNA structure and the free energy of the thermodynamic ensemble was -993.22 kcal/mol. The frequency of the MFE structure in the ensemble is 0.00 %. The ensemble diversity is 527.58. A minimum free energy of -792.30 kcal/mol was obtained for the centroid secondary structure. These data confirmed suitable folding of RNA (Mathews et al., 2004; Gruber et al., 2008).

Metallopeptidase physicochemical study

According to the physicochemical features calculated for MP (Table 1), it can be concluded that this protein is a stable and acidic molecule with 706 amino acids.

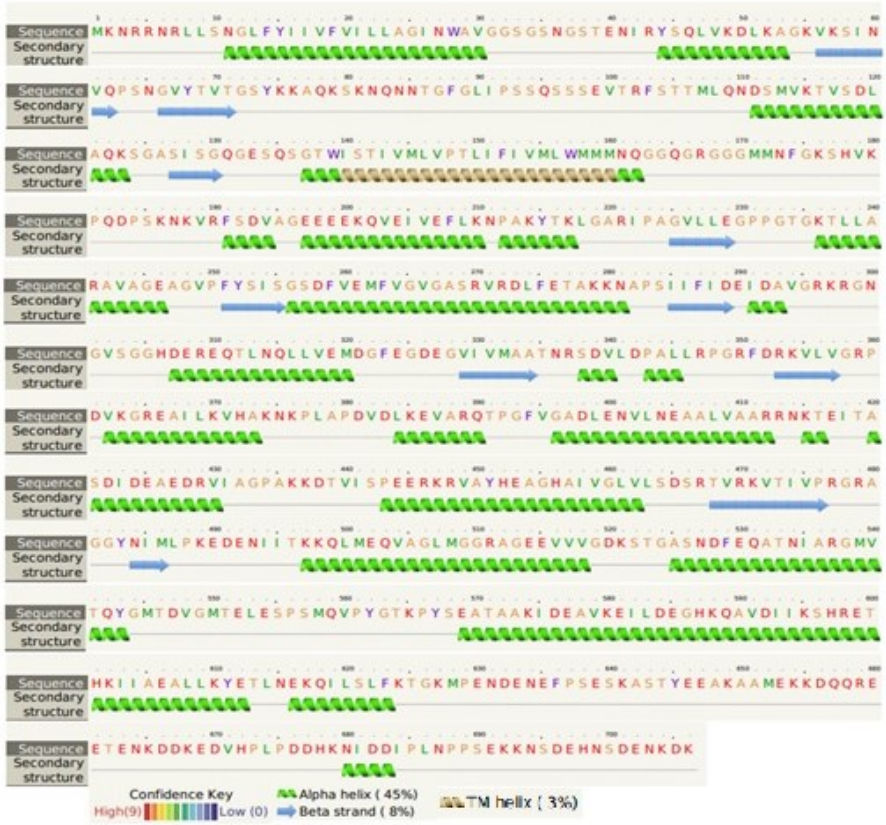

Figure 1. Secondary structure of MP protein based on Phyre2 analysis. It has 45% alpha helix and 8% beta strand, 31% Disordered and 3% TM helix.

Metallopeptidase secondary and tertiary structure

The secondary structure of MP protein with 568 aa, among 706 aa, was evaluated and identified by Phyre2 (Fig. 1) and the Expasy Protparam tool was applied for the analysis of protein physicochemical properties. Its molecular weight and PI values were 76941.05Da and 5.86, respectively. The total number of negatively (Asp+Glu) and positively (Arg+Lys) charged residues were 102 and 93, respectively. The estimated half-life of the protein is 30 hours (mammalian reticulocytes, *in vitro*), >20 hours (yeast, *in vivo*) and >10 hours (*Escherichia coli*, *in vivo*). It was considered as a stable protein due the instability index, which was estimated to be 35.20. The aliphatic index was 79.94, and the Grand average of hydropathicity (GRAVY) was -0.501. Based on the respective volume covered by aliphatic side chains (Ala, Val, ILeu, and Leu), it might be regarded as a thermostable protein.

We used the Phrye2 web server for generating a 3D model of MP protein sequence (Fig. 2) that it was carried out in 4 technical stages, 1) Gathering homologous sequences that captures the residue preferences at each position along its length using PSI-Blast14; 2) Fold library scanning of the predicted secondary structures in step1 and converting to a hidden Markov model. The alignment algorithm used in Phyre2 is HHsearch10; 3) Loop modelling using a library of fragments of known protein structures from lengths of 2-15 aa; 4) Sidechain placement is performed using the R3 protocol20 that involves a fast graph-based technique (Kelley et al., 2015).

Fig. 3 shows the predicted normalized B-factor; in which, negative values means the residue is relatively more stable in the structure. B-factor is a value to indicate the extent of the inherent thermal mobility of residues/atoms in proteins, which in I-TASSER, this value is deduced from threading template proteins from the PDB in combination with the sequence profiles derived from sequence reported B-factor profile that here, it corresponds to the normalized B-factor of the MP protein.

For MP protein, I-TASSER simulations could generate a large ensemble of structural conformations. To select the final models, it used the SPIKER program to cluster all of models based on the pair wise structure similarity. The estimated TM-score for final predicted MP model was 0.38±0.13, C-score was -3.17 and calculated RMSD was 15.9±3.2Aº.

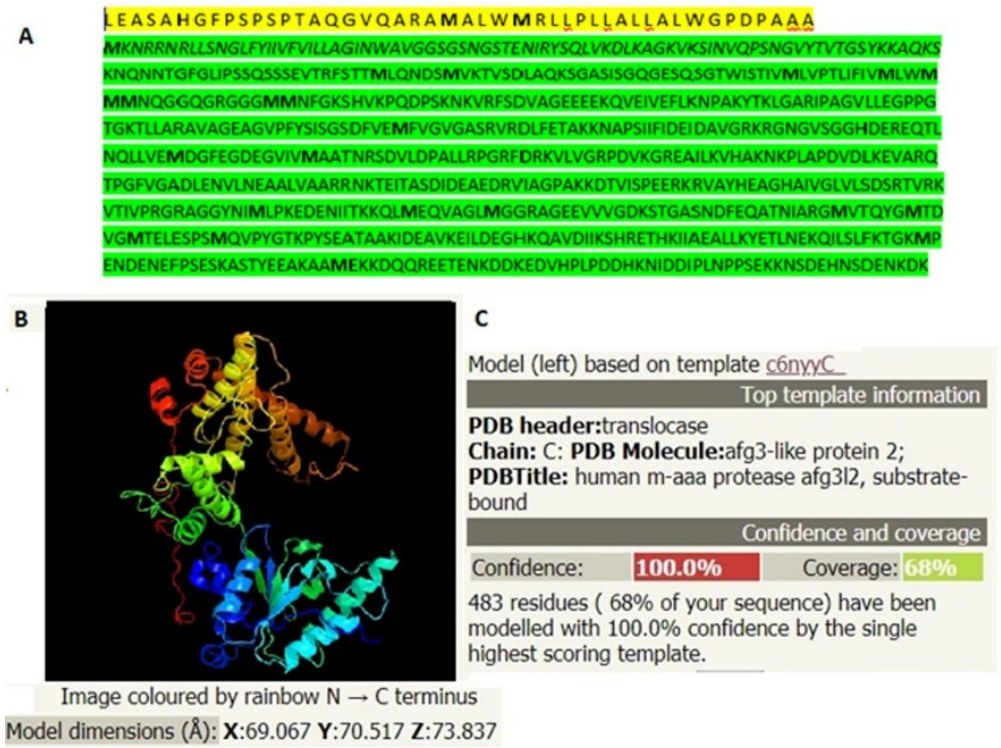

Figure 2. Schematic representation of the MP protein. A) Sequence of MP protein is shown; B) The Phyre2 protein model with 100% confidence and 68% coverage with human protease (C).

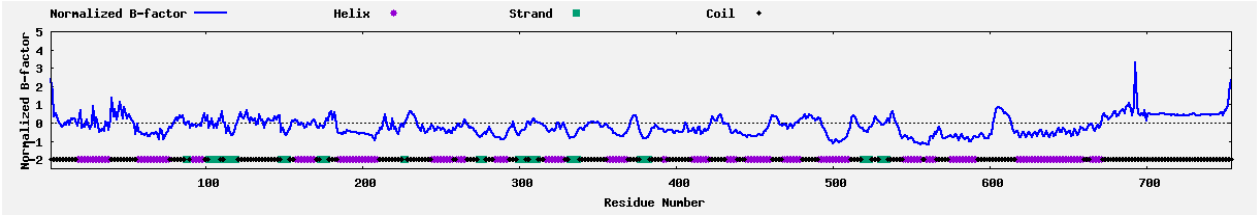

Figure 3. Predicted MP protein normalized B-factor**.** Negative values means the residue is relatively more stable in the structure.

Given a confident model produced by Phyre2, it is often desirable to perform more in-depth analyses of model quality, potential function and the effects of mutations. Model quality assessment by ProQ221 and catalytic site detection from the CSA24 were carried out for metallopeptidase protein sequence, which were presented in Fig. 4.

Fig. 4 shows Phyre2 investigator output in which R217, G63, K64, F27 and G58 amino acids in active site are identified to have interaction with the substrate of metallopeptidase enzyme.

Figure 4. Interaction between metallopeptidase (green color) and peptide (red color) by Phyre2 investigator. R217, G63, K64, F27 and G58 are shown in active site. Predicted active site residues are shown in purple.

Conclusion

Our *in silico* study on the metallopeptidase protein isolated from acidic condition culture showed that it is a stable and soluble protein with acceptable predicted 3D structure, predicted by I-TASSER and Phyre2 investigator. In sequence, analysis by RNA folding webserver showed that it has a proper RNA folding for production in prokaryotic and eukaryotic hosts.

Ethical Statement

This study does not involve human subjects and/or animals study.

Acknowledgement

This research is a part of student's thesis. The authors would like to thank the staff of Cellular and Molecular Research Center in Shahid Beheshti University of Medical Sciences.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

This study was funded by the Vice-Chancellor of Research, Shahid Beheshti University of Medical Sciences, Tehran, Iran (grant no. 12747).

Authors Contribution

Mojgan Bandehpour conceived and designed the study conception. Narges Dadfarma performed the experiments and analyzed the results. Mojgan Bandehpour, Narges Dadfarma and Jamileh Nowroozi prepared the draft manuscript. Mojgan Bandehpour supervised, revised and edited of the article. Bahram Kazemi scientifically consulted the work and finally, approved the manuscript.

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