Archives of Nutrition and Food Science

Park et al., Arch Nutr Food Sci.
2024;2(1):1-3

Commentary

Further comments on the single-cell protein ingredient manufactured from a non-GMO *Corynebacterium glutamicum* as an alternative protein nutrition

Sehyeon Park[1], Seungki Lee[2], Taeyeon Kim[1], Ahyoung Choi[2], Soyeon Lee[1], Pil Kim[1,2,*]

[1]Research Group of Novel Food Ingredients for Alternative Proteins, the Catholic University of Korea, Bucheon, Gyeonggi 14662, South Korea

[2]Dept. of Biotechnology, the Catholic University of Korea, Bucheon, Gyeonggi 14662, South Korea

*Author for correspondence:
Email: kimp@catholic.ac.kr

Received date: August 19, 2024
Accepted date: October 25, 2024

Commentary

In the previous study, Park *et al*. reported the efficacy of a single-cell protein manufactured from a growth-accelerated high-vital *Corynebacterium glutamicum* strain and potential strain selection strategy for development of non-GMO industrial strains harboring enriched target nutritional component, named as growth-acceleration-targeted evolution (G.A.T.E.) strategy [1]. This commentary manuscript provides further comments and discussions on the nutritional properties of the high-vital *C. glutamicum* protein (heme-SCP), plausible defects of G.A.T.E strategy and the possible overcome ways, the G.A.T.E. strategy in the viewpoint of regulation, and the differences of mouse gut microbiota by the supplementation of hemin and the heme-single-cell protein (SCP).

In the viewpoint of nutrition, protein extracts or biomasses from edible microbial cell cultures have high protein quantity (40-60%), one of nutritional factors of food ingredient. Amino acid score (AAS) is another factor that determine protein quality indicating the completeness of protein by their unique composition of essential amino acids, and a protein ingredient will meet all amino acid needs of the human body [2]. A food having AAS of 100 is considered as high-quality protein that does not require additional nutrition. The AAS of the *C. glutamicum* heme-SCP that has led the lowering blood lipids of mouse in the focal paper were as follows: His 115; Ile 96; Leu 92; Lys 111; SAA (sulfur containing AA) 135; AAA (aromatic AA) 154; Thr 144; Trp 132; Val 100 **(Table 1)**. Compared with the SCPs from yeast, spirulina, and chlorella [3-6], the *Corynebacterium glutamicum* heme-SCP in the focal paper is thought to be a good ingredient supplying a balanced amino acid for human need **(Table 1)**. Additionally, by using the target component responding promoters in the G.A.T.E. strategy of the focal paper, one would be able to isolate a natural mutant strain that enable us to manufacture a SCP that is fortified a specific nutritional component.

Though the G.A.T.E. strategy of the focal paper could evolve strains more effectively, design and construction of a proper-working genetic algorithm (combinations of a target component responding promoters and growth-stimulating genes) still requires much effort considering the complexity of microbial physiology. This plausible limitation could be overcome by introducing a biofoundry facility that combines artificial intelligence (AI) and robotics [8], which would allow to build optimal genetic algorithm combinations and evolution protocols even more efficient with less research labor. Even though the G.A.T.E. strategy does not perform artificial genetic manipulation, the possibility that plasmid DNA is inserted into the strain's genome during evolution cannot be ruled out. In addition, even if no foreign DNA is observed in the genome of the final mutant strain from which the plasmid

Table 1. The amino acid scores of the *C. glutamicum* comparing with other SCPs.

	C. glutamicum-SCP [3]	Yeast extract (*Saccharomyces cerevisiae*) [4]	Spirulina (*Arthrospira platensis*) [5]	*Chlorella vulgaris* [6]
Histidine	115	97	68	101
Isoleucine	96	130	100	112
Leucine	92	93	84	143
Lysine	111	129	62	119
SAA	135	86	125	181
AAA	154	141	136	277
Threonine	144	132	124	237
Tryptophan	132	145	447	35
Valine	100	108	91	177
SSA, Methionine + Cysteine; AAA, Phenylalanine + Tyrosine				

has been removed, food safety assessment may be varied depending on each country's regulation policy. For example, according to the cases of EFSA and FDA, the final product should be recombinant DNA-free. However, even if it is present, it is considered safe if it is not a gene that poses concerns about antibiotic resistance, toxicity, or pathogenicity [9]. The authors have reported in the focal paper that the *C. glutamicum* heme-SCP derived from a gradually increased growth rate in continuous culture showed a health-favorable effect on the ingested mice's blood fat levels along with slim bodies [1,7], and the possible reason of the lowering blood fat level by the *C. glutamicum* heme-SCP diet would be linked to the host intestinal health which led by flourishing health-beneficial anaerobic bacteria, especially anti-obese bacteria [7]. According to the recent Lee's Ph. D. dissertation research, the intestinal flora of the mice fed free heme showed different flora along with lower microbial diversity compared to those fed complex nutrition of the *C. glutamicum* heme-SCP [3] (**Tables 2**, quoted and rearranged from the Lee's Ph. D. dissertation). Therefore, the authors have reckoned that there should

Table 2. Effect of high-vital *Corynebacterium glutamicum*-SCP administration for 28 days (upper table) and for 10 days (lower table) on the fecal bacterial marker in the high-fat diet-induced obese mice.

Biomarker	Characteristic	HFD + 0 % heme-SCP	HFD + hemin 25 μM	HFD + 0.05 % heme-SCP	HFD + 0.5 % heme-SCP
Number of OTU		330	349	386	492
Shannon diversity index	Diversity	2.924	3.88	4.226	4.117
Lachnospiraceae	Produce butyrate and other SCFAs	6%	40%	36%	17%
Ruminococcaceae	Butyrate producers	5%	15%	17%	11%
Pseudoflavonifractor	Encompasses butyrate-producing bacteria	1%	5%	6%	3%
Christensenellaceae	Inversely related to host BMI	0.3%	0.3%	2%	3%
Oscillibacter	Anti-obesity	1%	0%	4%	3%
Akkermansia muciniphila	Prevents obesity	4%	3%	15%	11%
Bacteroides acidifaciens	Prevents obesity, improves insulin	0.2%	2%	1%	3%
Parabacteroides goldsteinii	Prevents obesity	0.2%	0.2%	0.3%	0.3%
Number of species		336	349	378	431

Shannon diversity index	Measuring biological diversity	3.7	3.8	4.024	4.187
Lachnospiraceae	Produce butyrate and other SCFAs	19%	40%	35%	43%
Ruminococcaceae	Butyrate producers	14%	15%	26%	26%
Pseudoflavonifractor	Encompasses butyrate-producing bacteria	5%	5%	12%	8%
Oscillibacter.	Prevents obesity	3%	5%	9%	10%
Bilophila wadsworthia	Aggravates high fat diet induced metabolic dysfunctions in mice	4%	1%	2%	3%
Bacteroides vulgatus	Inflammatory bowel disease	18%	6%	13%	1%
After obese C57BL/6N mice were fed a high-fat diet supplemented with h-SCP for 28 days and 10 days, feces were obtained and analyzed using 16srRNA. (Courtesy to HL) The feces were analyzed by mixing feces from each group rather than separating each mouse's feces. Data quoted from Lee's Ph. D. dissertation [3].					

have been unknown components in the *C. glutamicum* heme-SCP besides heme that has influenced on the construction of beneficial bacteria in gut. In this regard, it is necessary to identify additional interactions and molecular mechanisms between intestinal bacterial community. Consequently, the authors further suggest that 'high-vital *C. glutamicum* SCP' would be more appropriate term than the name of the *C. glutamicum* heme-SCP, considering there are unknown components beside heme affecting gut microbial flora among the components of the protein ingredient. The authors currently suspect that high amino acid scored sulfur containing AA (SAA) might be the one of the unknown factors in the ingredient for constructing beneficial anaerobic bacterial flora in the host gut because SAA could be used for cellular redox balancing component such as glutathione that could strengthen the oxidative stress in the strict anaerobic bacteria.

Credit Authorship Contribution Statement

Sehyeon Park: Investigation, Visualization, Writing-Original Draft. Seungki Lee: Investigation, Validation, Visualization. Taeyeon Kim: Investigation, Validation. Visualization. Soyeon Lee: Resources. Pil Kim: Conceptualization, Formal analysis, Writing-Review & Editing, Funding acquisition, Project administration.

Conflict of Interest Statement

The authors declare no conflict of interest. The funders had no role in the design of the study and the interpretation of data.

Funding Information

This research financially supported by the fundings from the Korean Ministry of Science and ICT (2022M3A9I3018121 and 2022R1A2C1005085).

References

1. Park S, Lee S, Kim T, Choi A, Lee S, Kim P. Development strategy of non-GMO organism for increased hemoproteins in Corynebacterium glutamicum: a growth-acceleration-targeted evolution. Bioprocess Biosyst Eng. 2024 Apr;47(4):549-556.
2. Millward DJ, Layman DK, Tomé D, Schaafsma G. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am J Clin Nutr. 2008 May;87(5):1576S-1581S.
3. Lee S. Heme-enriched single-cell protein supplementation improves body composition through gut microbiome modulation [dissertation]. [Bucheon]: The Catholic University of Korea; 2024.
4. Tomé D. Yeast extracts: Nutritional and flavoring food ingredients. ACS Food Science & Technology. 2021 Apr 28;1(4):487-94.
5. Grdeń AS, Sołowiej BG. Macronutrients, amino and fatty acid composition, elements, and toxins in high-protein powders of crickets, Arthrospira, single cell protein, potato, and rice as potential ingredients in fermented food products. Applied Sciences. 2022 Dec 14;12(24):12831.
6. Andreeva A, Budenkova E, Babich O, Sukhikh S, Ulrikh E, Ivanova S, et al. Production, purification, and study of the amino acid composition of microalgae proteins. Molecules. 2021 May 8;26(9):2767.
7. Lee S, Choi A, Park KH, Cho Y, Yoon H, Kim P. Single-cell hemoprotein diet changes adipose tissue distributions and re-shapes gut microbiota in high-fat diet-induced obese mice. Journal of Microbiology and Biotechnology. 2023 Dec 12;33(12):1648.
8. Holowko MB, Frow EK, Reid JC, Rourke M, Vickers CE. Building a biofoundry. Synthetic Biology. 2021 Jan 1;6(1):ysaa026.
9. Park S, Kim T, Lee S, Kim P. Safety Regulations of Edible Ingredients Derived from Corynebacterium glutamicum. Food Science & Nutrition Technology. 2024 Feb;9(1):000328.