# Purification and identification of the myostatin inhibitory peptides from mealworm (T*enebrio molitor* larvae) protein hydrolysate in C2C12 cells

**Eunyoung Oh* [1,2], Hyeon Soo Kim[3], Yookyung Kim [1]**

[1] Department of Human Ecology (Food Science and Nutrition), Graduate School, Korea University, Seoul 136-701, Republic of Korea
[2]Interdisciplinary Program in Sustainable Living System, Graduate School, Korea University, Seoul 136-701, Republic of Korea
[3]Department of Anatomy, Korea University College of Medicine, Korea University, Seoul 136-701, Republic of Korea

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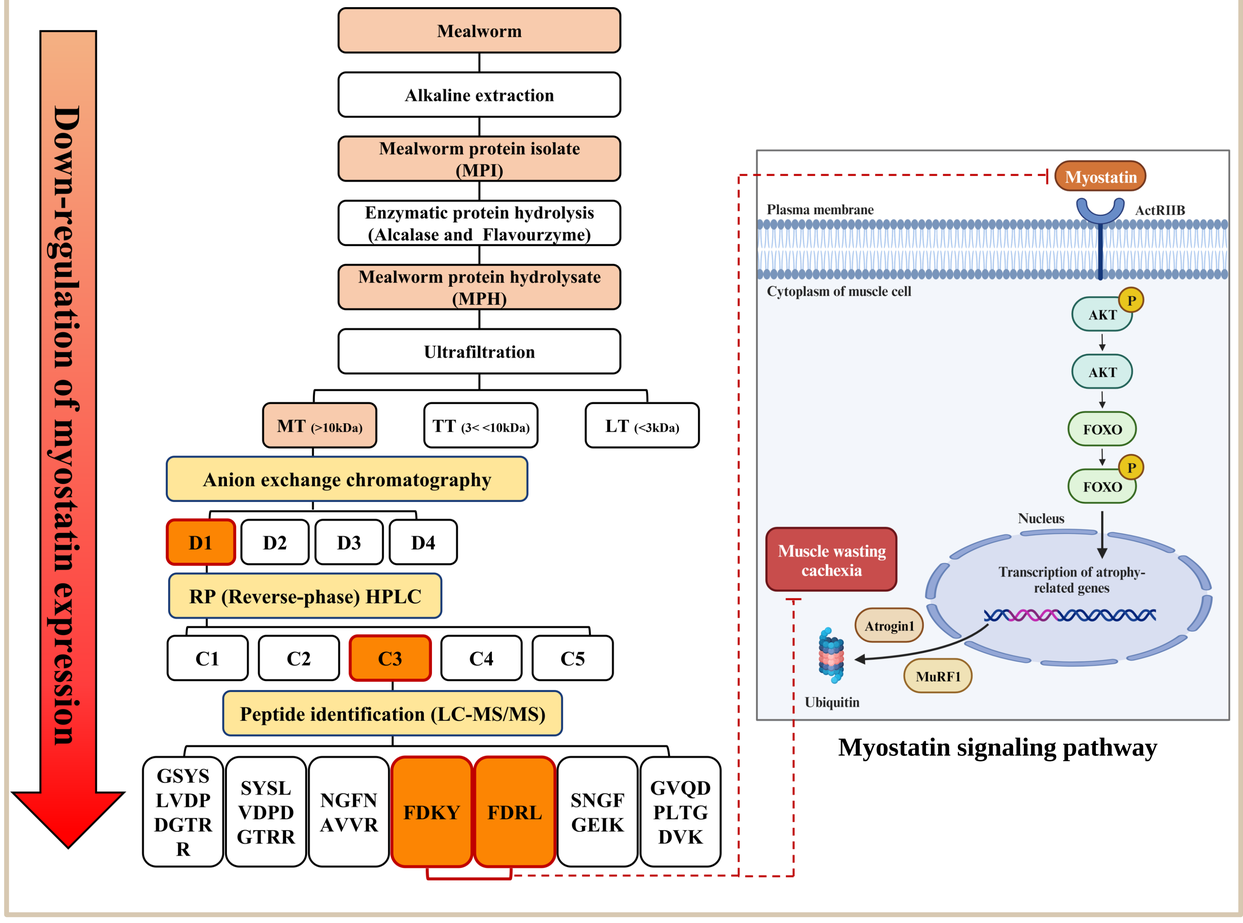
## Graphical abstract




Myostatin signaling pathway

## Introduction and Objective

**Mealworms** (*Tenebrio molitor* larvae) are considered a highly nutritious and sustainable protein source. **Bioactive peptides** produced from protein hydrolysis have recently gained interest as alternative chemotherapeutic agents due to their potential to improve physiological activities. Muscle atrophy, wasting or loss of muscle mass, is a prevalent problem limiting physical activity and reducing the overall quality of life. Nonetheless, there are currently few investigations on natural agents preventing **muscle atrophy**. In this study, two potential peptides, Phe-Asp-Lys-Tyr (**FDKY**) and Phe-Asp-Arg-Leu (**FDRL**), were purified and identified from mealworm protein hydrolysate (MPH), and their inhibitory potentials of **myostatin**, a major negative regulator of skeletal muscle, in C2C12 cells were determined.

**Keywords**: Mealworm, Bioactive peptides, Muscle atrophy, Myostatin

## Materials and Methods

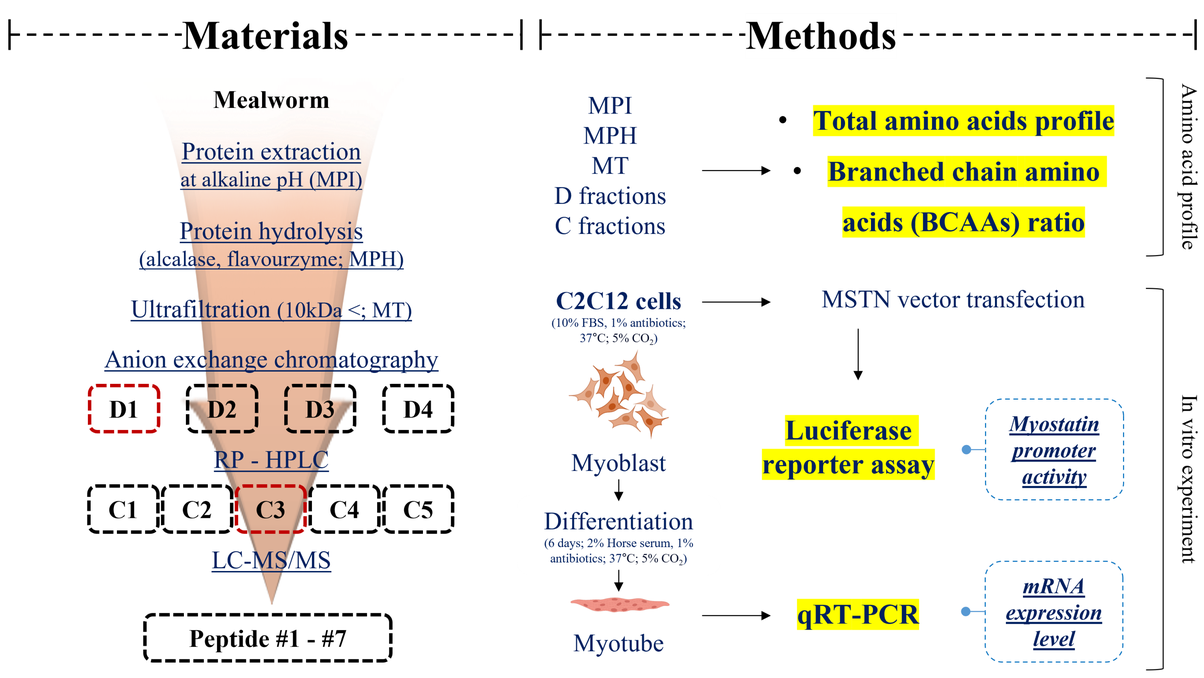



**Table 1. Best peptide sequence**

| No. | Best Sequence | Modifications | Original protein |
|---|---|---|---|
| 1 | **GSYSLVDPDGTRR** | | larval cuticle protein A3A-like |
| 2 | **SYSLVDPDGTRR** | | larval cuticle protein A3A-like |
| 3 | **NGFNAVVR** | Deamidated(N)@1, Deamidated(N)@4 | hypothetical protein GEV33_008419 |
| 4 | **FDKY** | | 86 kDa early-staged encapsulation inducing protein |
| 5 | **FDRL** | | 86 kDa early-staged encapsulation inducing protein |
| 6 | **SNGFGEIK** | Deamidated(N)@2 | 86 kDa early-staged encapsulation inducing protein |
| 7 | **GVQDPLTGDVK** | | hypothetical protein GEV33_008419 |

*Patent pending*

## Results

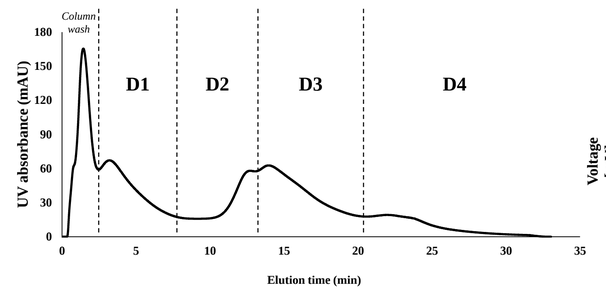



**Figure 1. Anion exchange chromatograph of MT**

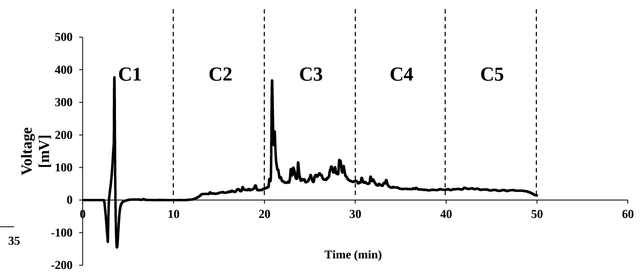



**Figure 2. Reverse phase chromatograph of D1**

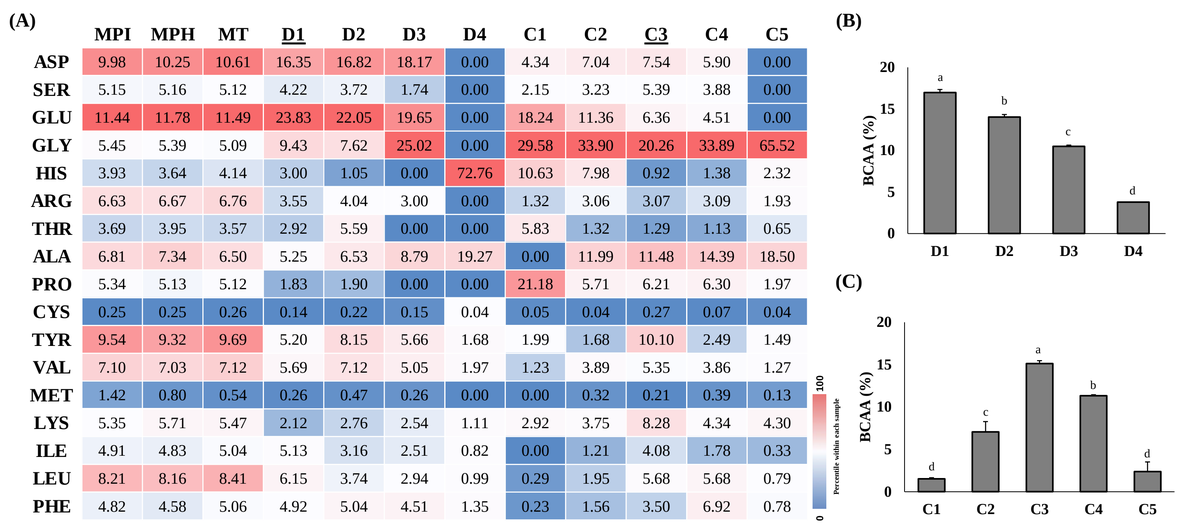



**Figure 3. (A) Heat-map analysis of amino acid, BCAAs ratio of (B) D fractions, and (C) C fractions**

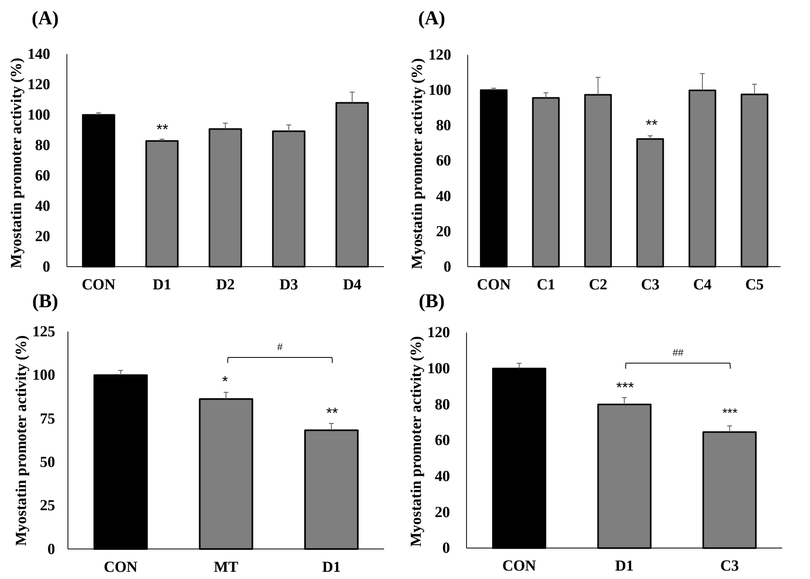



**Figure 4. Effects of (A) D fractions and (B) MT and D1 on myostatin promoter activity**




**Figure 5. Effects of (A) C fractions and (B) D1 and C3 on myostatin promoter activity**




**Figure 6. Effects of identified peptides on myostatin promoter activity**

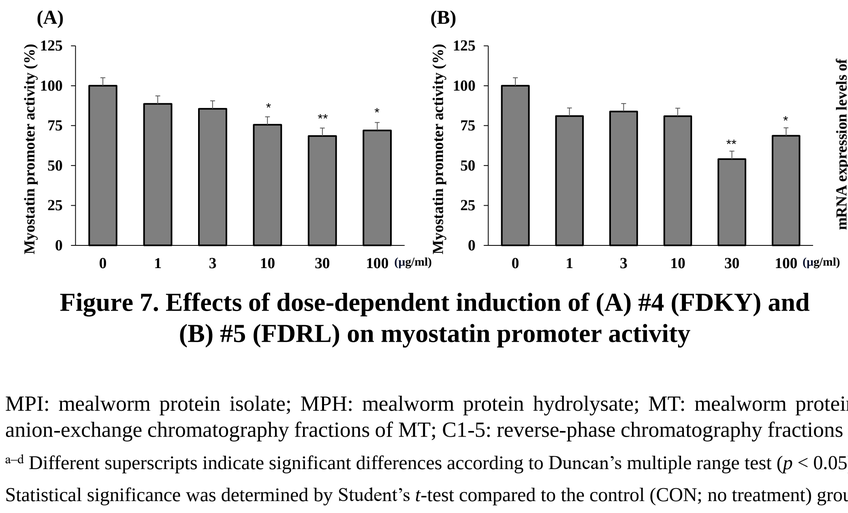



**Figure 7. Effects of dose-dependent induction of (A) #4 (FDKY) and (B) #5 (FDRL) on myostatin promoter activity**

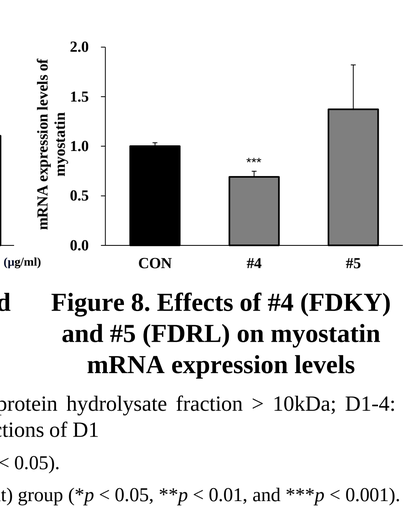



**Figure 8. Effects of #4 (FDKY) and #5 (FDRL) on myostatin mRNA expression levels**

MPI: mealworm protein isolate; MPH: mealworm protein hydrolysate; MT: mealworm protein hydrolysate fraction > 10kDa; D1-4: anion-exchange chromatography fractions of MT; C1-5: reverse-phase chromatography fractions of D1

[a–d] Different superscripts indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

Statistical significance was determined by Student's *t*-test compared to the control (CON; no treatment) group (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

Statistical significance was determined by Student's *t*-test, comparing to an upper-level fraction ($^{\#}p<0.05$, $^{\#\#}p<0.01$).

## Summary and Conclusions

- D1 and C3, which have the most significant potential for myostatin inhibition in each fractionation step, had the highest branched chain to total amino acid ratios.
- Among all the peptides, only the treatment of FDKY and FDRL significantly lowered the luciferase activity compared to the control group (no treatment) in a dose-dependent manner. FDKY treatment also decreased myostatin mRNA expression levels compared to the control group.
- The bioactive peptides from MPH could be an attractive natural agent that can be used as a drug or functional food ingredient to prevent muscle atrophy.

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## AUTHOR INFORMATION

- Ph.D Candidate **Eunyoung Oh**: oheuy0725@korea.ac.kr
- (Correspondence) Professor **Yookyung Kim**: yookyung_kim@korea.ac.kr

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