

Solution for Affordable Insulin: Efficient Production of Single Chain Insulin Through *P. fluorescens*

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Diabetes poses a significant global health challenge, emphasizing the urgent need for insulin. Insulin costs a quite demanding as it is needed every year through one's lifetime. Meanwhile, single-chain insulin (SCI) has been studied to have high stability and production efficiency. Also, *P. fluorescens* has an advantage in protein production as cell lysis process isn't needed. Thus, our study aimed to find the solution for affordable insulin by researching the efficient production of SCI through *P. fluorescens*. We developed vectors, pAU, pSD1, and pSD2 using restriction enzymes, and identified productivity of SCI production on *P. fluorescens* using western blotting. We also verified the conditions that SCI is produced well, attempted a large-scale production of SCI, and purified SCI through His-tag purification method. With the concentrated SCI, we tested its effect on mouse. Results showed successful, similar SCI production in pAD2 and pSD2. Also, SCI production was confirmed only in M9 media and showed more production in 3ml than 5ml. The change in blood glucose level after injection of SCI and Humulin in mouse showed Pearson correlation rate of $R^2=0.86$. This study has succeeded in developing the vector for SCI production in *P. fluorescens*, and we found out aeration is an important factor for insulin production in *P. fluorescens*, especially in large capacity. Also, we've identified that SCI has similar activation as Humulin in vertebrate. Thus, we believe that SCI would be a breakthrough for insulin treatment, and could progress factory production of SCI through further research.