93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more identical to SEQ ID NO:2, 4, 6, 8, 10, and/or 12. In some embodiments of the methods, the penicillin G acylase is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to SEQ ID NO:2, 4, 6, 8, 10, and/or 12. In some further embodiments of the methods, , the penicillin G acylase comprises SEQ ID NO:2, 4, 6, 8, 10, and/or 12. In some embodiments, the engineered penicillin G acylase produces more than 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more free insulin. The present invention also provides compositions comprising free insulin produced according to the method(s) provided herein.

[0009A] The invention also provides an engineered non-naturally occurring penicillin G acylase capable of removing the A1/B1/B29 tri-phenyl acetate protecting groups from insulin to produce free insulin, wherein said penicillin G acylase comprises SEQ ID NO:12.

BRIEF DESCRIPTION OF THE FIGURES

[0010] Figure 1 provides a graph showing the substrate inhibition activity observed with Variant 1.

[0011] Figure 2 provides a graph showing the amount of free insulin produced using seven variant PGAs.

[0012] Figure 3 provides a graph showing % yield of free insulin produced using three variant PGAs.

[0013] Figure 4 provides a graph showing % yield of free insulin produced using three variant PGAs in the presence of DMSO in the reactions.