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.

DESCRIPTION OF THE INVENTION

[0014] The present invention provides engineered penicillin G acylases (PGA) that are capable of cleaving penicillin to phenylacetic acid and 6-aminopenicillanic acid (6-APA), which is a key intermediate in the synthesis of a large variety of β-lactam antibiotics. In particular, the present invention provides engineered PGAs that are capable of removing the A1/B1/B29 tri-phenyl acetate protecting groups to release free insulin.

[0015] Generally, naturally occurring PGAs are heterodimeric enzymes composed of an alpha subunit and a beta-subunit. Wild-type PGA is naturally synthesized as a pre-pro-PGA polypeptide, containing an N-terminal signal peptide that mediates translocation to the periplasm and a linker region connecting the C-terminus of the alpha subunit to the N-terminus of the beta subunit. Proteolytic processing leads to the mature heterodimeric enzyme. The intermolecular linker region can also function in promoting proper folding of the enzyme. The PGAs described herein are based on the PGA from *Kluyvera citrophila* in which various modifications have been introduced to generate improved enzymatic properties as described in detail below.

[0016] For the descriptions provided herein, the use of the singular includes the plural (and vice versa) unless specifically stated otherwise. For instance, the singular forms “a”, “an” and “the”

[0017] include plural referents unless the context clearly indicates otherwise. Similarly, “comprise,” “comprises,” “comprising” “include,” “includes,” and “including” are interchangeable and not intended to be limiting. It is to be further understood that where descriptions of various embodiments

The Claims defining the Invention are as follows:

1. 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.

2. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase is encoded by the polynucleotide sequence set forth in SEQ ID NO:11.