CLAIMS

We claim:

1. An engineered penicillin G acylase capable of acylating insulin, wherein the polypeptide sequence of said penicillin G acylase is 90% or more identical to SEQ ID NO: 100, and wherein the residue at position X67 as compared to SEQ ID NO: 100 is an alanine.

2. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises a sequence that is at least 90% or more identical to at least one sequence set forth in Table 5.1, 5.2, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 7.1, 8.1, 9.1, 11.1, 12.1, 13.1, 14.1, 15.1, 16.1, 17.1, 18.1, 19.1, 20.1, 22.1, 23.1, and/or 23.2.

3. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises a sequence set forth in Table 5.1, 5.2, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 7.1, 8.1, 9.1, 11.1, 12.1, 13.1, 14.1, 15.1, 16.1, 17.1, 18.1, 19.1, 22.1, 23.1, and/or Table 23.2.

4. The engineered penicillin G acylase of Claim 1, wherein said engineered penicillin G acylase comprises a polypeptide sequence selected from SEQ ID NOS:100 and 110.