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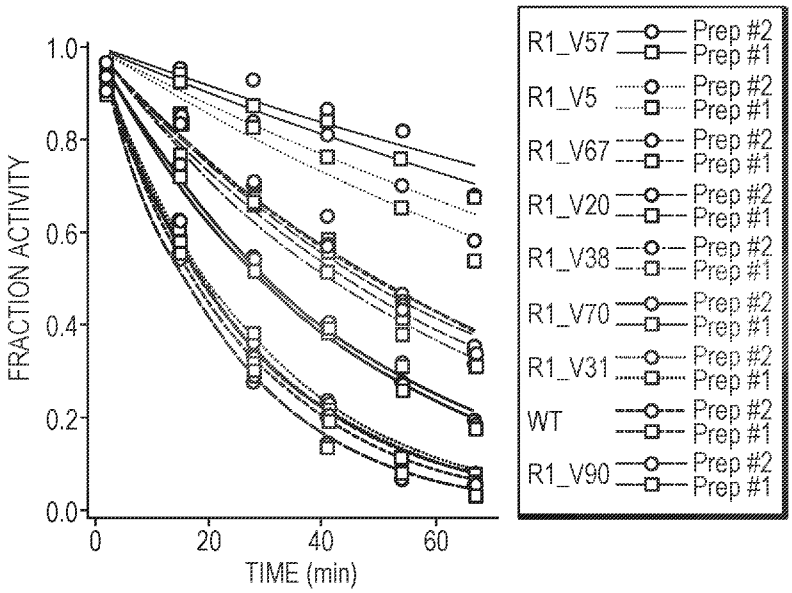

FIG. 2A

(57) Abstract: Disclosed are recombinant mutant *Candida utilis* uricase enzymes with improved pancreatin stability and/or activity, compositions containing such uricase enzymes, which can be used, among other things, to treat diseases or disorders associated with an elevated amount of uric acid, including, for example, hyperuricemia, hyperuricosuria, and gout.

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RECOMBINANT URICASE ENZYME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. application number 62/529,726, filed July 7, 2017, and U.S. application number 62/678,511, filed May 31, 2018, the contents of each of which are hereby incorporated by reference in their entireties for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates generally to methods and compositions for treating diseases or disorders associated with an elevated amount of uric acid, and, more particularly, the invention relates to recombinant mutant *Candida utilis* uricases and methods using, and compositions containing, such uricases for treating diseases or disorders associated with an elevated amount of uric acid.

BACKGROUND

[0003] Uric acid is the final oxidation product of purine metabolism in humans and higher primates. Uricase, or urate oxidase, is an enzyme that degrades uric acid into allantoin and carbon dioxide. Due to mutational silencing, humans and higher primates lack a functional uricase gene. Therefore, unlike certain other mammals, humans have lost the capacity to metabolize uric acid by hepatic uricase due to mutational silencing of the enzyme. Although humans produce large quantities of uric acid, the majority of the uric acid is excreted in urine. Nevertheless, increased production and/or decreased excretion of uric acid can result in high levels of uric acid in blood (hyperuricemia) and urine (hyperuricosuria). Hyperuricemia and hyperuricosuria can result, for example, as in inflammatory arthritis due to urate deposits in joints and cutaneous tissue.

[0004] Gout is a condition that affects an estimated 8 million Americans and is characterized by recurring attacks of joint inflammation (arthritis). The joint inflammation is precipitated by deposits of uric acid crystals in the joint fluid (synovial fluid) and joint lining (synovial lining). Intense joint inflammation occurs as white blood cells engulf the uric acid crystals and release inflammatory chemicals, causing pain, heat, and redness of the joint tissues. Chronic gout can additionally lead to decreased kidney function and kidney stones.

[0005] Limitations in efficacy and/or tolerance of existing therapies of gout such as oral xanthine oxidase inhibitors (for example, allopurinol), uricosurics, and intravenous uricase

agents, contribute to refractoriness to urate-lowering therapy (ULT) in gout. For example, delayed or insufficient dosing with allopurinol contributes to refractory gout. *See* Fels and Sundy (2008), *CURR. OPIN. RHEUMATOL.*, 20(2): 198-202. Renal excretion is the major route of uric acid elimination, but the gastrointestinal tract (GIT) plays an increasingly recognized role in urate homeostasis, especially in chronic kidney disease (CKD) where urate renal elimination is impaired.

[0006] Functional uricase enzymes can be found in a wide range of organisms, including animals, plants, bacteria and fungi, and, as such, exogenous uricase has been used in the treatment of diseases or disorders associated with an elevated amount of uric acid. Clinically approved uricases include Krystexxa® (pegloticase), which has been approved for the treatment of chronic refractory gout, and Elitek® (rasburicase), which has been approved for tumor lysis syndrome.

[0007] Although developments have been made to date, there is still an ongoing need for new and effective therapies for treating and managing diseases or disorders associated with an elevated amount of uric acid such as hyperuricemia and gout, and improved uricase enzymes for use in treating and managing such diseases or disorders.

SUMMARY OF THE INVENTION

[0008] The invention is based, in part, upon the discovery of recombinant uricase enzymes that are active in humans and have greater stability and/or activity than naturally occurring enzymes. In particular, the recombinant enzymes of the invention exhibit improved stability against proteolytic digestion by pancreatin (a collection of enzymes secreted by the pancreas) compared to naturally occurring versions of the enzyme. Furthermore, the recombinant enzymes of the invention may have greater specific activity than a wild type uricase enzyme. Furthermore, it is contemplated that the recombinant enzymes described herein, given their enhanced stability, may be suitable for oral administration, and therefore potentially safer and more tolerable than the commercially available, injectable forms of uricase (*e.g.*, Krystexxa® and Elitek®), because it is contemplated that the enzymes will remain active within the intestines and will not be absorbed through the intestinal wall.

[0009] In one aspect, the invention provides a recombinant mutant *Candida utilis* uricase enzyme that comprises at least one (for example, one, two, three, four, five, six, seven or eight) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is selected from: (a) at position 180, isoleucine is

substituted by valine or alanine (I180V or I180A), (b) at position 165, tyrosine is substituted by phenylalanine (Y165F), (c) at position 190, valine is substituted by glycine or alanine (V190G or V190A), (d) at position 51, glutamic acid is substituted by lysine (E51K), (e) at position 244, glutamine is substitute by lysine (Q244K), (f) at position 132, isoleucine is substituted by arginine or asparagine (I132R or I132N), (g) at position 97, valine is substituted by isoleucine (V97I), (h) at position 92, glutamic acid is substituted by asparagine (E92N), (i) at position 87, alanine is substituted by glycine (A87G), (j) at position 142, aspartic acid is substituted by glutamic acid (D142E), (k) at position 44, glycine is substituted by alanine (G44A), (l) at position 128, glycine is substituted by proline (G128P), (m) at position 236, alanine is substituted by asparagine (A236N), (n) at position 208, lysine is substituted by alanine (K208A), (o) at position 213, asparagine is substituted by alanine (N213A), (p) at position 140, serine is substituted by threonine (S140T), (q) at position 253, tyrosine is substituted by glutamine (Y253Q), (r) at position 84, alanine is substituted by serine (A84S), (s) at position 47, threonine is substituted by glutamic acid (T47E), (t) at position 95, serine is substituted by proline (S95P), (u) at position 103, lysine is substituted by threonine (K103T), (v) at position 134, aspartic acid is substituted by glutamic acid (D134E), (w) at position 136, tyrosine is substituted by arginine (Y136R), (x) at position 196, isoleucine is substituted by leucine (I196L), (y) at position 224, threonine is substituted by aspartic acid (T224D), (z) at position 285, proline is substituted by serine (P285S), and (aa) at position 296, valine is substituted by alanine (V296A).

[0010] In certain embodiments, the recombinant mutant *C. utilis* uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, V190A, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, G44A, G128P, A236N, K208A, N213A, S140T, Y253Q, and A84S. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, and G44A. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, I132R, and G44A. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, E51K, I132R, and G44A. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, and I132R.

[0011] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at

a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 132, and position 44. In certain embodiments, one or more mutations may be conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1.

[0012] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising at least one (for example, one, two, three, four, or five) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 51, position 132, and position 44. In certain embodiments, one or more mutations may be conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1.

[0013] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase comprising at least one (for example, one, two, three, four, or five) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 244, and position 132. In certain embodiments, one or more mutations may be conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1.

[0014] In certain embodiments, in any of the foregoing recombinant mutant *C. utilis* uricase enzymes, the uricase comprises two, three, four, five, six, seven, or eight mutations.

[0015] In certain embodiments, in any of the foregoing recombinant mutant *C. utilis* uricase enzymes, the uricase comprises the following substitutions (i) I180V, Y165F, E51K, I132R, and G44A, (ii) I180A, Y165F, E51K, I132R, and G44A, (iii) I180V, Y165F, V190G, E51K, I132R, and G44A, (iv) I180A, Y165F, V190G, E51K, I132R, and G44A, (v) I180V and Y165F, or (vi) I180V, Y165F, V190G, E51K, Q244K, and I132R, either alone or in combination with other substitutions.

[0016] In certain embodiments, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising three substitutions listed in a given row of **TABLE 1** hereinbelow. In

certain embodiments, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising five substitutions listed in a given row of **TABLE 2** hereinbelow.

[0017] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase having a half-life of at least 35 minutes in the presence of pancreatin, *e.g.,* a half-life of 35-200 minutes in the presence of pancreatin, for example, under the conditions set forth in Example 1.

[0018] It is contemplated that any of the foregoing recombinant mutant *Candida utilis* uricases may, for example, have 5-50 fold, 10-40 fold, 10-30 fold, 20-40 fold, or 20-30 fold, higher stability in the presence of pancreatin, compared to the wild-type uricase. The uricase may, for example, be more stable at a pH less than about 6.5 compared to the template (or reference) wild-type uricase.

[0019] It is contemplated that any of the foregoing recombinant mutant *Candida utilis* uricases may, for example, be conjugated to a water soluble polymer, *e.g.*, polyethylene glycol (PEG).

[0020] In certain embodiments, in any of the foregoing recombinant mutant *C. utilis* uricase enzymes, the uricase is isolated.

[0021] In another aspect, the invention provides an isolated nucleic acid comprising a nucleotide sequence encoding any one of the foregoing uricase enzymes. In certain embodiments, the nucleotide sequence is codon optimized for expression in a host cell, *e.g.,* an *Escherichia coli* cell. The invention also provides an expression vector that comprises any one of the foregoing nucleotide sequences. Similarly, the invention provides host cells, *e.g.,* *Escherichia coli* cells, comprising one or more of the foregoing expression vectors.

[0022] In another aspect, the invention provides a pharmaceutical composition comprising any one of the foregoing recombinant mutant *C. utilis* uricase enzymes and at least one pharmaceutically acceptable carrier and/or an excipient. The enzyme may be in a soluble form or in a crystal form. Furthermore, the composition may comprise a pH increasing agent. It is contemplated that the pharmaceutical composition may, for example, be formulated as an oral dosage form or a parenteral dosage form. In certain embodiments, the composition is a formulated as a powder, granulate, pellet, micropellet, or a minitablet. In certain embodiments, the composition is encapsulated in a capsule, *e.g.,* a hydroxypropyl methylcellulose (HPMC) capsule, soft gelatin capsule, or a hard gelatin capsule, or the composition is formulated as a tablet dosage form.

[0023] In another aspect, the invention provides a method of treating a disease or disorder associated with an elevated amount of uric acid in a subject in need thereof. In certain embodiments, the disease or disorder is associated with an elevated amount of uric acid in plasma or urine of the subject. The method comprises administering to the subject an effective amount of any of the uricase enzymes or compositions described herein, to treat the disease or disorder in the subject.

[0024] In another aspect, the invention provides a method of treating hyperuricemia and/or hyperuricosuria in a subject in need thereof. The method comprises administering to the subject an effective amount of any of the uricase enzymes or compositions described herein, to treat the hyperuricemia and/or hyperuricosuria in the subject.

[0025] In another aspect, the invention provides a method of treating gout in a subject in need thereof. The method comprises administering to the subject an effective amount of any of the uricase enzymes or compositions described herein, to treat the gout in the subject.

[0026] In certain embodiments, in any of the foregoing methods, the recombinant mutant *C. utilis* uricase is administered in combination with a xanthine oxidase inhibitor (*e.g.,* allopurinol or febuxostat), a uricosuric (*e.g.,* probenecid, benzbromarone, losartan or lesinurad), or a combination thereof.

[0027] These and other aspects and features of the invention are described in the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The invention can be more completely understood with reference to the following drawings.

[0029] FIGURE 1A is a SDS-PAGE gel depicting pancreatin, wild-type *C. utilis* uricase (His-UO), and wild-type *C. utilis* uricase following a 90 minute incubation with pancreatin. **FIGURE 1B** is a line graph depicting wild-type *C. utilis* uricase activity as measured by loss of substrate uric acid concentration following incubation of wild-type *C. utilis* uricase with pancreatin for the indicated time points. Uric acid concentration is measured by absorbance at 298 nm.

[0030] FIGURE 2A is a line graph depicting the activity of the indicated mutant *C. utilis* uricases in the presence of pancreatin. Data from two independent preparations are depicted for each uricase. Activity values are normalized to the activity in presence of pancreatin at

time zero. **FIGURE 2B** is a line graph demonstrating the reproducibility across each preparation for the data depicted in **FIGURE 2A**.

[0031] FIGURE 3 is a line graph depicting the activity of the R2_V79, R2_15, R2_V16 and R2_Parent mutant *C. utilis* uricases following incubation with pancreatin for the indicated time-points. Activity values are normalized to the activity in presence of pancreatin at time zero.

[0032] FIGURE 4 shows protein unfolding as determined by differential scanning fluorimetry (DSF) for wild-type *C. utilis* uricase and the indicated mutant *C. utilis* uricase enzymes.

[0033] FIGURE 5 is an SDS-PAGE gel showing the R2_V17, R2_V4 and R2_V79 mutant *C. utilis* uricases following incubation with pancreatin for the indicated timepoints.

[0034] FIGURE 6 is an SDS-PAGE gel showing the wild-type *C. utilis* uricase and R2_V17 mutant *C. utilis* uricase following incubation with pancreatin for the indicated timepoints.

[0035] FIGURE 7 is a bar graph showing the pancreatin stability of the indicated mutant *C. utilis* uricases relative to wild-type. R2 mutant *C. utilis* uricases described in Example 1, each containing five substitutions (right), and mutant *C. utilis* uricases described in Example 2, each containing a single substitution (left and middle), are depicted.

[0036] FIGURE 8 is a waterfall chart showing the pancreatin stability of the mutant *C. utilis* uricases described in Example 2, each containing a single substitution, relative to wild-type. Enzymes are ordered relative to their effect on stability.

[0037] FIGURE 9A is a bar graph showing the plasma urate levels (mg/dL) in Uricase knockout (UrOxKO) mice with severe hyperuricemia. Mean (SEM) of pre-treatment (plasma urate level was measured in samples collected on day 7 after removal of maintenance dose of allopurinol), treatment (plasma urate level was measured in samples collected on day 7 after administration of 50mg/L of allopurinol, 150mg/L of allopurinol, or 150mg/day mutant *C. utilis* uricase, respectively), and post-treatment (plasma urate level was measured in samples collected on day 7 after treatment was terminated) plasma urate levels are shown.

[0038] FIGURE 9B is a bar graph showing the urine uric acid levels (mg/dL) in UrOxKO mice with severe hyperuricosuria. Uric acid levels were measured in 24-hour urine samples collected during the last 3 days of pre-treatment and treatment periods, as indicated.

DETAILED DESCRIPTION

[0039] The invention is based, in part, upon the discovery of recombinant uricase enzymes that are active in humans and have greater stability and/or activity than naturally occurring enzymes. In particular, the recombinant enzymes of the invention exhibit improved stability against proteolytic digestion by pancreatin (a collection of enzymes secreted by the pancreas) compared to naturally occurring versions of the enzyme. Furthermore, the recombinant enzymes of the invention may have greater specific activity than a wild type uricase enzyme. Furthermore, it is contemplated that the recombinant enzymes described herein, given their enhanced stability, may be suitable for oral administration, and therefore potentially safer and more tolerable than the commercially available, injectable forms of uricase (*e.g.*, Krystexxa® and Elitek®), because it is contemplated that the enzymes will remain active within the intestines and will not be absorbed through the intestinal wall because the size of the recombinant enzyme would preclude passive absorption, and no receptor has been identified for active transport of the enzyme from the intestine.

[0040] Various features and aspects of the invention are discussed in more detail below.

I. Uric Acid And Uricase

[0041] Uric acid (also known as urate) is the final product of purine metabolism in humans and higher primates. Uricase (also known as urate oxidase or UrOx) degrades uric acid into allantoin by catalyzing the following reaction:

$$\text{Uric acid} + O_2 + H_2O \rightarrow \text{5-hydroxyisourate} + H_2O_2 \rightarrow \text{allantoin} + CO_2.$$

[0042] Due to mutational silencing, humans and higher primates lack a functional uricase gene. However, functional uricase enzymes can be found in a wide range of organisms, including animals, plants, bacteria and fungi. One such organism is the yeast *Candida utlilus* (also known as *Cyberlindnera jadinii* or Torula yeast). *C. utilis* uricase is a homo-tetrameric enzyme that does not require a metal atom or an organic co-factor for catalysis. The amino acid sequence of wild type *C. utilis* uricase is as follows:

MSTTLSSSTYGKDNVKFLKVKKDPQNPKKQEVMEATVTCLLEGGFDTSYTEADNSSIVPTDT
VKNTILVLAKTTEIWPIERFAAKLATHFVEKYSHVSGVSVKIVQDRWVKYAVDGKPHDHSFI
HEGGEKRITDLYYKRSGDYKLSSAIKDLTVLKSTGSMFYGYNKCDFTTLQPTTDRILSTDVD
ATWVWDNKKIGSVYDIAKAADKGIFDNVYNQAREITLTTFALENSPSVQATMFNMATQILEK
ACSVYSVSYALPNKHYFLIDLKWKGLENDNELFYPSPHPNGLIKCTVVRKEKTKL (SEQ ID NO: 1).

[0043] An exemplary nucleotide sequence encoding the wild type *C. utilis* uricase is as follows:

ATGTCGACGACCCTGAGCAGCAGCACCTATGGCAAAGATAATGTGAAATTTCTGAAAGTCAA
AAAAGACCCGCAGAACCCTAAGAAACAAGAGGTCATGGAAGCGACCGTTACGTGTCTGCTGG
AAGGCGGCTTCGACACCAGCTATACCGAAGCGGATAATTCCTCCATCGTTCCGACCGATACG
GTCAAGAACACCATTCTGGTTCTGGCCAAGACCACGGAAATCTGGCCAATTGAGCGCTTCGC
CGCGAAACTGGCGACCCATTTCGTTGAGAAGTACAGCCACGTGAGCGGCGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTCAAATATGCCGTGGATGGTAAGCCGCATGACCACAGCTTTATT
CACGAGGGTGGCGAGAAGCGTATCACTGACCTGTATTACAAGCGCAGCGGTGACTACAAATT
GAGCAGCGCAATCAAAGACCTGACGGTCCTGAAAAGCACCGGTTCTATGTTTTACGGTTACA
ATAAGTGCGACTTTACGACGCTCCAACCGACTACGGACCGTATCCTGTCTACCGATGTAGAC
GCGACCTGGGTCTGGGATAACAAGAAAATTGGCAGCGTGTACGATATTGCGAAAGCCGCTGA
CAAGGGTATCTTCGACAACGTCTATAATCAAGCGCGTGAGATCACCCTGACCACGTTTGCTC
TGGAGAATTCCCCGAGCGTTCAGGCGACCATGTTTAACATGGCAACGCAGATTTTGGAAAAG
GCATGTAGCGTGTACAGCGTGAGCTATGCATTGCCGAATAAGCACTACTTCCTGATTGATCT
GAAGTGGAAGGGTCTGGAGAACGATAACGAACTGTTCTATCCGAGCCCGCACCCGAATGGTC
TGATCAAGTGCACCGTTGTGCGTAAAGAAAAGACTAAACTG (SEQ ID NO: 7).

II. Recombinant Mutant *Candida Utilis* Uricase Enzymes

[0044] Among other things, the invention provides a family of recombinant mutant *Candida Utilis* uricase enzymes that, for example, are useful in treating disorders associated with elevated levels of uric acid in a subject, for example, disorders associated with elevated levels of uric acid in plasma of the subject. In certain embodiments, the recombinant mutant *C. Utilis* uricase enzymes described herein have higher stability compared to the wild-type *C. Utilis* uricase, *e.g.,* higher stability in the presence of pancreatin compared to the wild-type *C. Utilis* uricase, and are therefore better suited for oral delivery and activity in the intestines than wild-type *C. Utilis* uricase. Unless stated otherwise, as used herein, wild-type *C. Utilis* uricase refers a *C. Utilis* uricase having the amino acid sequence of SEQ ID NO: 1, or a functional fragment thereof that can catalyze the oxidation of uric acid to 5-hydroxyisourate. As used herein, the term "functional fragment" is understood to be a protein fragment of wild type *C. utilis* uricase of SEQ ID NO: 1 that has at least 50%, 60%, 70%, 80%, 90%, 95%, or 98% of the activity of wild type *C. utilis* uricase to catalyze the conversion of uric acid to 5-hydroxyisourate and/or allantoin.

[0045] In one aspect, the invention provides a recombinant mutant *Candida utilis* uricase enzyme that comprises at least one (for example, one, two, three, four, five, six, seven or eight) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is selected from: (a) at position 180, isoleucine is substituted by valine or alanine (I180V or I180A), (b) at position 165, tyrosine is substituted

by phenylalanine (Y165F), (c) at position 190, valine is substituted by glycine or alanine (V190G or V190A), (d) at position 51, glutamic acid is substituted by lysine (E51K), (e) at position 244, glutamine is substitute by lysine (Q244K), (f) at position 132, isoleucine is substituted by arginine or asparagine (I132R or I132N), (g) at position 97, valine is substituted by isoleucine (V97I), (h) at position 92, glutamic acid is substituted by asparagine (E92N), (i) at position 87, alanine is substituted by glycine (A87G), (j) at position 142, aspartic acid is substituted by glutamic acid (D142E), (k) at position 44, glycine is substituted by alanine (G44A), (l) at position 128, glycine is substituted by proline (G128P), (m) at position 236, alanine is substituted by asparagine (A236N), (n) at position 208, lysine is substituted by alanine (K208A), (o) at position 213, asparagine is substituted by alanine (N213A), (p) at position 140, serine is substituted by threonine (S140T), (q) at position 253, tyrosine is substituted by glutamine (Y253Q), (r) at position 84, alanine is substituted by serine (A84S), (s) at position 47, threonine is substituted by glutamic acid (T47E), (t) at position 95, serine is substituted by proline (S95P), (u) at position 103, lysine is substituted by threonine (K103T), (v) at position 134, aspartic acid is substituted by glutamic acid (D134E), (w) at position 136, tyrosine is substituted by arginine (Y136R), (x) at position 196, isoleucine is substituted by leucine (I196L), (y) at position 224, threonine is substituted by aspartic acid (T224D), (z) at position 285, proline is substituted by serine (P285S), and (aa) at position 296, valine is substituted by alanine (V296A).

[0046] In certain embodiments, the recombinant mutant *C. utilis* uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, V190A, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, G44A, G128P, A236N, K208A, N213A, S140T, Y253Q, and A84S. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, and G44A. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, I132R, and G44A. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, E51K, I132R, and G44A. In certain other embodiments, the uricase enzyme comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, and I132R.

[0047] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least

one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 132, and position 44. In certain embodiments, one or more mutations may be conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1.

[0048] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising at least one (for example, one, two, three, four, or five) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 51, position 132, and position 44. In certain embodiments, one or more mutations may be conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1. As used herein, the term "conservative substitution" refers to a substitution with a structurally similar amino acid. For example, conservative substitutions may include those within the following groups: Ser and Cys; Leu, Ile, and Val; Glu and Asp; Lys and Arg; Phe, Tyr, and Trp; and Gln, Asn, Glu, Asp, and His. Conservative substitutions may also be defined by the BLAST (Basic Local Alignment Search Tool) algorithm, the BLOSUM substitution matrix (e.g., BLOSUM 62 matrix), or the PAM substitution:p matrix (e.g., the PAM 250 matrix). Non conservative substitutions are amino acid substitutions that are not conservative substitutions.

[0049] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 244, and position 132. In certain embodiments, one or more mutations may be conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild type *C. utilis* uricase of SEQ ID NO: 1.

[0050] In certain embodiments, in any of the foregoing recombinant mutant *C. utilis* uricase enzymes, the uricase comprises two, three, four, five, six, seven, or eight mutations.

[0051] In certain embodiments, in any of the foregoing recombinant mutant *C. utilis* uricase enzymes, the uricase comprises the following substitutions (i) I180V, Y165F, E51K, I132R,

and G44A, (ii) I180A, Y165F, E51K, I132R, and G44A, (iii) I180V, Y165F, V190G, E51K, I132R, and G44A, (iv) I180A, Y165F, V190G, E51K, I132R, and G44A, (v) I180V and Y165F, or (vi) I180V, Y165F, V190G, E51K, Q244K, and I132R, either alone or in combination with other substitutions.

[0052] In one aspect, the invention provides a recombinant mutant *C. utilis* uricase enzyme comprising three substitutions listed in a given row of **TABLE 1**.

TABLE 1

1	K130T	I180V	V190A
2	E51K	H125K	Q217L
3	Y165F	D201E	A242C
4	A83G	V97I	D201E
5	T38C	G128P	S251L
6	H125K	G128P	I196L
7	I180V	V214A	A242C
8	K130T	F170Y	A236N
9	Y165F	I180V	G197A
10	Y165F	Q217L	T243Q
11	A83G	H119S	Y165F
12	E51K	Y137A	Y165F
13	E92N	S95A	K130T
14	E92D	I180V	F281Y
15	G44A	V97I	S256N
16	S95A	V185I	Q217L

[0053] In another aspect, the invention provides a recombinant mutant *C. utilis* uricase comprising five substitutions listed in a given row of **TABLE 2.**

TABLE 2

1	Y165F	I180V	Q25A	T47E	S256D
2	Y165F	I180V	D142Q	Q217L	A236N
3	Y165F	I180V	G128P	R139E	D142E
4	Y165F	I180V	E51K	V97I	A236N
5	Y165F	I180V	D134E	R139E	V296A
6	Y165F	I180V	A87G	E220A	T224D
7	Y165F	I180V	G44A	G128P	K270E
8	Y165F	I180V	D142Q	I149L	F165Y
9	Y165F	I180V	G44A	Y136R	Y253Q
10	Y165F	I180V	E51K	I149L	D268N
11	Y165F	I180V	D142E	Q174G	S254N
12	Y165F	I180V	E92N	I149L	Y253Q
13	Y165F	I180V	I132N	V190A	N213A
14	Y165F	I180V	E51K	D142E	S256N

15	Y165F	I180V	G44A	E51K	I132R
16	Y165F	I180V	K103T	D134E	V180I
17	Y165F	I180V	A52S	A236N	S256N
18	Y165F	I180V	G128P	Y253Q	P285S
19	Y165F	I180V	E51K	P118I	S147T
20	Y165F	I180V	A84S	S140T	K204A
21	Y165F	I180V	E51K	G128P	F170Y
22	Y165F	I180V	E51K	A87G	D142Q
23	Y165F	I180V	E51K	G128P	N213A
24	Y165F	I180V	V97I	K103T	N213A
25	Y165F	I180V	K103T	F165Y	K208A
26	Y165F	I180V	Q25A	E51K	V296A
27	Y165F	I180V	K85I	P118I	E220A
28	Y165F	I180V	E51K	Y253Q	K270E
29	Y165F	I180V	Q25A	S95P	D142E
30	Y165F	I180V	V97I	G128P	S140T
31	Y165F	I180V	G128P	N193R	S254N
32	Y165F	I180V	S95P	I132N	Y253Q
33	Y165F	I180V	T47E	E92N	V97I
34	Y165F	I180V	E51K	D142E	Q217L
35	Y165F	I180V	A52S	K85I	Q244K
36	Y165F	I180V	A84S	G128P	S256N
37	Y165F	I180V	A84S	V97I	Y253Q
38	Y165F	I180V	A87G	I196L	S256N
39	Y165F	I180V	E51K	G128P	Y253Q
40	Y165F	I180V	D142E	I196L	K208A
41	Y165F	I180V	E51K	V97I	I196L
42	Y165F	I180V	Q174G	T224D	Y253Q
43	Y165F	I180V	I132R	D142E	V296A
44	Y165F	I180V	V97I	D142E	Y253Q
45	Y165F	I180V	A84S	D142E	V190A
46	Y165F	I180V	E92N	F170Y	N193R
47	Y165F	I180V	G128P	V180I	Q217L
48	Y165F	I180V	V97I	F170Y	S254N
49	Y165F	I180V	E92N	G128P	D142E
50	Y165F	I180V	A52S	I196L	S254N
51	Y165F	I180V	S140T	T224D	S256N
52	Y165F	I180V	S95P	K103T	G128P
53	Y165F	I180V	Y136R	Q244K	L274I
54	Y165F	I180V	A84S	Q217L	Q244K
55	Y165F	I180V	S95P	S140T	L274I
56	Y165F	I180V	D142E	N193R	L274I
57	Y165F	I180V	G44A	K204A	P285S
58	Y165F	I180V	V97I	D134E	Y137R
59	Y165F	I180V	A52S	E92N	S256D
60	Y165F	I180V	V97I	I132N	T224D
61	Y165F	I180V	F170Y	Q217L	D268N
62	Y165F	I180V	S95P	Q217L	S254N

63	Y165F	I180V	G44A	S95P	V97I
64	Y165F	I180V	D142E	S147T	F170Y
65	Y165F	I180V	S140T	F165Y	A236N
66	Y165F	I180V	V97I	K208A	D268N
67	Y165F	I180V	V97I	G128P	V190A
68	Y165F	I180V	Y136R	N193R	K270E
69	Y165F	I180V	Q25A	G128P	I149L
70	Y165F	I180V	V97I	P118I	D142E
71	Y165F	I180V	I132R	Q217L	P285S
72	Y165F	I180V	T47E	I196L	Y253Q
73	Y165F	I180V	E51K	Y136R	V190A
74	Y165F	I180V	E92N	V180I	D268N
75	Y165F	I180V	A87G	K204A	L274I
76	Y165F	I180V	V97I	S147T	K270E
77	Y165F	I180V	R139E	Q174G	Q244K
78	Y165F	I180V	A84S	A236N	V296A
79	Y165F	I180V	E51K	K85I	P285S
80	Y165F	I180V	V180I	Y253Q	S256D
81	Y165F	I180V	E51K	S140T	D142E
82	Y165F	I180V	V97I	E220A	S256N
83	Y165F	I180V	Q174G	N213A	P285S
84	Y165F	I180V	P118I	G128P	I196L
85	Y165F	I180V	D134E	K208A	Y253Q

[0054] A recombinant mutant *Candida utilis* uricase disclosed herein may, for example, have higher specific activity than wild-type *C. utilis* uricase of SEQ ID NO.: 1. For example, a recombinant mutant *C. utilis* uricase may have from 5 to 50 fold higher specific activity than the wild-type *C. utilis* uricase. In certain embodiments, the uricase has from about 5 to about 50, from about 5 to about 40, from about 5 to about 30, from about 5 to about 20, from about 5 to about 10, from about 10 to about 50, from about 10 to about 40, from about 10 to about 30, from about 10 to about 20, from about 20 to about 50, from about 20 to about 40, from about 20 to about 30, from about 30 to about 50, from about 30 to about 40, from about 40 to about 50, about 5, about 10, about 20, about 30, about 40, or about 50 fold higher specific activity than wild-type *C. utilis* uricase.

[0055] Alternatively or in addition, the recombinant mutant *Candida utilis* uricase disclosed herein may, for example, have higher stability, *e.g.,* higher stability in the presence of pancreatin, compared to the wild-type *C. utilis* uricase. For example, a recombinant mutant *C. utilis* uricase may have from 5 to 50 fold higher stability in the presence of pancreatin compared to the wild-type *C. utilis* uricase. In certain embodiments, the uricase has from about 5 to about 50, from about 5 to about 40, from about 5 to about 30, from about 5 to about 20, from about 5 to about 10, from about 10 to about 50, from about 10 to about 40,

from about 10 to about 30, from about 10 to about 20, from about 20 to about 50, from about 20 to about 40, from about 20 to about 30, from about 30 to about 50, from about 30 to about 40, from about 40 to about 50, about 5, about 10, about 20, about 30, about 40, or about 50 fold higher stability in the presence of pancreatin compared to the wild-type *C. utilis* uricase.

[0056] Alternatively or in addition, the recombinant mutant *Candida utilis* uricase may, for example, have a half-life of at least 35 minutes in the presence of pancreatin. In certain embodiments, the uricase has a half-life of at least from about 35 to about 200 minutes, from about 35 to about 175 minutes, from about 35 to about 150 minutes, from about 35 to about 125 minutes, from about 35 to about 100 minutes, from about 35 to about 75 minutes, from about 35 to about 50 minutes, from about 50 to about 200 minutes, from about 50 to about 175 minutes, from about 50 to about 150 minutes, from about 50 to about 125 minutes, from about 50 to about 100 minutes, from about 50 to about 75 minutes, from about 75 to about 200 minutes, from about 75 to about 175 minutes, from about 75 to about 150 minutes, from about 75 to about 125 minutes, from about 75 to about 100 minutes, from about 100 to about 200 minutes, from about 100 to about 175 minutes, from about 100 to about 150 minutes, from about 100 to about 125 minutes, from about 125 to about 200 minutes, from about 125 to about 175 minutes, from about 125 to about 150 minutes, from about 150 to about 200 minutes, from about 150 to about 175 minutes, from about 175 to about 200 minutes, about 35 minutes, about 50 minutes, about 75 minutes, about 100 minutes, about 125 minutes, about 150 minutes, about 175 minutes, or about 200 minutes in the presence of pancreatin. Uricase stability or half-life may be measured by any method known in the art, including absorption based assays or SDS-PAGE as described in Example 1. Uricase half-life in the presence of pancreatin will depend upon the experimental conditions in which the half-life is measured, including, *e.g.*, the concentration of pancreatin. In certain embodiments, the half-life of a disclosed recombinant mutant *Candida utilis* uricase in the presence of pancreatin is measured in the presence of 20 ng/μL or 80 ng/μL pancreatin, *e.g.*, pancreatin available from Sigma-Aldrich (Cat No. P7545).

[0057] Alternatively or in addition, it is contemplated that a recombinant mutant *Candida utilis* uricase enzyme disclosed herein may, for example, have higher stability at a pH less than about 6.5 compared to the wild-type *C. utilis* uricase. For example, a recombinant mutant *C. utilis* uricase may have from 5 to 50 fold higher stability in the presence of pancreatin compared to the wild-type *C. utilis* uricase. In certain embodiments, the uricase enzyme has from about 5 to about 50, from about 5 to about 40, from about 5 to about 30,

from about 5 to about 20, from about 5 to about 10, from about 10 to about 50, from about 10 to about 40, from about 10 to about 30, from about 10 to about 20, from about 20 to about 50, from about 20 to about 40, from about 20 to about 30, from about 30 to about 50, from about 30 to about 40, from about 40 to about 50, about 5, about 10, about 20, about 30, about 40, or about 50 fold higher stability at a pH less than about 6.5 compared to the wild-type *C. utilis* uricase. Uricase stability or half-life may be measured by any method known in the art, including absorption based assays or SDS-PAGE as described in Example 1.

[0058] The invention further provides a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, G44A, E51K, and I132R, *e.g.,* a recombinant mutant *C. Utilis* uricase comprising the following amino acid sequence, *e.g.,* a recombinant mutant uricase referred to as R2_V17 herein:

MSTTLSSSTYGKDNVKFLKVKKDPQNPKKQEVMEATVTCLLEGAFDTSYTKADNSSIVPTDT
VKNTILVLAKTTEIWPIERFAAKLATHFVEKYSHVSGVSVKIVQDRWVKYAVDGKPHDHSFI
HEGGEKRRTDLYYKRSGDYKLSSAIKDLTVLKSTGSMFYGFNKCDFTTLQPTTDRVLSTDVD
ATWVWDNKKIGSVYDIAKAADKGIFDNVYNQAREITLTTFALENSPSVQATMFNMATQILEK
ACSVYSVSYALPNKHYFLIDLKWKGLENDNELFYPSPHPNGLIKCTVVRKEKTKL (SEQ ID NO: 2).

[0059] The invention further provides a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, E51K, V97I, and A236N, *e.g.,* a recombinant mutant *C. Utilis* uricase comprising the following amino acid sequence, *e.g.,* a recombinant mutant uricase referred to as R2_V4 herein:

MSTTLSSSTYGKDNVKFLKVKKDPQNPKKQEVMEATVTCLLEGGFDTSYTKADNSSIVPTDT
VKNTILVLAKTTEIWPIERFAAKLATHFVEKYSHISGVSVKIVQDRWVKYAVDGKPHDHSFI
HEGGEKRITDLYYKRSGDYKLSSAIKDLTVLKSTGSMFYGFNKCDFTTLQPTTDRVLSTDVD
ATWVWDNKKIGSVYDIAKAADKGIFDNVYNQAREITLTTFALENSPSVQNTMFNMATQILEK
ACSVYSVSYALPNKHYFLIDLKWKGLENDNELFYPSPHPNGLIKCTVVRKEKTKL (SEQ ID NO: 3).

[0060] The invention further provides a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, I132R, Q217L, and P285S, *e.g.,* a recombinant mutant *C. Utilis* uricase comprising the following amino acid sequence, *e.g.,* a recombinant mutant uricase referred to as R2_V79 herein:

MSTTLSSSTYGKDNVKFLKVKKDPQNPKKQEVMEATVTCLLEGGFDTSYTEADNSSIVPTDT
VKNTILVLAKTTEIWPIERFAAKLATHFVEKYSHVSGVSVKIVQDRWVKYAVDGKPHDHSFI
HEGGEKRRTDLYYKRSGDYKLSSAIKDLTVLKSTGSMFYGFNKCDFTTLQPTTDRVLSTDVD
ATWVWDNKKIGSVYDIAKAADKGIFDNVYNLAREITLTTFALENSPSVQATMFNMATQILEK
ACSVYSVSYALPNKHYFLIDLKWKGLENDNELFYPSSHPNGLIKCTVVRKEKTKL (SEQ ID NO: 4).

[0061] The invention further provides a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, E51K, V97I, and I196L, *e.g.,* a recombinant mutant *C. Utilis* uricase comprising the following amino acid sequence, *e.g.,* a recombinant mutant uricase referred to as R2_V47 herein:

MSTTLSSSTYGKDNVKFLKVKKDPQNPKKQEVMEATVTCLLEGGFDTSYTKADNSSIVPTDT
VKNTILVLAKTTEIWPIERFAAKLATHFVEKYSHISGVSVKIVQDRWVKYAVDGKPHDHSFI
HEGGEKRITDLYYKRSGDYKLSSAIKDLTVLKSTGSMFYGFNKCDFTTLQPTTDRVLSTDVD
ATWVWDNKKLGSVYDIAKAADKGIFDNVYNQAREITLTTFALENSPSVQATMFNMATQILEK
ACSVYSVSYALPNKHYFLIDLKWKGLENDNELFYPSPHPNGLIKCTVVRKEKTKL (SEQ ID NO: 5).

[0062] The invention further provides a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, E51K, D142E, and Q217L, *e.g.,* a recombinant mutant *C. Utilis* uricase comprising the following amino acid sequence, *e.g.,* a recombinant mutant uricase referred to as R2_V39 herein:

MSTTLSSSTYGKDNVKFLKVKKDPQNPKKQEVMEATVTCLLEGGFDTSYTKADNSSIVPTDT
VKNTILVLAKTTEIWPIERFAAKLATHFVEKYSHVSGVSVKIVQDRWVKYAVDGKPHDHSFI
HEGGEKRITDLYYKRSGEYKLSSAIKDLTVLKSTGSMFYGFNKCDFTTLQPTTDRVLSTDVD
ATWVWDNKKIGSVYDIAKAADKGIFDNVYNLAREITLTTFALENSPSVQATMFNMATQILEK
ACSVYSVSYALPNKHYFLIDLKWKGLENDNELFYPSPHPNGLIKCTVVRKEKTKL (SEQ ID NO: 6).

[0063] The invention further provides a recombinant mutant *C. Utilis* uricase that has at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a *C. Utilis* uricase disclosed herein, and has at least 60% specific activity and/or 5 fold higher stability as wild type *C. Utilis* uricase. Sequence identity may be determined in various ways that are within the skill in the art, e.g., using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul, (1993) J. MOL. EVOL. 36, 290-300; Altschul *et al.*, (1997) NUCLEIC ACIDS RES. 25:3389-3402, incorporated by reference) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases, see Altschul *et al.*, (1994) NATURE GENETICS 6:119-129, which is fully incorporated by reference. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default

settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919, fully incorporated by reference). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (e.g.: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and –Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, *e.g.*, Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0064] It is contemplated that a disclosed recombinant mutant *C. Utilis* uricase may be modified, engineered or chemically conjugated. For example, it is contemplated that a disclosed recombinant mutant *C. Utilis* uricase can be conjugated to an effector agent using standard *in vitro* conjugation chemistries. If the effector agent is a polypeptide, the uricase enzyme can be chemically conjugated to the effector or joined to the effector as a fusion protein. Construction of fusion proteins is within ordinary skill in the art.

[0065] In certain embodiments, depending upon a particular mode of administration or site of activity, a disclosed recombinant mutant *C. Utilis* uricase can be modified with a moiety that improves its stabilization and/or retention in circulation, *e.g.*, in blood, serum, or other tissues. For example, a disclosed recombinant mutant *C. Utilis* uricase enzyme may be conjugated to a polymer, *e.g.*, a substantially non-antigenic polymer, such as a polyalkylene oxide or a polyethylene oxide. In certain embodiments, a disclosed recombinant mutant *C.*

Utilis uricase enzyme is conjugated to a water soluble polymer, *e.g.*, a hydrophilic polyvinyl polymer, *e.g.*, polyvinylalcohol or polyvinylpyrrolidone. Examples of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof. Additional useful polymers include polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene, polymethacrylates, carbomers, and branched or unbranched polysaccharides.

III. Uricase Production

[0066] Methods for producing uricase enzymes of the invention are known in the art. For example, DNA molecules encoding a uricase enzyme can be chemically synthesized using the sequence information provided herein. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, *e.g.*, expression control sequences, to produce conventional gene expression constructs encoding the desired uricase enzyme.

[0067] Nucleic acids encoding desired uricase enzymes can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the uricase enzyme.

[0068] Nucleic acids encoding recombinant mutant *C. Utilis* uricases of the invention may be generated by mutating a nucleotide sequence encoding the wild type *C. utilis* uricase, *e.g.*, SEQ ID NO: 7 disclosed herein, using methods known in the art. Furthermore, in certain embodiments, nucleic acids encoding recombinant mutant *C. Utilis* uricases of the invention may be codon optimized for expression in a heterologous cell, *e.g.,* an *E. coli* cell, using methods known in the art.

[0069] In one embodiment, an exemplary nucleotide sequence encoding a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, G44A, E51K, and I132R, *e.g.,* a recombinant mutant *C. Utilis* uricase referred to as R2_V17 herein, is as follows:

ATGTCGACGACCCTGAGCAGCAGCACCTATGGCAAAGATAATGTGAAATTTCTGAAAGTCAA
AAAAGACCCGCAGAACCCTAAGAAACAAGAGGTCATGGAAGCGACCGTTACGTGTCTGCTGG
AAGGCGCGTTCGACACCAGCTATACCAAAGCGGATAATTCCTCCATCGTTCCGACCGATACG
GTCAAGAACACCATTCTGGTTCTGGCCAAGACCACGGAAATCTGGCCAATTGAGCGCTTCGC
CGCGAAACTGGCGACCCATTTCGTTGAGAAGTACAGCCACGTGAGCGGCGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTCAAATATGCCGTGGATGGTAAGCCGCATGACCACAGCTTTATT
CACGAGGGTGGCGAGAAGCGTCGTACTGACCTGTATTACAAGCGCAGCGGTGACTACAAATT

GAGCAGCGCAATCAAAGACCTGACGGTCCTGAAAAGCACCGGTTCTATGTTTTACGGTTTCA
ATAAGTGCGACTTTACGACGCTCCAACCGACTACGGACCGTGTTCTGTCTACCGATGTAGAC
GCGACCTGGGTCTGGGATAACAAGAAAATTGGCAGCGTGTACGATATTGCGAAAGCCGCTGA
CAAGGGTATCTTCGACAACGTCTATAATCAAGCGCGTGAGATCACCCTGACCACGTTTGCTC
TGGAGAATTCCCCGAGCGTTCAGGCGACCATGTTTAACATGGCAACGCAGATTTTGGAAAAG
GCATGTAGCGTGTACAGCGTGAGCTATGCATTGCCGAATAAGCACTACTTCCTGATTGATCT
GAAGTGGAAGGGTCTGGAGAACGATAACGAACTGTTCTATCCGAGCCCGCACCCGAATGGTC
TGATCAAGTGCACCGTTGTGCGTAAAGAAAAGACTAAACTG (SEQ ID NO: 8).

[0070] An exemplary nucleotide sequence encoding a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, E51K, V97I , and A236N, *e.g.*, a recombinant mutant *C. Utilis* uricase referred to as R2_V4 herein, is as follows:

ATGTCGACGACCCTGAGCAGCAGCACCTATGGCAAAGATAATGTGAAATTTCTGAAAGTCAA
AAAAGACCCGCAGAACCCTAAGAAACAAGAGGTCATGGAAGCGACCGTTACGTGTCTGCTGG
AAGGCGGCTTCGACACCAGCTATACCAAAGCGGATAATTCCTCCATCGTTCCGACCGATACG
GTCAAGAACACCATTCTGGTTCTGGCCAAGACCACGGAAATCTGGCCAATTGAGCGCTTCGC
CGCGAAACTGGCGACCCATTTCGTTGAGAAGTACAGCCACATCAGCGGCGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTCAAATATGCCGTGGATGGTAAGCCGCATGACCACAGCTTTATT
CACGAGGGTGGCGAGAAGCGTATCACTGACCTGTATTACAAGCGCAGCGGTGACTACAAATT
GAGCAGCGCAATCAAAGACCTGACGGTCCTGAAAAGCACCGGTTCTATGTTTTACGGTTTCA
ATAAGTGCGACTTTACGACGCTCCAACCGACTACGGACCGTGTTCTGTCTACCGATGTAGAC
GCGACCTGGGTCTGGGATAACAAGAAAATTGGCAGCGTGTACGATATTGCGAAAGCCGCTGA
CAAGGGTATCTTCGACAACGTCTATAATCAAGCGCGTGAGATCACCCTGACCACGTTTGCTC
TGGAGAATTCCCCGAGCGTTCAGAACACCATGTTTAACATGGCAACGCAGATTTTGGAAAAG
GCATGTAGCGTGTACAGCGTGAGCTATGCATTGCCGAATAAGCACTACTTCCTGATTGATCT
GAAGTGGAAGGGTCTGGAGAACGATAACGAACTGTTCTATCCGAGCCCGCACCCGAATGGTC
TGATCAAGTGCACCGTTGTGCGTAAAGAAAAGACTAAACTG (SEQ ID NO: 9).

[0071] An exemplary nucleotide sequence encoding a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, I132R, Q217L, and P285S, *e.g.*, a recombinant mutant *C. Utilis* uricase referred to as R2_V79 herein, is as follows:

ATGTCGACGACCCTGAGCAGCAGCACCTATGGCAAAGATAATGTGAAATTTCTGAAAGTCAA
AAAAGACCCGCAGAACCCTAAGAAACAAGAGGTCATGGAAGCGACCGTTACGTGTCTGCTGG
AAGGCGGCTTCGACACCAGCTATACCGAAGCGGATAATTCCTCCATCGTTCCGACCGATACG
GTCAAGAACACCATTCTGGTTCTGGCCAAGACCACGGAAATCTGGCCAATTGAGCGCTTCGC
CGCGAAACTGGCGACCCATTTCGTTGAGAAGTACAGCCACGTGAGCGGCGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTCAAATATGCCGTGGATGGTAAGCCGCATGACCACAGCTTTATT
CACGAGGGTGGCGAGAAGCGTCGTACTGACCTGTATTACAAGCGCAGCGGTGACTACAAATT
GAGCAGCGCAATCAAAGACCTGACGGTCCTGAAAAGCACCGGTTCTATGTTTTACGGTTTCA
ATAAGTGCGACTTTACGACGCTCCAACCGACTACGGACCGTGTTCTGTCTACCGATGTAGAC
GCGACCTGGGTCTGGGATAACAAGAAAATTGGCAGCGTGTACGATATTGCGAAAGCCGCTGA
CAAGGGTATCTTCGACAACGTCTATAATCTGGCGCGTGAGATCACCCTGACCACGTTTGCTC
TGGAGAATTCCCCGAGCGTTCAGGCGACCATGTTTAACATGGCAACGCAGATTTTGGAAAAG
GCATGTAGCGTGTACAGCGTGAGCTATGCATTGCCGAATAAGCACTACTTCCTGATTGATCT
GAAGTGGAAGGGTCTGGAGAACGATAACGAACTGTTCTATCCGAGCAGCCACCCGAATGGTC
TGATCAAGTGCACCGTTGTGCGTAAAGAAAAGACTAAACTG (SEQ ID NO: 10).

[0072] An exemplary nucleotide sequence encoding a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, E51K, V97I , and I196L, *e.g.,* a recombinant mutant *C. Utilis* uricase referred to as R2_V47 herein, is as follows:

ATGTCGACGACCCTGAGCAGCAGCACCTATGGCAAAGATAATGTGAAATTTCTGAAAGTCAA
AAAAGACCCGCAGAACCCTAAGAAACAAGAGGTCATGGAAGCGACCGTTACGTGTCTGCTGG
AAGGCGGCTTCGACACCAGCTATACCAAAGCGGATAATTCCTCCATCGTTCCGACCGATACG
GTCAAGAACACCATTCTGGTTCTGGCCAAGACCACGGAAATCTGGCCAATTGAGCGCTTCGC
CGCGAAACTGGCGACCCATTTCGTTGAGAAGTACAGCCACATCAGCGGCGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTCAAATATGCCGTGGATGGTAAGCCGCATGACCACAGCTTTATT
CACGAGGGTGGCGAGAAGCGTATCACTGACCTGTATTACAAGCGCAGCGGTGACTACAAATT
GAGCAGCGCAATCAAAGACCTGACGGTCCTGAAAAGCACCGGTTCTATGTTTTACGGTTTCA
ATAAGTGCGACTTTACGACGCTCCAACCGACTACGGACCGTGTTCTGTCTACCGATGTAGAC
GCGACCTGGGTCTGGGATAACAAGAAACTGGGCAGCGTGTACGATATTGCGAAAGCCGCTGA
CAAGGGTATCTTCGACAACGTCTATAATCAAGCGCGTGAGATCACCCTGACCACGTTTGCTC
TGGAGAATTCCCCGAGCGTTCAGGCGACCATGTTTAACATGGCAACGCAGATTTTGGAAAAG
GCATGTAGCGTGTACAGCGTGAGCTATGCATTGCCGAATAAGCACTACTTCCTGATTGATCT
GAAGTGGAAGGGTCTGGAGAACGATAACGAACTGTTCTATCCGAGCCCGCACCCGAATGGTC
TGATCAAGTGCACCGTTGTGCGTAAAGAAAAGACTAAACTG (SEQ ID NO: 11).

[0073] An exemplary nucleotide sequence encoding a recombinant mutant *C. Utilis* uricase that comprises the following substitutions: Y165F, I180V, E51K, D142E, and Q217L, *e.g.,* a recombinant mutant *C. Utilis* uricase referred to as R2_V39 herein, is as follows:

ATGTCGACGACCCTGAGCAGCAGCACCTATGGCAAAGATAATGTGAAATTTCTGAAAGTCAA
AAAAGACCCGCAGAACCCTAAGAAACAAGAGGTCATGGAAGCGACCGTTACGTGTCTGCTGG
AAGGCGGCTTCGACACCAGCTATACCAAAGCGGATAATTCCTCCATCGTTCCGACCGATACG
GTCAAGAACACCATTCTGGTTCTGGCCAAGACCACGGAAATCTGGCCAATTGAGCGCTTCGC
CGCGAAACTGGCGACCCATTTCGTTGAGAAGTACAGCCACGTGAGCGGCGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTCAAATATGCCGTGGATGGTAAGCCGCATGACCACAGCTTTATT
CACGAGGGTGGCGAGAAGCGTATCACTGACCTGTATTACAAGCGCAGCGGTGAGTACAAATT
GAGCAGCGCAATCAAAGACCTGACGGTCCTGAAAAGCACCGGTTCTATGTTTTACGGTTTCA
ATAAGTGCGACTTTACGACGCTCCAACCGACTACGGACCGTGTTCTGTCTACCGATGTAGAC
GCGACCTGGGTCTGGGATAACAAGAAAATTGGCAGCGTGTACGATATTGCGAAAGCCGCTGA
CAAGGGTATCTTCGACAACGTCTATAATCTGGCGCGTGAGATCACCCTGACCACGTTTGCTC
TGGAGAATTCCCCGAGCGTTCAGGCGACCATGTTTAACATGGCAACGCAGATTTTGGAAAAG
GCATGTAGCGTGTACAGCGTGAGCTATGCATTGCCGAATAAGCACTACTTCCTGATTGATCT
GAAGTGGAAGGGTCTGGAGAACGATAACGAACTGTTCTATCCGAGCCCGCACCCGAATGGTC
TGATCAAGTGCACCGTTGTGCGTAAAGAAAAGACTAAACTG (SEQ ID NO: 12).

[0074] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it can be cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. The expressed secreted protein accumulates in refractile or inclusion bodies, and can be harvested

after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art.

[0075] A uricase enzyme can be produced by growing (culturing) a host cell transfected with an expression vector encoding such uricase enzyme, under conditions that permit expression of the uricase enzyme. Following expression, the uricase enzyme can be harvested and purified or isolated using techniques known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) and histidine tags. An exemplary expression and purification protocol for a uricase enzyme is described in Liu *et al.* (2011) APPL. MICROBIOL. BIOTECHNOL. 92(3):529-37.

IV. Pharmaceutical Compositions

[0076] For therapeutic use, a recombinant uricase enzyme described herein preferably is combined with a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0077] The term "pharmaceutically acceptable carrier" as used herein refers to buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see, *e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975]. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[0078] In certain embodiments, the uricase enzymes can be formulated, or co-administered (either at the same time or sequentially), for example, by an enteral route (e.g., orally), with a

pH increasing agent, for example, a protein pump inhibitor (PPI), to enhance the stability of the uricase enzyme, for example, in an acidic environment, for example, in the gastrointestinal tract.

[0079] Proton pump inhibitors are a group of drugs whose main action is pronounced and long-lasting reduction of gastric acid production. Proton pump inhibitors act by blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H^+/K^+ ATPase, or more commonly just gastric proton pump) of the gastric parietal cell. The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H^+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion. Examples of proton pump inhibitors include: Omeprazole (brand names: LOSEC®, PRILOSEC®, ZEGERID®); Lansoprazole (brand names: PREVACID®, ZOTON®, INHIBITOL®); Esomeprazole (brand names: NEXIUM®); and Pantoprazole (brand names: PROTONIX®, SOMAC®, PANTOLOC®).

[0080] Pharmaceutical compositions containing a recombinant uricase enzyme disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions, dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form will depend upon the intended mode of administration and therapeutic application.

[0081] Although the compositions preferably are formulated for administration enterally (for example, orally), such compositions can be administered by a parenteral mode (e.g., intravenous, subcutaneous, intraperitoneal, or intramuscular injection). The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and infrasternal injection and infusion.

[0082] The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile

injectable solutions can be prepared by incorporating an agent described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating an agent described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yield a powder of an agent described herein plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0083] Depending upon the mode of administration, for example, by parenteral administration, it may be desirable to produce a pharmaceutical formulation that is sterile. Sterilization can be accomplished by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[0084] In certain embodiments, a disclosed composition comprises a polyionic reagent which may, *e.g.,* coat the uricase (*e.g.,* the composition comprises a polyionic coating). Exemplary polyionic reagents include PSS (poly(Sodium 4-styrenesulfonate), PAA (poly Acrylic acid sodium salt), PMG (poly(methylene-co-guanidine) hydrochloride), DS (dextran sulfate), PMA (poly(methyl acrylate)), or PVS (polyvinylsiloxane).

V. Therapeutic Uses

[0085] The recombinant uricase enzymes disclosed herein can be used to treat various diseases or disorders associated with an elevated amount of uric acid in a subject. As used herein, "elevated amount of uric acid in a subject" may refer to an elevated amount of uric acid in a body fluid (*e.g.,* blood, plasma, serum, or urine), tissue and/or cell in a subject, relative to a subject without the disease or disorder. In human blood, uric acid concentrations between 2.4-6 mg/dL for females and 3.4-7.2 mg/dL for males are considered normal by the Clinical Mayo Reference laboratory.

[0086] The invention provides a method of treating a disease or disorder associated with an elevated amount of uric acid in a subject. In certain embodiments, the disease or disorder is associated with an elevated amount of uric acid in plasma of the subject. The method comprises administering to the subject an effective amount of a disclosed recombinant uricase, either alone or in a combination with another therapeutic agent to treat the disease or disorder in the subject. The term "effective amount" as used herein refers to the amount of an active agent (*e.g.*, a recombinant uricase of the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[0087] In certain embodiments, the method comprises orally administering to the subject an effective amount of a disclosed recombinant uricase, either alone or in a combination with another therapeutic agent to treat the disease or disorder in the subject. It is contemplated that, in certain embodiments, the orally administered recombinant uricase may avoid passive absorption in the intestine due to its size, and if metabolized, the novel recombinant uricase of the present invention orally administered with food would be metabolized in a manner similar to that of any other ingested protein.

[0088] As used herein, "treat", "treating" and "treatment" mean the treatment of a disease in a subject, *e.g.*, in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its development; and (b) relieving the disease, *i.e.*, causing regression of the disease state. As used herein, the terms "subject" and "patient" refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[0089] Examples of diseases or disorders associated with an elevated amount of uric acid include a metabolic disorder, *e.g.*, metabolic syndrome, hyperuricemia, gout (*e.g.*, gouty arthritis), Lesch-Nyhan syndrome, cardiovascular disease, diabetes, hypertension, renal disease, metabolic syndrome, uric acid nephrolithiasis (or kidney stones (*see* Wiederkehr et al. (2011), *Clin. Rev. Bone. Miner. Metab.*, 9(3-4):207-217 ("Uric acid nephrolithiasis is characteristically a manifestation of a systemic metabolic disorder. It has a prevalence of about 10% among all stone formers, the third most common type of kidney stone in the industrialized world."))), tumor lysis syndrome, and hyperuricosuria.

[0090] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered "in combination," as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, such that the effects of the treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In certain embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[0091] In certain embodiments, a method or composition described herein, is administered in combination with one or more additional therapies selected from a xanthine-oxidase inhibitor (*e.g.*, allopurinol, TEI-6720 (2-(3-cyano-4-isobutoxyphenyl)-4-methyl-5-thiazolecarboxylic acid), febuxostat (2-[3-cyano-4-isobutoxyphenyl]-4-methylthiazole-5-carboxylic acid), oxypurinol, or pteridylaldehyde), a uricosuric (*e.g.*, probenecid, lesinurad, sulfinpyrazone, sulfinpyrazone, or fenofibrate), ethylenediaminetetraacetic acid, acetazolamide, a potassium supplement, and any combination thereof.

[0092] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[0093] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the

element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[0094] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

[0095] It should be understood that the expression "at least one of" includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression "and/or" in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[0096] The use of the term "include," "includes," "including," "have," "has," "having," "contain," "contains," or "containing," including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[0097] Where the use of the term "about" is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term "about" refers to a ±10% variation from the nominal value unless otherwise indicated or inferred.

[0098] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[0099] The use of any and all examples, or exemplary language herein, for example, "such as" or "including," is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

EXAMPLES

[0100] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1 – Recombinant mutant *Candida utilis* uricase design and testing

[0101] This example describes the design and testing of recombinant mutant *Candida utilis* uricases with improved pancreatin stability.

[0102] 95 mutant *C. utilis* uricases were designed each with three amino acid substitutions relative to the wild-type sequence. The mutant *C. utilis* uricases are indicated as R1_V1 - R1_V95.

[0103] Briefly, DNA fragments encoding the 95 mutant *C. utilis* uricases were cloned into a rhamanose pD861-NH expression vector (ATUM, Newark, CA) that encodes a N-terminal His-tag. All constructs were confirmed by sequencing. Following expression in *Escherichia coli* cells, each recombinant mutant *C. utilis* uricase enzyme was bound to a Ni-NTA column and eluted in a buffer containing 25 mM Tris-HCl pH 8.0, 100 mM NaCl, 200 mM imidazole, and 50% (v/v) glycerol.

[0104] The purified recombinant mutant *C. utilis* uricases were tested for enzymatic activity in the presence of pancreatin (Sigma-Aldrich Cat No. P7545; which converts at least 25 times its weight of potato starch into soluble carbohydrates in 5 minutes in water at 40°C, digests at least 25 times its weight of casein in 60 minutes at pH 7.5 at 40°C, and releases at least 2 microequivalents of acid per minute per mg pancreatin from olive oil at pH 9.0 at 37°C) to determine pancreatin stability. Briefly, 25 ng/μL of uricase was incubated with 20 ng/μL of pancreatin at 37°C for up to 200 minutes. The assay was performed in simulated intestinal fluid (SIF) buffer (50mM potassium phosphate, pH 6.8) in 96 well plates. Following incubation with pancreatin for the indicated time points, enzymatic activity was monitored using an absorption based assay. Uric acid has a strong absorbance at 293nm, and the enzymatic oxidation of uric acid to 5-hydroxyisourate by uricase results in a corresponding drop in 293nm absorbance over time.

[0105] Results for *C. utilis* uricase mutants with the most improved pancreatin stability were confirmed over multiple protein preparations. Representative data for wild type *C. utilis* uricase is depicted in **FIGURE 1,** and representative data for a subset of mutant *C. utilis* uricases is depicted in **FIGURE 2.**

[0106] **TABLE 3** depicts the amino acid substitutions for the 95 recombinant mutant *C. utilis* uricases, as well as the specific activity (μM/minute per 1.2ng/μl of uricase), pancreatin stability (half-life, minutes) and expression yield (μg/ml) for each enzyme. "nd" indicates that activity and stability measurements were not determined due to insufficient expression yield.

TABLE 3

Clone	Specific Activity*	Pancreatin Stability#	Expression (μg/ml)	Substitutions		
WT	4-4.9	10-19	200-299			
R1_V1	>5	0-9	>300	G44A	S95P	P285S
R1_V2	4-4.9	0-9	200-299	S140T	Y163H	S254N
R1_V3	3-3.9	0-9	200-299	T38C	H119S	T243Q
R1_V4	4-4.9	0-9	100-199	E92D	Y137A	K167R
R1_V5	>5	>50	10-99	K130T	I180V	V190A
R1_V6	4-4.9	0-9	200-299	Y136H	I196L	K208G
R1_V7	4-4.9	10-19	200-299	V97I	Y136H	V185I
R1_V8	4-4.9	0-9	200-299	T62S	I196L	D201E
R1_V9	4-4.9	0-9	200-299	V69I	Q244D	H286C
R1_V10	0-2.9	0-9	200-299	H125K	Y163H	Y253Q
R1_V11	0-2.9	0-9	100-199	A84S	H125K	N276D
R1_V12	3-3.9	0-9	100-199	H119S	Y136D	S254N
R1_V13	>5	0-9	>300	K130T	D142E	F239Y
R1_V14	4-4.9	0-9	100-199	K167R	T243Q	S254N
R1_V15	0-2.9	0-9	10-99	Y136H	Y143A	M161Q
R1_V16	3-3.9	20-49	200-299	E51K	H125K	Q217L
R1_V17	>5	0-9	>300	D142E	Y163H	D201E
R1_V18	4-4.9	0-9	200-299	G44A	E51K	G159N
R1_V19	4-4.9	0-9	200-299	Y136D	I196L	S251L
R1_V20	4-4.9	20-49	200-299	Y165F	D201E	A242C
R1_V21	>5	10-19	10-99	A83G	V97I	D201E
R1_V22	4-4.9	10-19	200-299	E92N	Q244D	Y253Q
R1_V23	4-4.9	0-9	200-299	D46E	V97I	H286C
R1_V24	3-3.9	0-9	200-299	D46E	V69I	G159N
R1_V25	4-4.9	0-9	200-299	F170Y	S198G	V214A
R1_V26	4-4.9	0-9	200-299	S95A	A113E	H286C
R1_V27	4-4.9	0-9	100-199	E92D	Y136D	H286A
R1_V28	0-2.9	0-9	200-299	Y136D	Y163H	N276D
R1_V29	nd	nd	0-9	Y143A	S251L	H286C
R1_V30	nd	nd	0-9	A83G	Y163H	G197A

Clone	Specific Activity*	Pancreatin Stability#	Expression (μg/ml)	Substitutions		
R1_V31	>5	10-19	200-299	T38C	G128P	S251L
R1_V32	3-3.9	10-19	200-299	H125K	G128P	I196L
R1_V33	4-4.9	0-9	10-99	L70E	V190A	A236N
R1_V34	4-4.9	10-19	100-199	A242C	Q244D	P285S
R1_V35	3-3.9	0-9	100-199	E92N	D201E	E229D
R1_V36	4-4.9	0-9	200-299	Y136H	S256N	F281Y
R1_V37	4-4.9	0-9	10-99	L70E	V105I	Q217L
R1_V38	4-4.9	20-49	200-299	I180V	V214A	A242C
R1_V39	nd	nd	0-9	V105I	Y143A	A236N
R1_V40	4-4.9	10-19	200-299	K130T	F170Y	A236N
R1_V41	nd	nd	0-9	G44A	S140T	Y143A
R1_V42	4-4.9	10-19	200-299	V105I	S140T	D142E
R1_V43	4-4.9	10-19	10-99	A83G	A84S	V185I
R1_V44	4-4.9	10-19	100-199	A113E	K208G	Q244D
R1_V45	3-3.9	0-9	200-299	V69I	F239Y	S251L
R1_V46	3-3.9	0-9	100-199	D46E	T62S	A242C
R1_V47	4-4.9	0-9	>300	S95P	F281Y	H286A
R1_V48	3-3.9	0-9	200-299	T62S	Q244D	F281Y
R1_V49	4-4.9	0-9	200-299	A113E	Y253Q	S256N
R1_V50	4-4.9	0-9	200-299	V105I	G128P	E229D
R1_V51	4-4.9	10-19	200-299	A84S	S140T	F281Y
R1_V52	>5	0-9	100-199	G197A	Y253Q	H286A
R1_V53	4-4.9	10-19	200-299	G44A	G128P	V185I
R1_V54	4-4.9	0-9	10-99	V97I	G197A	V214A
R1_V55	0-2.9	0-9	200-299	T62S	H119S	M161Q
R1_V56	4-4.9	0-9	200-299	V69I	A84S	A236N
R1_V57	>5	>50	10-99	Y165F	I180V	G197A
R1_V58	4-4.9	20-49	200-299	Y165F	Q217L	T243Q
R1_V59	3-3.9	0-9	200-299	G159N	K167R	F239Y
R1_V60	4-4.9	10-19	200-299	E92D	S140T	P285S
R1_V61	4-4.9	0-9	10-99	V69I	L70E	E92N
R1_V62	4-4.9	0-9	10-99	L70E	K130T	T243Q
R1_V63	3-3.9	0-9	200-299	H119S	K208G	H286A
R1_V64	0-2.9	20-49	100-199	A83G	H119S	Y165F
R1_V65	4-4.9	10-19	100-199	T38C	M161Q	S254N
R1_V66	4-4.9	10-19	200-299	S95P	Q217L	A236N
R1_V67	4-4.9	20-49	100-199	E51K	Y137A	Y165F
R1_V68	4-4.9	10-19	100-199	E92N	S95A	K130T
R1_V69	4-4.9	0-9	100-199	A84S	G159N	S198G
R1_V70	4-4.9	20-49	200-299	E92D	I180V	F281Y
R1_V71	0-2.9	0-9	100-199	G159N	F170Y	N276D
R1_V72	4-4.9	20-49	200-299	G44A	V97I	S256N
R1_V73	4-4.9	10-19	10-99	E92D	V190A	S198G
R1_V74	4-4.9	0-9	200-299	S95A	F239Y	S256N
R1_V75	4-4.9	0-9	200-299	K208G	V214A	H286C
R1_V76	4-4.9	0-9	200-299	T38C	D142E	E229D
R1_V77	0-2.9	0-9	200-299	H125K	Y136H	V214A
R1_V78	4-4.9	0-9	200-299	S95P	Y137A	D142E

Clone	Specific Activity*	Pancreatin Stability#	Expression (μg/ml)	Substitutions		
R1_V79	4-4.9	10-19	200-299	S95A	V185I	Q217L
R1_V80	4-4.9	10-19	200-299	E51K	F170Y	T243Q
R1_V81	nd	nd	0-9	T38C	Y136D	Y143A
R1_V82	4-4.9	10-19	200-299	M161Q	S198G	A242C
R1_V83	4-4.9	0-9	200-299	S95P	V105I	S256N
R1_V84	nd	nd	0-9	L70E	Y137A	F170Y
R1_V85	4-4.9	0-9	100-199	D46E	V190A	E229D
R1_V86	4-4.9	0-9	200-299	S95A	K167R	P285S
R1_V87	4-4.9	0-9	200-299	V190A	F239Y	H286A
R1_V88	3-3.9	0-9	100-199	T62S	E92N	Y137A
R1_V89	4-4.9	0-9	200-299	D46E	A113E	G128P
R1_V90	>5	10-19	200-299	I196L	Y253Q	P285S
R1_V91	4-4.9	10-19	200-299	E51K	M161Q	V185I
R1_V92	0-2.9	0-9	10-99	A83G	A113E	N276D
R1_V93	4-4.9	0-9	10-99	G197A	K208G	S251L
R1_V94	4-4.9	0-9	200-299	K167R	I180V	E229D
R1_V95	0-2.9	0-9	10-99	S198G	S254N	N276D

*Specific Activity unit: μM/minute per 1.2ng/μl of uricase; #Pancreatin Stability unit: half-life, minutes

[0107] An analysis of the 95 recombinant mutant *C. utilis* uricases using protein modeling tools identified Y165F and I180V as key substitutions contributing towards improved pancreatin stability. As a result, a mutant *C. utilis* uricase enzyme containing these two substitutions was used a parent in the design of a second round of *C. utilis* uricases.

[0108] Unless otherwise indicated, the second round of mutational design, expression, purification, and pancreatin stability assays were all conducted as described above. The process resulted in 95 mutant *C. utilis* uricases each with five amino substitutions relative to the wild-type sequence, two of which in each case were the Y165F and I180V substitutions. The mutant *C. utilis* uricases are indicated as R2_V1 – R2_V95 in **TABLE 4**.

[0109] **TABLE 4** depicts the amino acid substitutions for the 95 mutant *C. utilis* uricases, as well as the specific activity (μM/minute per 1.2ng/μl of uricase), pancreatin stability (half-life, minutes) and expression yield (μg/ml) for each enzyme. Pancreatin stability was assayed at 80 ng/μL soluble pancreatin. "nd" indicates that activity and stability measurements were not determined due to insufficient expression yield.

TABLE 4

Clone	Specific Activity*	Pancreatin Stability#	Expression (μg/ml)	Substitutions				
R2 Parent	4-4.9	30-49	200-299	Y165F	I180V			
R2_V1	4-4.9	10-29	>300	Y165F	I180V	Q25A	T47E	S256D
R2_V2	4-4.9	30-49	200-299	Y165F	I180V	D142Q	Q217L	A236N
R2_V3	4-4.9	30-49	200-299	Y165F	I180V	G128P	R139E	D142E
R2_V4	4-4.9	>50	200-299	Y165F	I180V	E51K	V97I	A236N
R2_V5	3-3.9	0-9	200-299	Y165F	I180V	E51K	F170Y	W271R
R2_V6	4-4.9	10-29	100-199	Y165F	I180V	D134E	R139E	V296A
R2_V7	4-4.9	10-29	200-299	Y165F	I180V	A87G	E220A	T224D
R2_V8	3-3.9	10-29	>300	Y165F	I180V	G44A	G128P	K270E
R2_V9	4-4.9	10-29	200-299	Y165F	I180V	D142Q	I149L	F165Y
R2_V10	4-4.9	30-49	>300	Y165F	I180V	G44A	Y136R	Y253Q
R2_V11	3-3.9	0-9	200-299	Y165F	I180V	I132R	S256D	W271R
R2_V12	>5	30-49	100-199	Y165F	I180V	E51K	I149L	D268N
R2_V13	>5	0-9	200-299	Y165F	I180V	D142E	Q174G	S254N
R2_V14	4-4.9	>50	200-299	Y165F	I180V	E92N	I149L	Y253Q
R2_V15	4-4.9	>50	10-99	Y165F	I180V	I132N	V190A	N213A
R2_V16	4-4.9	30-49	200-299	Y165F	I180V	E51K	D142E	S256N
R2_V17	4-4.9	>50	200-299	Y165F	I180V	G44A	E51K	I132R
R2_V18	4-4.9	0-9	>300	Y165F	I180V	K103T	D134E	V180I
R2_V19	0-2.9	0-9	10-99	Y165F	I180V	K85I	S147T	Q217L
R2_V20	nd	nd	0-9	Y165F	I180V	E51K	Y137R	S254N
R2_V21	4-4.9	10-29	200-299	Y165F	I180V	A52S	A236N	S256N
R2_V22	4-4.9	30-49	>300	Y165F	I180V	G128P	Y253Q	P285S
R2_V23	4-4.9	10-29	10-99	Y165F	I180V	E51K	P118I	S147T
R2_V24	4-4.9	30-49	200-299	Y165F	I180V	A84S	S140T	K204A
R2_V25	4-4.9	30-49	>300	Y165F	I180V	E51K	G128P	F170Y
R2_V26	4-4.9	30-49	100-199	Y165F	I180V	E51K	A87G	D142Q
R2_V27	4-4.9	30-49	200-299	Y165F	I180V	E51K	G128P	N213A
R2_V28	4-4.9	30-49	200-299	Y165F	I180V	V97I	K103T	N213A
R2_V29	4-4.9	10-29	200-299	Y165F	I180V	K103T	F165Y	K208A
R2_V30	>5	30-49	200-299	Y165F	I180V	Q25A	E51K	V296A
R2_V31	4-4.9	0-9	100-199	Y165F	I180V	K85I	P118I	E220A
R2_V32	3-3.9	10-29	200-299	Y165F	I180V	E51K	Y253Q	K270E
R2_V33	nd	nd	0-9	Y165F	I180V	G128P	Y137R	A236N
R2_V34	4-4.9	10-29	>300	Y165F	I180V	Q25A	S95P	D142E
R2_V35	4-4.9	30-49	>300	Y165F	I180V	V97I	G128P	S140T
R2_V36	4-4.9	30-49	100-199	Y165F	I180V	G128P	N193R	S254N
R2_V37	4-4.9	30-49	200-299	Y165F	I180V	S95P	I132N	Y253Q
R2_V38	4-4.9	30-49	>300	Y165F	I180V	T47E	E92N	V97I
R2_V39	4-4.9	>50	>300	Y165F	I180V	E51K	D142E	Q217L
R2_V40	3-3.9	10-29	>300	Y165F	I180V	A52S	K85I	Q244K
R2_V41	4-4.9	10-29	200-299	Y165F	I180V	A84S	G128P	S256N
R2_V42	4-4.9	30-49	>300	Y165F	I180V	A84S	V97I	Y253Q
R2_V43	4-4.9	10-29	100-199	Y165F	I180V	A87G	I196L	S256N
R2_V44	nd	nd	0-9	Y165F	I180V	Y137R	D142Q	K204A

Clone	Specific Activity*	Pancreatin Stability#	Expression (μg/ml)	Substitutions				
R2_V45	4-4.9	>50	>300	Y165F	I180V	E51K	G128P	Y253Q
R2_V46	4-4.9	30-49	>300	Y165F	I180V	D142E	I196L	K208A
R2_V47	4-4.9	>50	>300	Y165F	I180V	E51K	V97I	I196L
R2_V48	3-3.9	0-9	>300	Y165F	I180V	Q174G	T224D	Y253Q
R2_V49	4-4.9	30-49	>300	Y165F	I180V	I132R	D142E	V296A
R2_V50	3-3.9	0-9	>300	Y165F	I180V	G44A	D142E	W271R
R2_V51	4-4.9	30-49	>300	Y165F	I180V	V97I	D142E	Y253Q
R2_V52	4-4.9	30-49	200-299	Y165F	I180V	A84S	D142E	V190A
R2_V53	4-4.9	30-49	200-299	Y165F	I180V	E92N	F170Y	N193R
R2_V54	3-3.9	0-9	>300	Y165F	I180V	G128P	V180I	Q217L
R2_V55	4-4.9	30-49	200-299	Y165F	I180V	V97I	F170Y	S254N
R2_V56	3-3.9	30-49	>300	Y165F	I180V	E92N	G128P	D142E
R2_V57	4-4.9	10-29	100-199	Y165F	I180V	A52S	I196L	S254N
R2_V58	4-4.9	10-29	200-299	Y165F	I180V	S140T	T224D	S256N
R2_V59	4-4.9	10-29	>300	Y165F	I180V	S95P	K103T	G128P
R2_V60	4-4.9	30-49	>300	Y165F	I180V	Y136R	Q244K	L274I
R2_V61	4-4.9	>50	200-299	Y165F	I180V	A84S	Q217L	Q244K
R2_V62	4-4.9	10-29	>300	Y165F	I180V	S95P	S140T	L274I
R2_V63	4-4.9	30-49	>300	Y165F	I180V	D142E	N193R	L274I
R2_V64	4-4.9	30-49	>300	Y165F	I180V	G44A	K204A	P285S
R2_V65	0-2.9	10-29	10-99	Y165F	I180V	V97I	D134E	Y137R
R2_V66	4-4.9	10-29	100-199	Y165F	I180V	A52S	E92N	S256D
R2_V67	nd	nd	0-9	Y165F	I180V	D142E	F165Y	P285S
R2_V68	4-4.9	30-49	100-199	Y165F	I180V	V97I	I132N	T224D
R2_V69	4-4.9	10-29	100-199	Y165F	I180V	F170Y	Q217L	D268N
R2_V70	4-4.9	30-49	200-299	Y165F	I180V	]S95P	Q217L	S254N
R2_V71	4-4.9	30-49	>300	Y165F	I180V	G44A	S95P	V97I
R2_V72	3-3.9	10-29	100-199	Y165F	I180V	D142E	S147T	F170Y
R2_V73	4-4.9	10-29	>300	Y165F	I180V	S140T	F165Y	A236N
R2_V74	4-4.9	10-29	100-199	Y165F	I180V	V97I	K208A	D268N
R2_V75	4-4.9	30-49	>300	Y165F	I180V	V97I	G128P	V190A
R2_V76	4-4.9	0-9	100-199	Y165F	I180V	Y136R	N193R	K270E
R2_V77	4-4.9	30-49	>300	Y165F	I180V	Q25A	G128P	I149L
R2_V78	4-4.9	10-29	>300	Y165F	I180V	V97I	P118I	D142E
R2_V79	4-4.9	>50	200-299	Y165F	I180V	I132R	Q217L	P285S
R2_V80	3-3.9	30-49	>300	Y165F	I180V	T47E	I196L	Y253Q
R2_V81	4-4.9	30-49	200-299	Y165F	I180V	E51K	Y136R	V190A
R2_V82	3-3.9	0-9	100-199	Y165F	I180V	E92N	V180I	D268N
R2_V83	nd	nd	0-9	Y165F	I180V	I132N	R139E	E220A
R2_V84	4-4.9	30-49	100-199	Y165F	I180V	A87G	K204A	L274I
R2_V85	0-2.9	0-9	10-99	Y165F	I180V	V97I	S147T	K270E
R2_V86	4-4.9	0-9	100-199	Y165F	I180V	R139E	Q174G	Q244K
R2_V87	4-4.9	30-49	200-299	Y165F	I180V	A84S	A236N	V296A
R2_V88	0-2.9	0-9	>300	Y165F	I180V	T47E	G128P	W271R
R2_V89	4-4.9	30-49	100-199	Y165F	I180V	E51K	K85I	P285S
R2_V90	4-4.9	0-9	>300	Y165F	I180V	V180I	Y253Q	S256D
R2_V91	4-4.9	>50	>300	Y165F	I180V	E51K	S140T	D142E
R2_V92	4-4.9	30-49	100-199	Y165F	I180V	V97I	E220A	S256N

Clone	Specific Activity*	Pancreatin Stability#	Expression (μg/ml)	Substitutions				
R2_V93	4-4.9	0-9	>300	Y165F	I180V	Q174G	N213A	P285S
R2_V94	4-4.9	10-29	>300	Y165F	I180V	P118I	G128P	I196L
R2_V95	4-4.9	30-49	>300	Y165F	I180V	D134E	K208A	Y253Q

*Specific Activity unit: μM/min per 1.2ng/μl of uricase; #Pancreatin stability unit: half-life, minutes

[0110] Representative pancreatin stability data for a subset of the mutant *C. utilis* uricases is depicted in **FIGURE 3.** A subset of mutant *C. utilis* uricases were further tested for thermal stability by differential scanning fluorimetry (DSF). DSF is a method to evaluate thermal stability by heating a protein in the presence of a fluorescent dye which will increase its fluorescence upon binding to the exposed hydrophobic interior of the protein after protein unfolding. Protein unfolding curves are depicted in **FIGURE 4.** As can be seen, R2_V17 has the highest melting temperature among those tested, with a 5°C increase relative to wild type uricase.

[0111] A subset of mutant *C. utilis* uricase enzymes were further tested for pancreatin stability by SDS-PAGE. **FIGURE 5** shows the analysis of R2_V17, R2_V4, and R2_V79 *C. utilis* uricase enzymes by SDS-PAGE following incubation of 144 ng/μL of uricase with 80 ng/μL of pancreatin in SIF buffer at 37°C for the indicated time points. **FIGURE 6** shows the analysis of wild type and R2_V17 *C. utilis* uricase enzymes by SDS-PAGE following incubation of 100 ng/μL of uricase with 320 ng/μL of pancreatin in SIF buffer at 37°C for the indicated time points. The results from the SDS-PAGE analysis are consistent with the activity assay data. In particular, the R2_V17, R2_V4 and R2_V79 mutants show increased stability in the presence of pancreatin relative to wild type.

[0112] Together, these results identify mutant *C. utilis* uricase enzymes with increased stability against pancreatin compared to the wild-type *C. utilis* uricase and without significantly decreased specific activity.

Example 2 – Identification of individual substitutions that improve *Candida utilis* uricase stability

[0113] This example describes the testing of individual substitutions included in the recombinant mutant *Candida utilis* uricases described in Example 1.

[0114] Among the various substitutions included in the mutant *Candida utilis* uricases described in Example 1, a set of individual substitutions were selected for testing by protein modeling tools. In certain instances, conservative substitutions were tested along with the

original substitution that was identified in Example 1. In total, 51 mutant *C. utilis* uricases, each with one amino acid substitution relative to the wild-type sequence, were designed and tested. The 51 mutant *C. utilis* uricases containing one amino acid substitution are indicated by the individual substitution in **TABLE 5**. The mutant *C. utilis* uricases were tested in a pancreatin stability assay along with a subset of the mutant *C. utilis* uricases described in Example 1. The subset of mutant *C. utilis* uricases described in Example 1 that were tested, containing five substitutions, are as set forth in **TABLE 3.** Results are summarized in **TABLE 5**, **FIGURE** 7, and **FIGURE 8.**

[0115] TABLE 5 depicts the amino acid substitutions for the mutant *C. utilis* uricases, as well as the specific activity (μM/minute per 1.2μM of uricase), pancreatin stability (half-life, minutes. ± SEM), and expression yield (μg/ml) for each enzyme. Pancreatin stability was assayed at 40 ng/μL soluble pancreatin. "nd" indicates that activity and stability measurements were not determined due to insufficient expression yield.

TABLE 5

Clone	Pancreatin Stability (half-life, minutes)	Expression (μg/ml)	Specific Activity (μM/minute per 1.2μM of uricase)
R2_V17	>125	200-299	100-124
R2_V4	>125	200-299	>150
R2_V79	>125	100-199	0-99
R2_V47	>125	200-299	125-149
R2_V91	>125	>300	100-124
R2_V39	>125	200-299	0-99
R2_V75	>125	200-299	125-149
R2_V51	>125	>300	125-149
R2_V26	100-124	100-199	125-149
R2_V27	100-124	200-299	125-149
R2_V71	100-124	>300	100-124
R2_V56	100-124	>300	100-124
R2_V45	100-124	200-299	100-124
R2_V28	100-124	200-299	125-149
R2_V61	100-124	200-299	100-124
R2_V68	100-124	10-99	125-149
R2_V2	100-124	200-299	100-124
R2_V95	100-124	200-299	125-149
R2_V81	100-124	100-199	125-149
R2_V15	50-99	10-99	125-149
R2_V64	50-99	>300	125-149
R2_V42	50-99	>300	100-124
R2_V14	50-99	100-199	125-149

Clone	Pancreatin Stability (half-life, minutes)	Expression (μg/ml)	Specific Activity (μM/minute per 1.2μM of uricase)
R2_V10	50-99	200-299	100-124
R2_V24	50-99	200-299	0-99
R2_V22	50-99	>300	125-149
R2_Parent	50-99	>300	125-149
R2_V30	50-99	200-299	125-149
R2_V38	50-99	200-299	100-124
I180V	10-49	200-299	125-149
I180A	10-49	10-99	>150
Y165F	10-49	200-299	0-99
V190G	10-49	100-199	100-124
E51K	10-49	200-299	125-149
Q244K	10-49	200-299	100-124
I132R	5-9.9	100-199	125-149
V97I	5-9.9	200-299	125-149
E92N	5-9.9	200-299	125-149
A87G	5-9.9	200-299	125-149
D142E	5-9.9	>300	125-149
G44A	5-9.9	>300	100-124
G128P	5-9.9	>300	100-124
A236N	5-9.9	>300	100-124
K208A	5-9.9	>300	100-124
N213A	5-9.9	200-299	125-149
V190A	5-9.9	200-299	125-149
S140T	5-9.9	>300	125-149
Y253Q	5-9.9	200-299	125-149
A84S	5-9.9	>300	125-149
V190D	5-9.9	200-299	125-149
WT	5-9.9	>300	100-124
V190I	5-9.9	200-299	125-149
A87V	5-9.9	200-299	125-149
N193R	5-9.9	>300	100-124
Q25A	5-9.9	>300	125-149
K204A	5-9.9	200-299	125-149
I149L	5-9.9	>300	100-124
G44S	5-9.9	200-299	125-149
Q217L	5-9.9	200-299	>150
D142Q	5-9.9	200-299	125-149
V190L	5-9.9	200-299	100-124
G44L	5-9.9	200-299	>150
A87S	5-9.9	200-299	125-149
I180L	5-9.9	200-299	125-149
I149A	0-4.9	10-99	125-149
G44V	0-4.9	200-299	125-149
V97L	0-4.9	200-299	125-149

Clone	Pancreatin Stability (half-life, minutes)	Expression (μg/ml)	Specific Activity (μM/minute per 1.2μM of uricase)
G44I	0-4.9	200-299	125-149
I149V	0-4.9	10-99	100-124
A87I	0-4.9	10-99	125-15
V97A	0-4.9	100-199	100-124
I180G	nd	10-99	nd
Y165W	0-4.9	100-199	0-99
V97G	nd	10-99	nd
A87L	nd	0-9	nd
I149E	nd	0-9	nd
Y165K	0-4.9	200-299	0-99
I180E	nd	10-99	nd
V97D	nd	0-9	nd
I149G	nd	0-9	nd

[0116] Together, these results identify mutant *C. utilis* uricases with increased stability against pancreatin compared to the wild-type *C. utilis* uricase and without significantly decreased specific activity, and identify single substitutions that are sufficient to increase *C. utilis* uricase stability.

Example 3 - Recombinant mutant *Candida utilis* uricase reduces severe hyperuricemia and normalizes hyperuricosuria in nephropathic UrOx knockout (*UrOxKO*) mice

[0117] In this example, the effect of targeted gut elimination of urate (uric acid) by oral administration of recombinant mutant *Candida utilis* uricase on hyperuricemia (excessive amounts of urate in blood) and hyperuricosuria (excessive amounts of uric acid in urine) was investigated. The *UrOxKO* mice, generated with a targeted mutation at the urate oxidase locus by gene targeting in ES cells (following the method described in Wu *et al.*, *PROC. NAT. ACAD. SCI. USA* (1994), 91:742-746), develop severe hyperuricemia, hyperuricosuria, and uric acid crystalline obstructive nephropathy, and, therefore, is a suitable model to investigate hyperuricemia and associated disorders mimicking the human conditions.

[0118] An expression vector comprising a codon-optimized nucleic acid sequence of SEQ ID NO: 13, which encodes a mutant *Candida utilis* uricase, was expressed in *E. coli*, and the expressed recombinant mutant uricase was isolated and purified.

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ATGAGCACCACACTGAGCAGCAGCACCTATGGTAAAGATAATGTGAAATTCCTGAAAGTGAA
AAAAGATCCGCAGAACCCGAAAAAACAAGAAGTTATGGAAGCAACCGTTACCTGTCTGCTGG
AAGGTGCATTTGATACCAGCTATACCAAAGCAGATAATAGCAGCATTGTTCCGACCGATACC
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GTGAAAAATACCATTCTGGTTCTGGCAAAAACCACCGAAATTTGGCCGATTGAACGTTTTGC
AGCCAAACTGGCAACCCATTTTGTTGAGAAATATTCTCATGTTAGCGGTGTGAGCGTTAAAA
TTGTTCAGGATCGTTGGGTTAAATATGCCGTTGATGGTAAACCGCATGATCACAGCTTTATT
CATGAAGGTGGTGAAAAACGTCGTACCGATCTGTATTACAAACGTAGCGGTGATTATAAACT
GTCCAGCGCAATTAAAGATCTGACCGTTCTGAAAAGCACCGGCAGCATGTTTTATGGTTTTA
ACAAATGCGATTTCACAACCCTGCAGCCGACCACCGATCGTGTTCTGAGCACCGATGTTGAT
GCAACCTGGGTTTGGGATAATAAGAAAATTGGTAGCGTGTACGATATTGCCAAAGCAGCAGA
TAAAGGCATCTTCGATAATGTGTATAATCAGGCACGTGAAATTACCCTGACCACCTTTGCAC
TGGAAAATAGCCCGAGCGTTCAGGCAACCATGTTTAATATGGCGACCCAGATTCTGGAAAAA
GCGTGTAGCGTTTATAGCGTTAGCTATGCACTGCCGAACAAACACTATTTTCTGATTGACCT
GAAATGGAAGGGCCTTGAAAATGATAACGAACTGTTTTATCCGAGTCCGCATCCGAATGGTC
TGATTAAATGTACCGTTGTGCGTAAAGAGAAAACCAAACTG (SEQ ID NO: 13)

[0119] The study used *UrOxKO* mice in three parallel arms in three study periods – a pre-treatment arm, a treatment arm, and a follow-up arm, each lasting 7 days. All mice received 150mg/L allopurinol (ALLO) prior to initiation of the study; this phase is the maintenance dose of ALLO. During the "pre-treatment" period the mice were not administered the maintenance dose of ALLO or any other therapeutic agent for treating severe hyperuricemia, hyperuricosuria, and uric acid crystalline obstructive nephropathy.

[0120] Eight (8) mice were selected in the treatment arm for treatment with recombinant mutant uricase, and, as a positive control, seventeen (17) mice were selected for treatment with allopurinol (ALLO) (n = 9 for ALLO 150mg/L, and n = 8 for ALLO 50mg/L); measurements of plasma urate levels were taken from the same group of mice (*i.e.*, closed cohort) before starting treatment (on day 7 of removal of ALLO maintenance dose, or day 7 of the pre-treatment period), during treatment (on day 7 of treatment (spray dried powder of 25% Uricase and 75% trehalose, mixed with 3.5g food, was administered each day for 7 days, and measurements were taken on day 7 of the treatment)), and in the follow-up arm, 7 days after termination of treatment. In both the recombinant mutant uricase and ALLO cohorts, mice received a maintenance dose of 150mg/L ALLO before initiation of the respective pre-treatment observation period.

[0121] At the start of the pre-treatment period, the maintenance dose of 150mg/L ALLO was removed. The plasma urate levels were measured in plasma samples collected on day 7 after removal of the maintenance dose of ALLO, and urine uric acid levels were measured in 24-hour urine samples collected during the last 3 days of the pre-treatment period. Plasma urate levels and urine uric acid levels were measured following the Liquick Cor-UA 30 plus protocol by Cormay, Poland (Liquick Cor-UA 30 plus, kit size 5 x 30 ml, Cat. No. 2-260.

[0122] Mice treated with the recombinant mutant uricase (n = 8) orally received approximately 62mg/day (or 1,500U/day) recombinant mutant uricase mixed with food (spray dried powder of 25% Uricase and 75% trehalose, mixed with 3.5 g food). In the control group, mice (n = 17) were administered 150mg/L of ALLO (n = 9) and 50mg/L of ALLO (n = 8), supplemented in water. The plasma urate levels were measured in blood samples collected from the mice on day 7 of treatment with recombinant mutant uricase, ALLO 150mg/L, and ALLO 50mg/L, respectively, and urine uric acid levels were measured in 24-hour urine samples collected during the last 3 days of the treatment period.

[0123] In the follow-up period, plasma urate levels were measured in blood samples collected from the mice on day 7 after termination of treatment with recombinant mutant uricase, ALLO 150mg/L, and ALLO 50mg/L, respectively.

[0124] The assay for urine uric acid was performed according to the manufacturer's instructions (Liquick Cor-UA 30 plus protocol by Cormay, Poland (Liquick Cor-UA 30 plus, kit size 5 x 30 ml, Cat. No. 2-260)). For example, urine samples were diluted 1:4, 1:9, or 1:14 depending on groups of animals and the time of collection. To prevent precipitation of salts of uric acid, 1 drop of NaOH (500g/L) was added to the collection tube before collection of a 24-hour specimen.

[0125] Plasma urate levels were also measured according to manufacturer's instructions (Liquick Cor-UA 30 plus protocol). Urate levels in the blood samples were measured without dilution or diluted 1:1 with double-distilled water (ddH_2O).

[0126] The measured plasma urate levels and the urine uric acid levels demonstrated that hyperuricemia (*i.e.*, excess of uric acid in the blood) was reduced significantly ($p<0.001$) and hyperuricosuria (*i.e.*, the presence of excessive amounts of uric acid in the urine) normalized in 7 days after oral administration of the recombinant mutant uricase (Figures 9A and 9B). Mice treated with recombinant mutant uricase had a plasma urate decrease by 44% from pre-treatment (standard of mean (SEM) 14.5±0.9 to 8.1±0.5 mg/dL), which was similar to 51% decrease observed in the 50mg/L ALLO mice (mean (SEM) 13.2±2.6 to 6.5±1.1 mg/dL); p=NS. The result demonstrated that there was no significant difference between the effects of ALLO 50mg/L and recombinant mutant uricase on plasma urate levels. The highest reduction of 69% was observed in mice treated with ALLO 150mg/L (mean (SEM) 13.8±1.7 to 4.3±0.6 mg/dL).

[0127] The removal of recombinant mutant uricase or ALLO resulted in hyperuricemia returning to approximately the pre-treatment levels. This was studied as follows.

[0128] Urine uric acid excretion normalized (<2mg/24 hour) with recombinant mutant uricase with 86% reduction (mean (SEM) 4.7±0.6 to 0.7±0.1mg/24h); while in mice treated with ALLO 50mg/L and 150mg/L, reduction was 34% (mean (SEM) 4.9±0.4 to 3.2±0.3mg/24h) and 66% (mean (SEM) 6.4±0.7 to 2.2±0.3mg/24h), respectively. Analysis of digesta (the semifluid mass into which food is converted by gastric secretion and which passes from the stomach into the small intestine) from different parts of the gastrointestinal tract (GIT) indicated the uric acid is present along the whole gut, confirming secretion of the urate from circulation.

[0129] The results presented in this example demonstrated that targeting enteric uric acid (uric acid secrete from circulation into intestine), by orally administered recombinant mutant uricase successfully lowered serum uric acid level, and normalized urinary uric acid in nephropathic *UrOxKO* mice.

NUMBERED EMBODIMENTS

[0130] It is to be understood that while the present disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is presented by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[0131] Embodiments disclosed herein include embodiments P1 to P53, as provided in the numbered embodiments of the disclosure:

[0132] Embodiment P1: A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, five, six, seven or eight) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is selected from: (a) at position 180, isoleucine is substituted by valine or alanine (I180V or I180A), (b) at position 165, tyrosine is substituted by phenylalanine (Y165F), (c) at position 190, valine is substituted by glycine or alanine (V190G or V190A), (d) at position 51, glutamic acid is substituted by lysine (E51K), (e) at position 244, glutamine is substitute by lysine (Q244K), (f) at position 132, isoleucine is substituted by arginine or asparagine (I132R or I132N), (g) at position 97, valine is substituted by isoleucine (V97I), (h) at position 92, glutamic acid is substituted by asparagine (E92N), (i) at position

87, alanine is substituted by glycine (A87G), (j) at position 142, aspartic acid is substituted by glutamic acid (D142E), (k) at position 44, glycine is substituted by alanine (G44A), (l) at position 128, glycine is substituted by proline (G128P), (m) at position 236, alanine is substituted by asparagine (A236N), (n) at position 208, lysine is substituted by alanine (K208A), (o) at position 213, asparagine is substituted by alanine (N213A), (p) at position 140, serine is substituted by threonine (S140T), (q) at position 253, tyrosine is substituted by glutamine (Y253Q), (r) at position 84, alanine is substituted by serine (A84S), (s) at position 47, threonine is substituted by glutamic acid (T47E), (t) at position 95, serine is substituted by proline (S95P), (u) at position 103, lysine is substituted by threonine (K103T), (v) at position 134, aspartic acid is substituted by glutamic acid (D134E), (w) at position 136, tyrosine is substituted by arginine (Y136R), (x) at position 196, isoleucine is substituted by leucine (I196L), (y) at position 224, threonine is substituted by aspartic acid (T224D), (z) at position 285, proline is substituted by serine (P285S), and (aa) at position 296, valine is substituted by alanine (V296A).

[0133] Embodiment P2: The recombinant mutant *C. utilis* uricase of embodiment P1, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, V190A, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, G44A, G128P, A236N, K208A, N213A, S140T, Y253Q, and A84S.

[0134] Embodiment P3: The recombinant mutant *C. utilis* uricase of embodiment P1 or P2, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, and G44A.

[0135] Embodiment P4: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P3, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, I132R, and G44A.

[0136] Embodiment P5: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P4, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, E51K, I132R, and G44A.

[0137] Embodiment P6: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P5, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, and I132R.

[0138] Embodiment P7: A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one

mutation is present at a position selected from position 180, position 165, position 190, position 51, position 132, and position 44.

[0139] Embodiment P8: A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, or five) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 51, position 132, and position 44.

[0140] Embodiment P9: A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 244, and position 132.

[0141] Embodiment P10: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P9, wherein the uricase comprises two, three, four, five, six, seven, or eight mutations.

[0142] Embodiment P11: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P10, wherein the uricase comprises the following substitutions: I180V, Y165F, E51K, I132R, and G44A.

[0143] Embodiment P12: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P10, wherein the uricase comprises the following substitutions: I180A, Y165F, E51K, I132R, and G44A.

[0144] Embodiment P13: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P10, wherein the uricase comprises the following substitutions: I180V, Y165F, V190G, E51K, I132R, and G44A.

[0145] Embodiment P14: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P10, wherein the uricase comprises the following substitutions: I180A, Y165F, V190G, E51K, I132R, and G44A.

[0146] Embodiment P15: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P10, wherein the uricase comprises the following substitutions: I180V and Y165F.

[0147] Embodiment P16: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P10, wherein the uricase comprises the following substitutions: I180V, Y165F, V190G, E51K, Q244K, and I132R.

[0148] Embodiment P17: A recombinant mutant *C. utilis* uricase comprising a substitution listed in TABLE 1 or TABLE 2.

[0149] Embodiment P18: A recombinant mutant *Candida utilis* uricase having a half-life of at least 35 minutes in the presence of pancreatin.

[0150] Embodiment P19: The recombinant mutant *C. utilis* uricase of embodiment P17, wherein the half-life is 35–200 minutes in the presence of pancreatin.

[0151] Embodiment P20: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P19, wherein the uricase has 5-50 fold higher stability in the presence of pancreatin, compared to the wild-type uricase.

[0152] Embodiment P21: The recombinant mutant *C. utilis* uricase of embodiment P20, wherein the uricase has 20-30 fold higher stability in the presence of pancreatin, compared to the wild-type uricase.

[0153] Embodiment P22: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P21, wherein the uricase is isolated.

[0154] Embodiment P23: The recombinant mutant *C. utilis* uricase of any one of embodiments P1-P22, wherein the uricase is conjugated to a water soluble polymer.

[0155] Embodiment P24: The recombinant mutant *C. utilis* uricase of embodiment P23, wherein the uricase is conjugated to polyethylene glycol (PEG).

[0156] Embodiment P25: An expression vector comprising a nucleic acid sequence encoding the recombinant mutant *C. utilis* uricase of any one of embodiments P1-P24.

[0157] Embodiment P26: The expression vector of embodiment P25, wherein the nucleic acid sequence encoding the recombinant mutant uricase is codon optimized for expression in a heterologous cell.

[0158] Embodiment P27: The expression vector of embodiment P26, wherein the heterologous cell is *Escherichia coli*.

[0159] Embodiment P28: A cell comprising the expression vector of any one of embodiments P25-P27.

[0160] Embodiment P29: The cell of embodiment 28, wherein the cell is *Escherichia coli*.

[0161] Embodiment P30: A pharmaceutical composition comprising the recombinant mutant *C. utilis* uricase of any one of embodiments P1-P24.

[0162] Embodiment P31: The pharmaceutical composition of embodiment P30, further comprising a pharmaceutically acceptable carrier and/or an excipient.

[0163] Embodiment P32: The pharmaceutical composition of embodiment P30 or P31, wherein the composition is formulated as an oral dosage form or a parenteral dosage form.

[0164] Embodiment P33: The pharmaceutical composition of embodiment P32, wherein the composition is formulated as an oral dosage form.

[0165] Embodiment P34: The pharmaceutical composition of any one of embodiments P30-P33, wherein the composition is a formulated as a powder, granulate, pellet, micropellet, or a minitablet.

[0166] Embodiment P35: The pharmaceutical composition of any one of embodiments P30-P34, wherein the composition is encapsulated in a capsule or formulated as a tablet dosage form.

[0167] Embodiment P36: The pharmaceutical composition of embodiment P35, wherein the capsule is a hydroxypropyl methylcellulose (HPMC) capsule, soft gelatin capsule, or a hard gelatin capsule.

[0168] Embodiment P37: The pharmaceutical composition of embodiment P32, wherein the composition is formulated as a parenteral dosage form.

[0169] Embodiment P38: The pharmaceutical composition of embodiment P37, wherein the composition is formulated as an intravenous dosage form.

[0170] Embodiment P39: A method of treating a disease or disorder associated with an elevated amount of uric acid in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of embodiments P1-P24, thereby treating the disease or disorder in the subject.

[0171] Embodiment P40: The method of embodiment P39, wherein the disease or disorder is associated with an elevated amount of uric acid in plasma of the subject.

[0172] Embodiment P41: A method of treating hyperuricemia in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of embodiments P1-P24, thereby treating hyperuricemia in the subject.

[0173] Embodiment P42: A method of treating gout in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of embodiments P1-P24, thereby to treat gout in the subject.

[0174] Embodiment P43: A method of treating hyperuricemia in a subject in need thereof, the method comprising administering to the subject an effective amount of the

pharmaceutical composition of any one of embodiments P30-P38, thereby to treat hyperuricemia in the subject.

[0175] Embodiment P44: A method of treating gout in a subject in need thereof, the method comprising administering to the subject an effective amount of the pharmaceutical composition of any one of embodiments P30-P38, thereby to treat gout in the subject.

[0176] Embodiment P45: The method of any one of embodiments P39-P44, wherein the recombinant mutant *C. utilis* uricase is administered in combination with a xanthine oxidase inhibitor, a uricosuric, or a combination thereof.

[0177] Embodiment P46: The method of embodiment P45, wherein the xanthine oxidase inhibitor is selected from allopurinol and febuxostat.

[0178] Embodiment P47: The method of embodiment P45, wherein the uricosuric is selected from probenecid, benzbromarone, losartan and lesinurad.

[0179] Embodiment P48: A method of treating hyperuricosuria in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of embodiments P1-P24, thereby treating hyperuricosuria in the subject.

[0180] Embodiment P49: A method of treating hyperuricosuria in a subject in need thereof, the method comprising administering to the subject an effective amount of the pharmaceutical composition of any one of embodiments P30-P38, thereby to treat hyperuricosuria in the subject.

[0181] Embodiment P50: The method of embodiment P48 or P49, wherein the recombinant mutant *C. utilis* uricase is administered in combination with a xanthine oxidase inhibitor, a uricosuric, or a combination thereof.

[0182] Embodiment P51: The method of embodiment P48 or P49, wherein the recombinant mutant *C. utilis* uricase is administered subsequent to administration of a xanthine oxidase inhibitor, a uricosuric, or a combination thereof.

[0183] Embodiment P52: The method of embodiment P50 or P51, wherein the xanthine oxidase inhibitor is selected from allopurinol and febuxostat.

[0184] Embodiment P53: The method of embodiment P50 or P51, wherein the uricosuric is selected from probenecid, benzbromarone, losartan and lesinurad.

INCORPORATION BY REFERENCE

[0185] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0186] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, five, six, seven or eight) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is selected from: (a) at position 180, isoleucine is substituted by valine or alanine (I180V or I180A), (b) at position 165, tyrosine is substituted by phenylalanine (Y165F), (c) at position 190, valine is substituted by glycine or alanine (V190G or V190A), (d) at position 51, glutamic acid is substituted by lysine (E51K), (e) at position 244, glutamine is substitute by lysine (Q244K), (f) at position 132, isoleucine is substituted by arginine or asparagine (I132R or I132N), (g) at position 97, valine is substituted by isoleucine (V97I), (h) at position 92, glutamic acid is substituted by asparagine (E92N), (i) at position 87, alanine is substituted by glycine (A87G), (j) at position 142, aspartic acid is substituted by glutamic acid (D142E), (k) at position 44, glycine is substituted by alanine (G44A), (l) at position 128, glycine is substituted by proline (G128P), (m) at position 236, alanine is substituted by asparagine (A236N), (n) at position 208, lysine is substituted by alanine (K208A), (o) at position 213, asparagine is substituted by alanine (N213A), (p) at position 140, serine is substituted by threonine (S140T), (q) at position 253, tyrosine is substituted by glutamine (Y253Q), (r) at position 84, alanine is substituted by serine (A84S), (s) at position 47, threonine is substituted by glutamic acid (T47E), (t) at position 95, serine is substituted by proline (S95P), (u) at position 103, lysine is substituted by threonine (K103T), (v) at position 134, aspartic acid is substituted by glutamic acid (D134E), (w) at position 136, tyrosine is substituted by arginine (Y136R), (x) at position 196, isoleucine is substituted by leucine (I196L), (y) at position 224, threonine is substituted by aspartic acid (T224D), (z) at position 285, proline is substituted by serine (P285S), and (aa) at position 296, valine is substituted by alanine (V296A).

2. The recombinant mutant *C. utilis* uricase of claim 1, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, V190A, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, G44A, G128P, A236N, K208A, N213A, S140T, Y253Q, and A84S.

3. The recombinant mutant *C. utilis* uricase of claim 1 or 2, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, I132R, V97I, E92N, A87G, D142E, and G44A.

4. The recombinant mutant *C. utilis* uricase of any one of claims 1-3, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, I132R, and G44A.

5. The recombinant mutant *C. utilis* uricase of any one of claims 1-4, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, E51K, I132R, and G44A.

6. The recombinant mutant *C. utilis* uricase of any one of claims 1-5, wherein the uricase comprises at least one mutation selected from: I180V, I180A, Y165F, V190G, E51K, Q244K, and I132R.

7. A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 132, and position 44.

8. A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, or five) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 51, position 132, and position 44.

9. A recombinant mutant *Candida utilis* uricase comprising at least one (for example, one, two, three, four, five, or six) mutation(s) at a position corresponding to wild type *C. utilis* uricase of SEQ ID NO: 1, wherein the at least one mutation is present at a position selected from position 180, position 165, position 190, position 51, position 244, and position 132.

10. The recombinant mutant *C. utilis* uricase of any one of claims 1-9, wherein the uricase comprises two, three, four, five, six, seven, or eight mutations.

11. The recombinant mutant *C. utilis* uricase of any one of claims 1-10, wherein the uricase comprises the following substitutions: I180V, Y165F, E51K, I132R, and G44A.

12. The recombinant mutant *C. utilis* uricase of any one of claims 1-10, wherein the uricase comprises the following substitutions: I180A, Y165F, E51K, I132R, and G44A.

13. The recombinant mutant *C. utilis* uricase of any one of claims 1-10, wherein the uricase comprises the following substitutions: I180V, Y165F, V190G, E51K, I132R, and G44A.

14. The recombinant mutant *C. utilis* uricase of any one of claims 1-10, wherein the uricase comprises the following substitutions: I180A, Y165F, V190G, E51K, I132R, and G44A.

15. The recombinant mutant *C. utilis* uricase of any one of claims 1-10, wherein the uricase comprises the following substitutions: I180V and Y165F.

16. The recombinant mutant *C. utilis* uricase of any one of claims 1-10, wherein the uricase comprises the following substitutions: I180V, Y165F, V190G, E51K, Q244K, and I132R.

17. A recombinant mutant *C. utilis* uricase comprising a substitution listed in TABLE 1 or TABLE 2.

18. A recombinant mutant *Candida utilis* uricase having a half-life of at least 35 minutes in the presence of pancreatin.

19. The recombinant mutant *C. utilis* uricase of claim 17, wherein the half-life is 35–200 minutes in the presence of pancreatin.

20. The recombinant mutant *C. utilis* uricase of any one of claims 1-19, wherein the uricase has 5-50 fold higher stability in the presence of pancreatin, compared to the wild-type uricase.

21. The recombinant mutant *C. utilis* uricase of claim 20, wherein the uricase has 20-30 fold higher stability in the presence of pancreatin, compared to the wild-type uricase.

22. The recombinant mutant *C. utilis* uricase of any one of claims 1-21, wherein the uricase is isolated.

23. The recombinant mutant *C. utilis* uricase of any one of claims 1-22, wherein the uricase is conjugated to a water soluble polymer.

24. The recombinant mutant *C. utilis* uricase of claim 23, wherein the uricase is conjugated to polyethylene glycol (PEG).

25. An expression vector comprising a nucleic acid sequence encoding the recombinant mutant *C. utilis* uricase of any one of claims 1-24.

26. The expression vector of claim 25, wherein the nucleic acid sequence encoding the recombinant mutant uricase is codon optimized for expression in a heterologous cell.

27. The expression vector of claim 26, wherein the heterologous cell is *Escherichia coli*.

28. A cell comprising the expression vector of any one of claims 25-27.

29. The cell of claim 28, wherein the cell is *Escherichia coli*.

30. A pharmaceutical composition comprising the recombinant mutant *C. utilis* uricase of any one of claims 1-24.

31. The pharmaceutical composition of claim 30, further comprising a pharmaceutically acceptable carrier and/or an excipient.

32. The pharmaceutical composition of claim 30 or 31, wherein the composition is formulated as an oral dosage form or a parenteral dosage form.

33. The pharmaceutical composition of claim 32, wherein the composition is formulated as an oral dosage form.

34. The pharmaceutical composition of any one of claims 30-33, wherein the composition is a formulated as a powder, granulate, pellet, micropellet, or a minitablet.

35. The pharmaceutical composition of any one of claims 30-34, wherein the composition is encapsulated in a capsule or formulated as a tablet dosage form.

36. The pharmaceutical composition of claim 35, wherein the capsule is a hydroxypropyl methylcellulose (HPMC) capsule, soft gelatin capsule, or a hard gelatin capsule.

37. The pharmaceutical composition of claim 32, wherein the composition is formulated as a parenteral dosage form.

38. The pharmaceutical composition of claim 37, wherein the composition is formulated as an intravenous dosage form.

39. A method of treating a disease or disorder associated with an elevated amount of uric acid in a subject in need thereof, the method comprising administering to the subject an

effective amount of the recombinant mutant *C. utilis* uricase of any one of claims 1-24, thereby treating the disease or disorder in the subject.

40. The method of claim 39, wherein the disease or disorder is associated with an elevated amount of uric acid in plasma of the subject.

41. A method of treating hyperuricemia in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of claims 1-24, thereby treating hyperuricemia in the subject.

42. A method of treating gout in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of claims 1-24, thereby to treat gout in the subject.

43. A method of treating hyperuricemia in a subject in need thereof, the method comprising administering to the subject an effective amount of the pharmaceutical composition of any one of claims 30-38, thereby to treat hyperuricemia in the subject.

44. A method of treating gout in a subject in need thereof, the method comprising administering to the subject an effective amount of the pharmaceutical composition of any one of claims 30-38, thereby to treat gout in the subject.

45. The method of any one of claims 39-44, wherein the recombinant mutant *C. utilis* uricase is administered in combination with a xanthine oxidase inhibitor, a uricosuric, or a combination thereof.

46. The method of claim 45, wherein the xanthine oxidase inhibitor is selected from allopurinol and febuxostat.

47. The method of claim 45, wherein the uricosuric is selected from probenecid, benzbromarone, losartan and lesinurad.

48. A method of treating hyperuricosuria in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant mutant *C. utilis* uricase of any one of claims 1-24, thereby treating hyperuricosuria in the subject.

49. A method of treating hyperuricosuria in a subject in need thereof, the method comprising administering to the subject an effective amount of the pharmaceutical composition of any one of claims 30-38, thereby to treat hyperuricosuria in the subject.

50. The method of claim 48 or 49, wherein the recombinant mutant *C. utilis* uricase is administered in combination with a xanthine oxidase inhibitor, a uricosuric, or a combination thereof.

51. The method of claim 48 or 49, wherein the recombinant mutant *C. utilis* uricase is administered subsequent to administration of a xanthine oxidase inhibitor, a uricosuric, or a combination thereof.

52. The method of claim 50 or 51, wherein the xanthine oxidase inhibitor is selected from allopurinol and febuxostat.

53. The method of claim 50 or 51, wherein the uricosuric is selected from probenecid, benzbromarone, losartan and lesinurad.

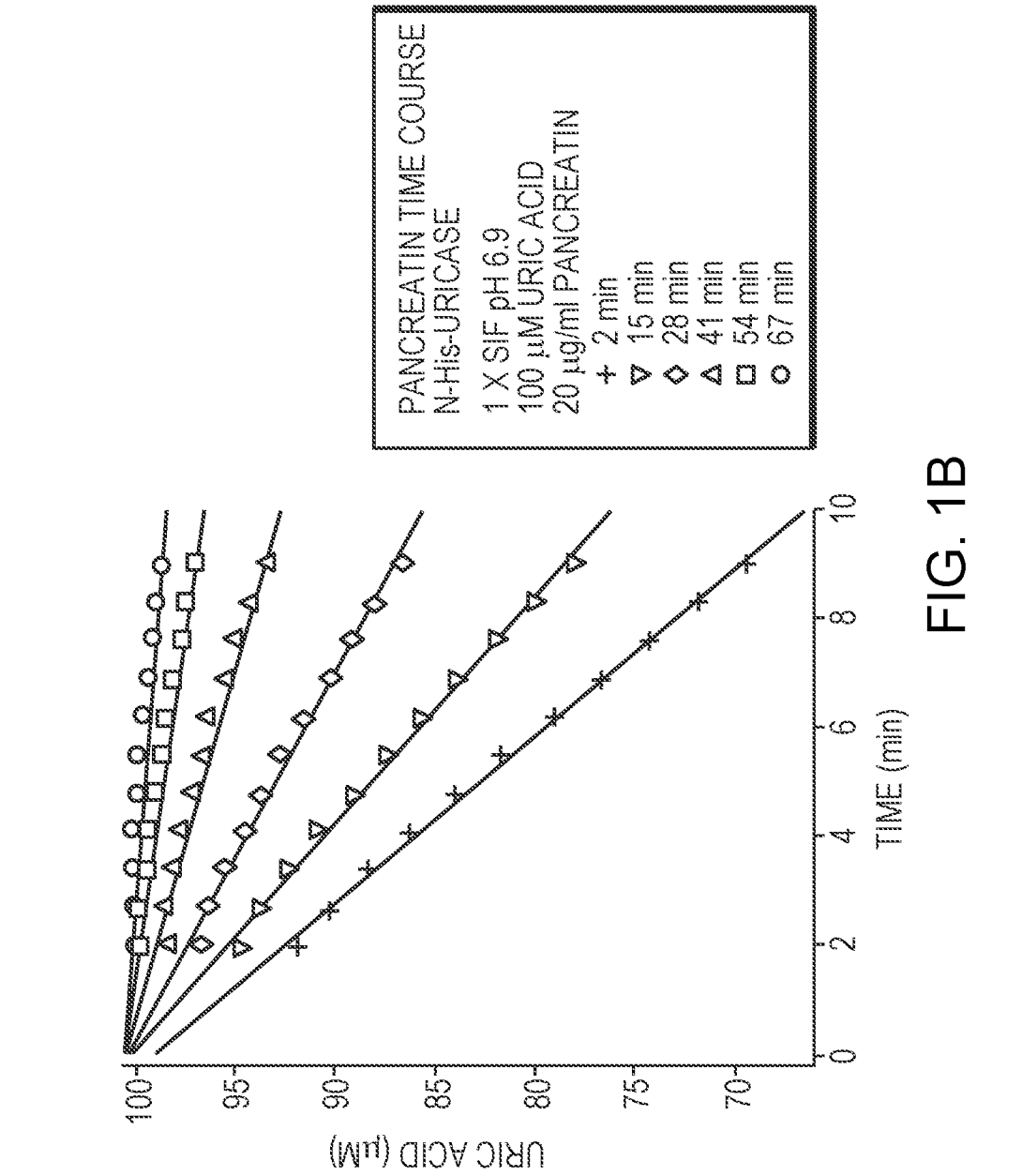
PANCREATIN TIME COURSE
N-His-URICASE
1 X SIF pH 6.9
100 µM URIC ACID
20 µg/ml PANCREATIN
2 min
15 min
28 min
41 min
54 min
67 min
URIC ACID (µM)
100
95
90
85
80
75
70
0
2
4
6
8
10
TIME (min)

FIG. 1B

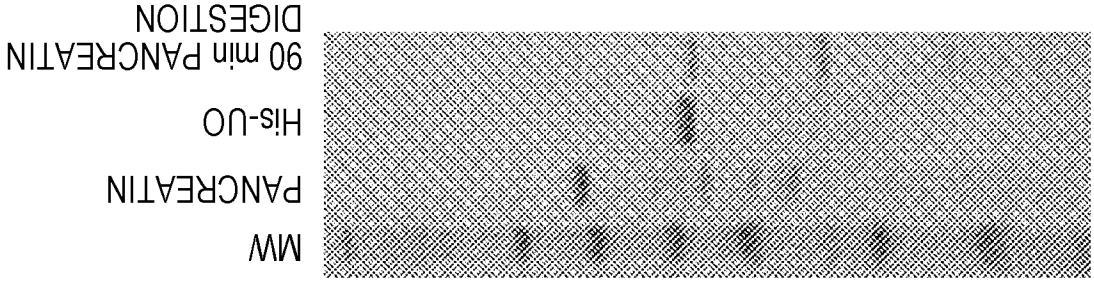
MW
PANCREATIN
His-UO
90 min PANCREATIN DIGESTION

FIG. 1A

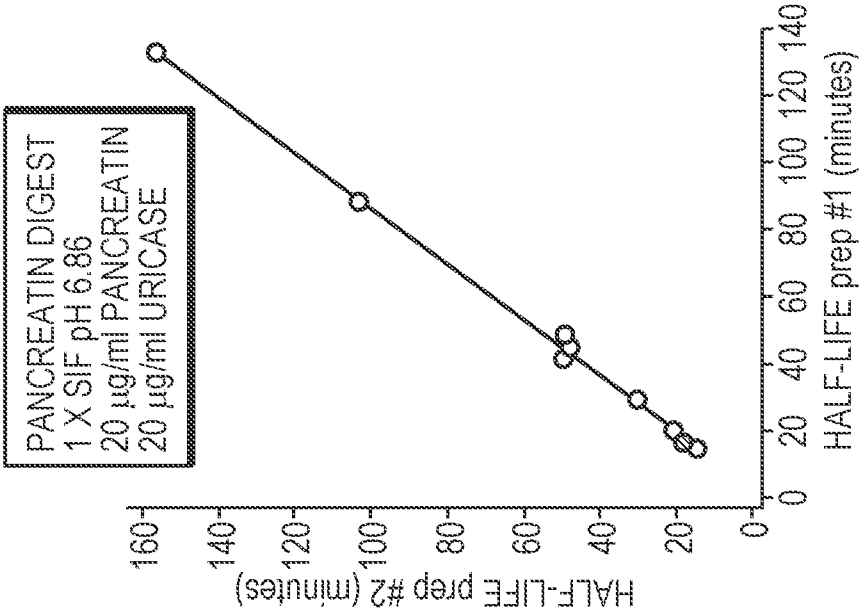
PANCREATIN DIGEST
1 X SIF pH 6.86
20 µg/ml PANCREATIN
20 µg/ml URICASE
HALF-LIFE prep #2 (minutes)
HALF-LIFE prep #1 (minutes)
0
20
40
60
80
100
120
140
160

FIG. 2B

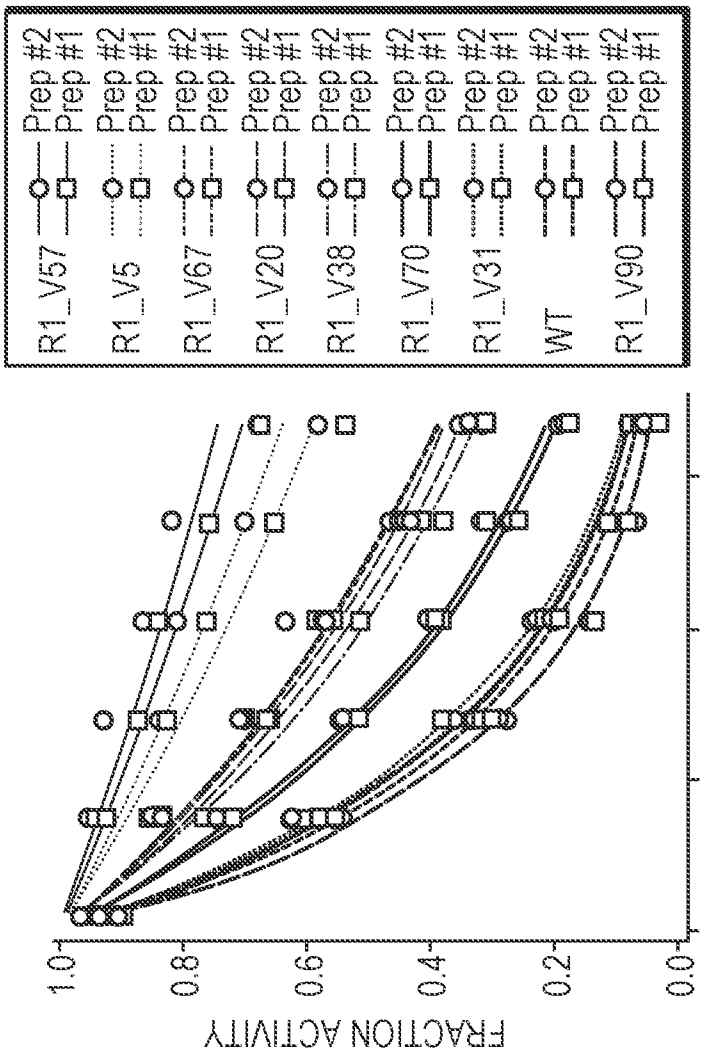
R1_V57 Prep #2 Prep #1
R1_V5 Prep #2 Prep #1
R1_V67 Prep #2 Prep #1
R1_V20 Prep #2 Prep #1
R1_V38 Prep #2 Prep #1
R1_V70 Prep #2 Prep #1
R1_V31 Prep #2 Prep #1
WT Prep #2 Prep #1
R1_V90 Prep #2 Prep #1
FRACTION ACTIVITY
TIME (min)
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60

FIG. 2A

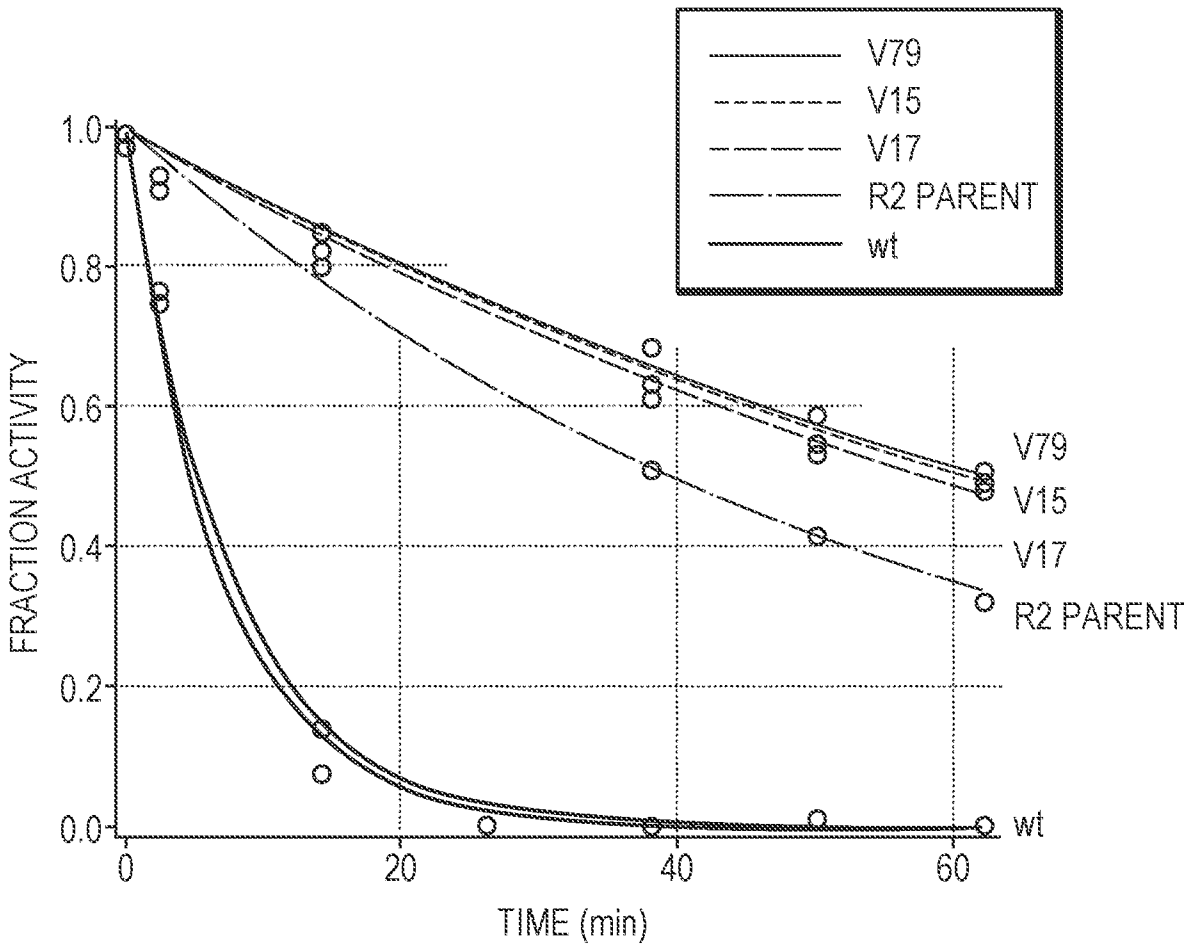
V79
V15
V17
R2 PARENT
wt
1.0
0.8
0.6
0.4
0.2
0.0
FRACTION ACTIVITY
0
20
40
60
TIME (min)
V79
V15
V17
R2 PARENT
wt

FIG. 3

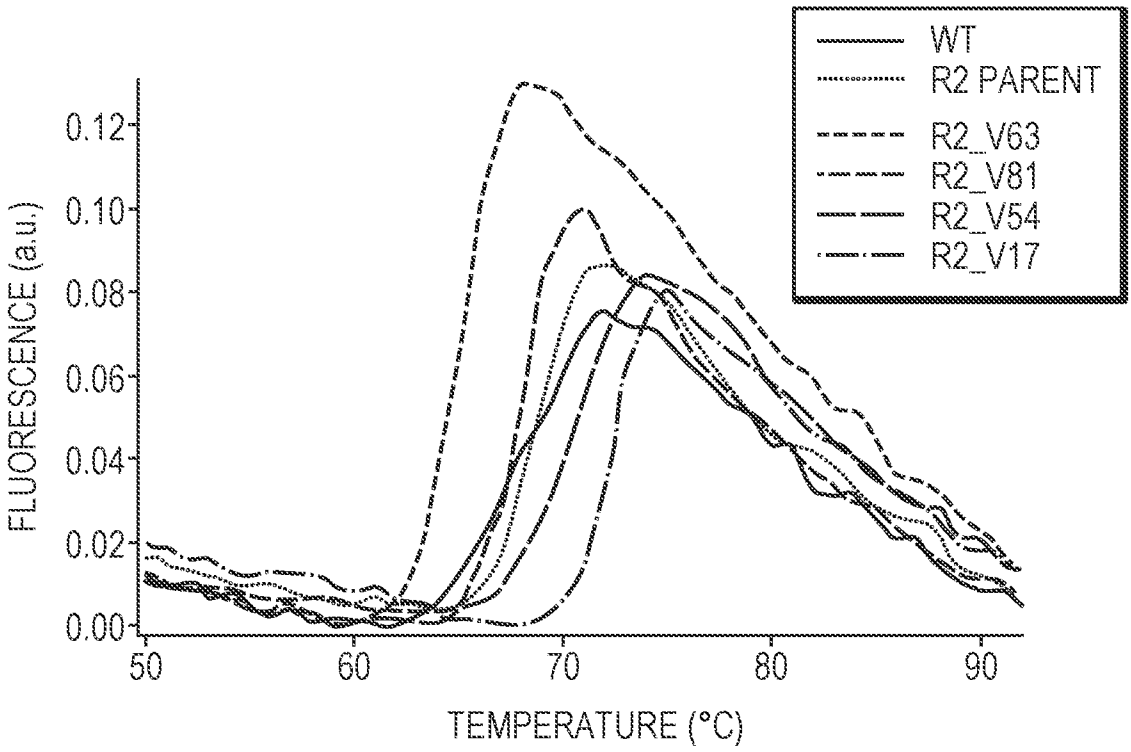
WT
R2 PARENT
R2_V63
R2_V81
R2_V54
R2_V17
FLUORESCENCE (a.u.)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
50
60
70
80
90
TEMPERATURE (°C)

FIG. 4

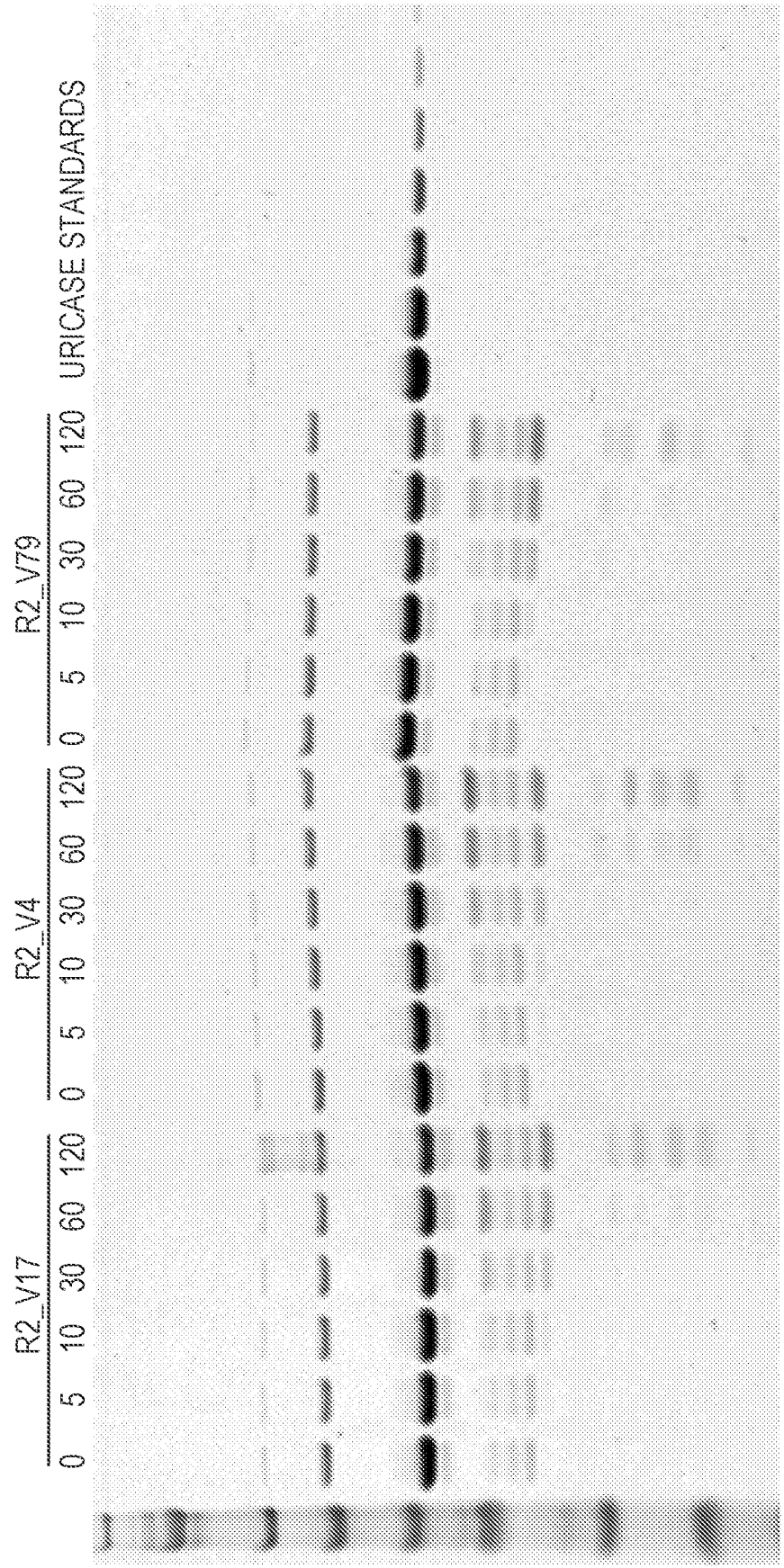
R2_V17
0
5
10
30
60
120
R2_V4
0
5
10
30
60
120
R2_V79
0
5
10
30
60
120
URICASE STANDARDS

FIG. 5

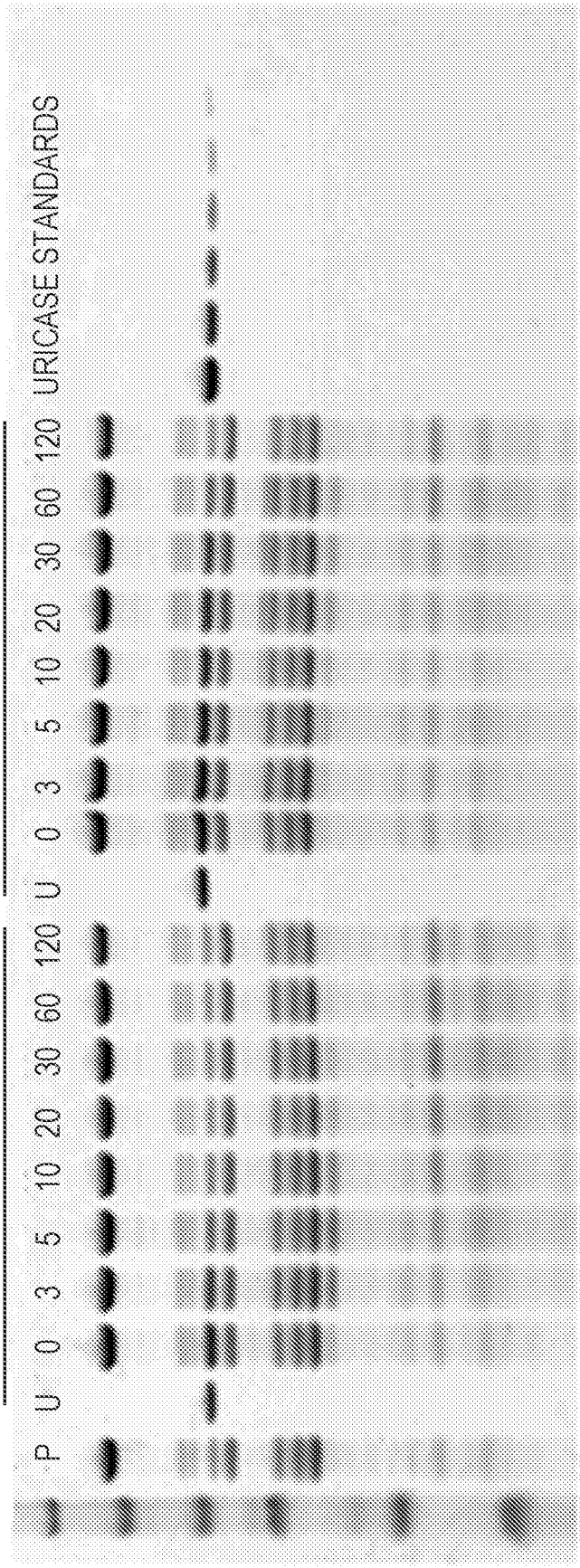
WT URICASE
R2_V17
P
U
0
3
5
10
20
30
60
120
U
0
3
5
10
20
30
60
120
URICASE STANDARDS

FIG. 6

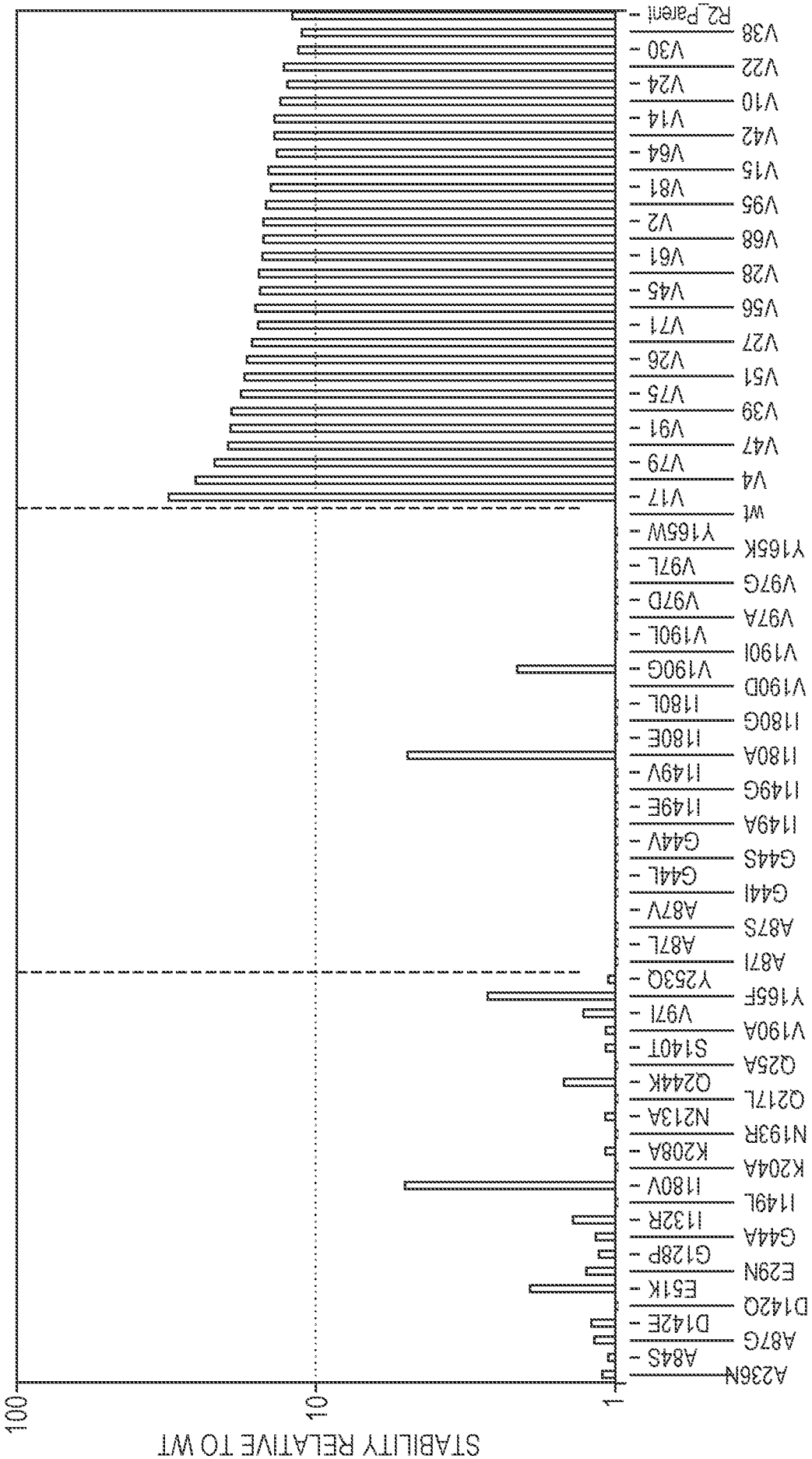
STABILITY RELATIVE TO WT
100
10
1
R2_Parent
V38
V30
V22
V24
V10
V14
V42
V64
V15
V81
V95
V2
V68
V61
V28
V45
V56
V71
V27
V26
V51
V75
V39
V91
V47
V79
V4
V17
wt
Y165W
Y165K
V97L
V97G
V97D
V97A
V190L
V190I
V190G
V190D
I180L
I180G
I180E
I180A
I149V
I149G
I149E
I149A
G44V
G44S
G44L
G44I
A87V
A87S
A87L
A87I
Y253Q
Y165F
V97I
S140T
V190A
Q244K
Q25A
N213A
Q217L
K208A
N193R
I180V
K204A
I132R
I149L
G128P
G44A
E51K
E29N
D142E
D142Q
A87G
A84S
A236N

FIG. 7

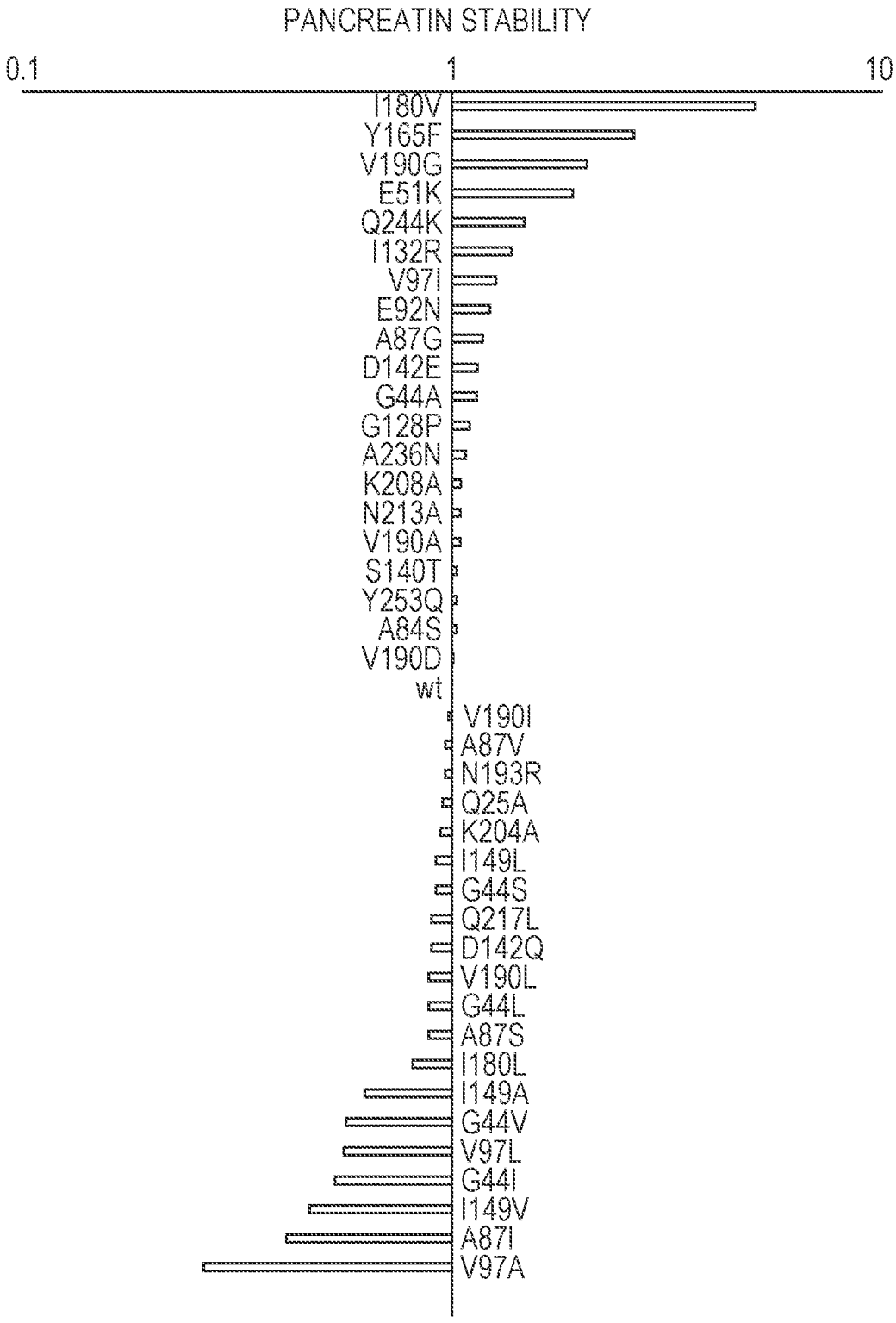
PANCREATIN STABILITY
0.1
1
10
I180V
Y165F
V190G
E51K
Q244K
I132R
V97I
E92N
A87G
D142E
G44A
G128P
A236N
K208A
N213A
V190A
S140T
Y253Q
A84S
V190D
wt
V190I
A87V
N193R
Q25A
K204A
I149L
G44S
Q217L
D142Q
V190L
G44L
A87S
I180L
I149A
G44V
V97L
G44I
I149V
A87I
V97A

FIG. 8

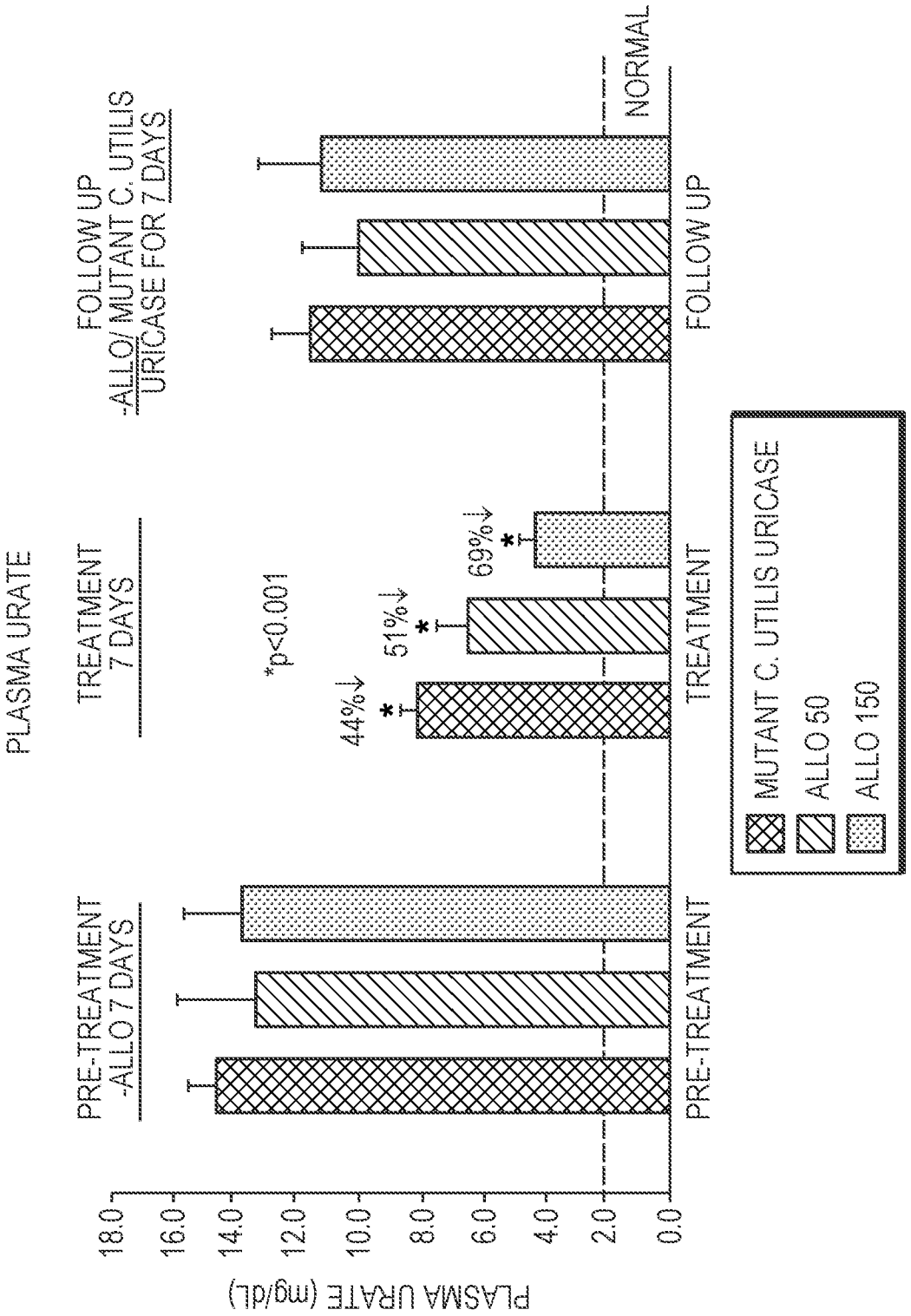
PLASMA URATE
PRE-TREATMENT
-ALLO 7 DAYS
TREATMENT
7 DAYS
FOLLOW UP
-ALLO/ MUTANT C. UTILIS
URICASE FOR 7 DAYS
*p<0.001
44%↓
51%↓
69%↓
NORMAL
PRE-TREATMENT
TREATMENT
FOLLOW UP
PLASMA URATE (mg/dL)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
MUTANT C. UTILIS URICASE
ALLO 50
ALLO 150

FIG. 9A

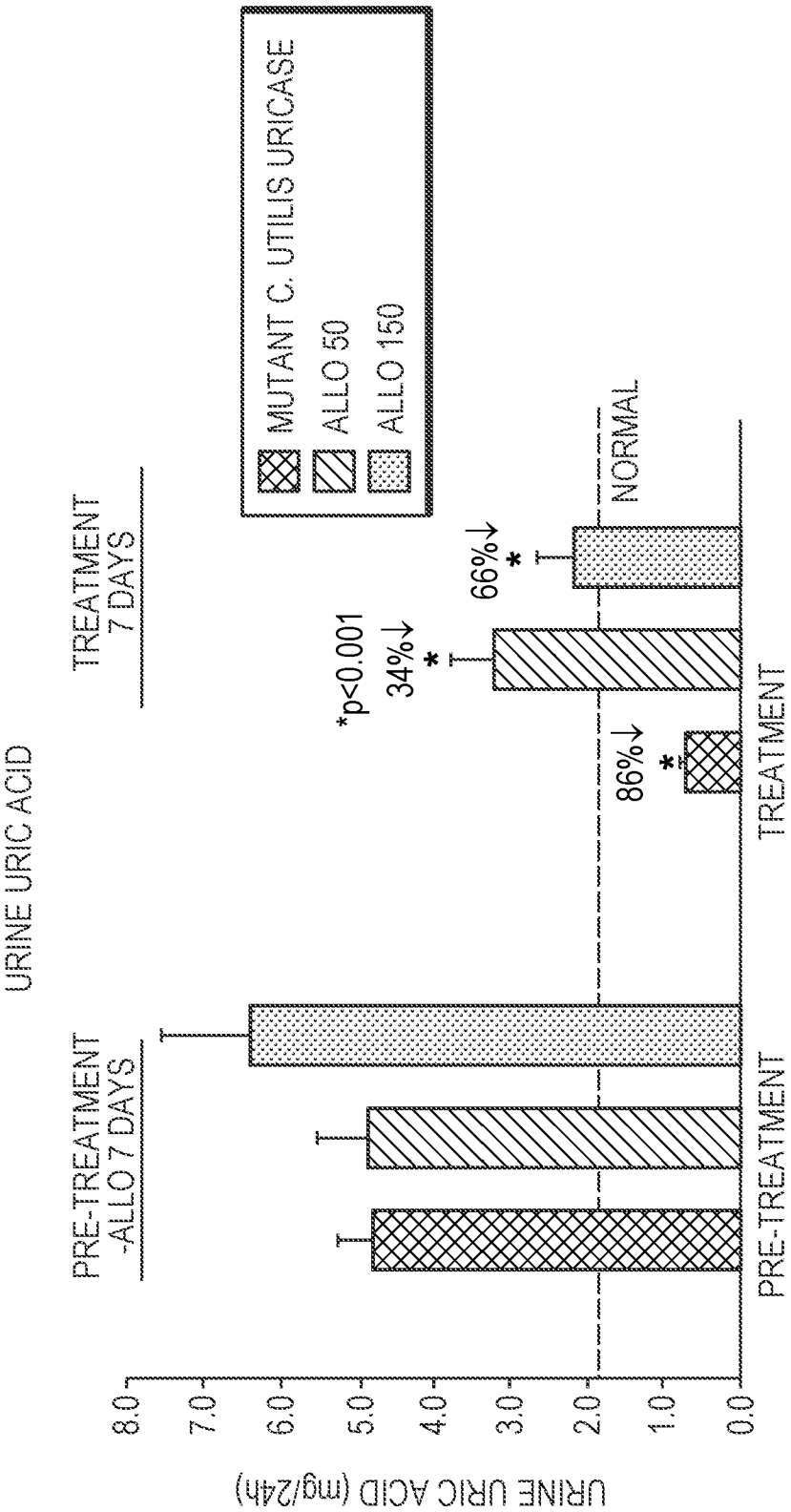
URINE URIC ACID
PRE-TREATMENT
-ALLO 7 DAYS
TREATMENT
7 DAYS
MUTANT C. UTILIS URICASE
ALLO 50
ALLO 150
*p<0.001
34%↓
66%↓
86%↓
NORMAL
PRE-TREATMENT
TREATMENT
URINE URIC ACID (mg/24h)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

FIG. 9B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/41015

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 9/06, C12N 1/20 (2018.01)
CPC - C12N 9/0046

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,700,674 A (KOYAMA etal.) 23 December 1997 (23.12.1997) abstract; SEQ ID NO: 1; col 1, ln 29-35; col 2, ln 1-27; col 4, ln 25-28; col 6, ln 10-16; Table 1.	1-3, 7-9, 17 --- 18, 19
Y	TRUSZKOWSKI et al. "Uricase and its action: Extraction and precipitation of ox kidney uricase." Biochemical Journal, December 1935, Vol 29, No 12, pages 2787-2797. p. 2796, para 3.	18, 19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2018	26 SEP 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/41015

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-6, 10-16, 20-53
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.