Advanced Patent Prosecution Workshop 2021:

*Claim Drafting & Amendment Writing*

**Biotechnology**In-Class Problems 8-10

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**PLI Biotech Practice****In Class Problem 8**

On October 17, 2012**,** a compound known as "A1,"

A1:

which is an inhibitor of enzyme E and potentially useful for treatment of Alzheimer's Disease, was disclosed in the U.S. on a Power Point slide at a conference by a University graduate student. The student had obtained information about A1 from the lead inventor, who is a professor at University. The disclosure was not authorized by the University or the professor.

On December 20, 2012, a third party researcher who was present at the October 17, 2012 conference disclosed the graduate student's material (including the structure of A1) in a blog entry about novel methods of treating Alzheimer's Disease. The third party researcher went on to speculate about additional structurally similar compounds which may be active as inhibitors of enzyme E and for treatment of Alzheimer's Disease:

 I suspect that derivatives with other halogen or small

alkoxy groups at the phenyl group would also be active against

Enzyme E. I’m thinking of filing a patent on this once I figure

out how to make them. Does anyone know how to file a patent?

On April 30, 2013, University files a first provisional patent application 001P, claiming novel compounds of genus A, and discloses their utility for inhibiting enzyme E and for treatment of Alzheimer's Disease. Application 001P discloses species A1-A50, all of which are within genus A.

**GENUS A:**

X, Y and Z are each independently selected from the group consisting of C1-4 alkyl and C1-4 alkoxy, each of which are optionally substituted with halogen and cyano;

R1, R2 and R3 are each independently selected from the group consisting of hydrogen, halogen, C1-4 alkyl, and C1-4 alkoxy.

On April 30, 2014, University files a PCT application and a US non-provisional application 001NP, which are identical to 001P.

**Questions:**

1. Does the University have a valid claim to species A1 in the US? What about in the EPO and Japan? What about a claim to genus A?
2. Would the answers change if 001P was filed 3/3/2013?
3. Assume for this answer that 001P was filed 3/3/2013. In early July 2013, your client comes to you and says his group has identified a new species A51:

He suggests that you “supplement the application that we already filed to add this compound.” How do you respond to the inventor’s request on A51? What’s the best strategy for obtaining patent protection for A51?

1. Your application was filed 4/3/2013. It is later discovered that Innovator Company independently synthesized species A1 on 12/20/2012 and filed a Japanese patent application on 1/2/2013. Who has patent rights to species A1?
2. Does your answer change if your application was filed 3/3/2013?

**PLI Biotech Practice****In Class Problem 9**

Dr. Movreek at Virus-Cure discovered that four particular 2’-methyl modified nucleosides (shown below) are highly effective in the treatment of Virus C.

These nucleosides have the basic structure shown below. At each numbered position on the sugar ring, substituents can be in the “up” or “down” position. Dr. Movreek believes based on this data that any purine or pyrimidine β-D-2’-methyl-ribofuranosyl nucleoside (or phosphate thereof, or a salt or ester thereof) having the 2’ methyl in the up position will exhibit this enhanced activity for Virus-C.

Dr. Movreek recognizes that modifications at other positions on the sugar moiety could be made. For example, the 2’ down and 3’ down positions (where hydroxy is present in the structures above) can be replaced, for example, with halogen and unsubstituted and substituted alkyl groups.

Please draft broad and intermediate scope claims covering these new compositions of matter and methods of treatment.

Does the broadest claim meet the enablement requirement?

**PLI Biotech Practice****In Class Problem 10**

You are the attorney of record prosecuting United States patent application Serial No. 15/666,666, which is the U.S. National Phase of PCT application No. PCT/DE2015/111111 filed July 2, 2015 and titled “VARIANT AMYLASE ENZYMES.” The PCT application claims priority to an application that was filed in Germany on July 2, 2014. The German priority application is written in the German language. However, the PCT application is an English language translation of the German priority document, and published in English on January 2, 2016, as publication number WO 2016/000011. A copy of the published PCT application is attached, including a copy of the original claims which are now pending and under examination in the USPTO.

The USPTO issued a first Office Action for the U.S. national phase application on June 1, 2019. On June 9, 2019, you received a letter from the inventor (Dr. Iam Goode) commenting on the Examiner’s rejections.

Draft a complete response to the Office Action, including any amendments, arguments and supporting evidence you believe necessary to overcome the Examiner’s rejections and comply with the inventors’ comments and instructions.

VARIANT AMYLASE ENZYMES

Field of the Invention

The present invention relates to amylase variants having improved properties relate to the parent enzyme from which they are derived, such as improved thermal and/or oxidation stability and/or reduced calcium ion dependency. The invention also relates to DNA constructs encoding the variant amylases, and to vectors and cells harboring the DNA constructs. The invention further relates to methods of producing amylase variants, and to industrial methods and compositions that use the variant amylases.

Background of the Invention

Amylase enzymes have been used industrially for a number of years and for a variety of different purposes, including starch liquefaction, textile desizing, starch modification and for brewing and baking. A further use of amylases is for the removal of starchy stains during washing of clothes and textiles and/or for dishwashing.

In recent years attempts have been made to construct variant amylase enzymes that have improved properties with respect to specific uses such as starch liquefaction and textile desizing. For instance, Suzuki *et al.* (1989) disclose chimeric amylases in which a specific region of an amylase enzyme from the bacteria species *Bacillus amyloliquefaciens* are modified to improve thermostability. Among the variant *B. amyloliquefaciens* amylases reported to have improved thermostability is a variant in which amino acid residues 176 and 177 are deleted.

Bisgaard-Frantzen (WO 95/10603) also describes amylase variants from different *Bacillus* species, including *B. amyloliquefaciens*, *B. stearothermophilus* and *B. licheniformis*. Bisgaard-Frantzen also provides an alignment of the amino acid sequences for these two amylase enzymes, showing that they are highly homologous. For example, the alignment shows that amino acids 176 and 177 of the *B. amyloliquefaciens* amylase (*i.e.*, the amino acid residues deleted by Suzuki) correspond to residues 179 and 180 of the *B. stearothermophilus* amylase.

It is an object of the present invention to provide variant amylase enzymes which – relative to their parent amylase – possess improved properties, such as increased thermal stability, increased stability towards oxidation, reduced dependency on Calcium or other ions, and/or improved activities in conditions or either acid or alkaline pH relative to industrial and commercial applications such as laundry washing and dishwashing.

Summary and Detailed Description

Amylase variants of the invention are suitably prepared on the basis of a “parent” or wild-type amylase. For example, SEQ ID NO:1 and SEQ ID NO:2 provide amino acid sequences for parent amylases obtainable from alkalophilic *Bacillus* strains NCIB 12512 and NCIB 12513, respectively, described in EP 0 277 216 B1. The amino acid sequence of a parent amylase obtainable from *Bacillus stearothermophilus*, described in *J. Bacteriol.* 166 (1986) pp. 635-643, is shown in SEQ ID NO:3. Another preferred parent amylase, having the amino acid sequence in SEQ ID NO:4, is obtainable from *Bacdillus* sp. #707 described by Tsukamoto *et al.*, *Biochem. Biophys. Res. Commun.* 151 (1988) pp. 25-31. Other variants according to the invention include variants of parent amylases which have amino acid sequences exhibiting a high degree of sequence identity to one of the parent amylase sequences shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. For purposes of describing and claiming the present invention, an amino acid sequence is considered to be X% identity to a parent amylase sequence if a comparison of the respective amino acid sequences, performed using the GAP computer program (GCG package, version 7.3 available from the Genetic Computer Group, Madison, WI) reveals an identity of X%. Preferred parent amylases used in accordance with the invention have at least 70%, preferably at least 80%, more preferably at least 85%, and even more preferably at least 90% identity to at least one of the parent amylase sequences set forth in SEQ ID NOS:1-4. In particularly preferred embodiments a parent amylase will have at least 95% identity to at least one of the parent amylase sequences in SEQ ID NOS:1-4.

The invention provides a variety of amylase variants, including but not limited to the specific variants listed in Table 1 below.

[TABLE 1 OMITTED FOR BREVITY]

Particularly preferred amylase variants of the invention are variants of the parent amylase from *B. stearothermophilus* having the amino acid sequence set forth in SEQ ID NO:3. Such variants preferably have an amino acid sequence that is at least 80% identical, and is more preferably at least 85%, 90% or 95% identical to SEQ ID NO:3. Moreover, the preferred variants, when aligned with the parent amylase sequence using the GAP computer program (Genetic Computer Group, Madison, WI) comprise a deletion of the amino acids residues at positions 179 and 180 of SEQ ID NO:3. Preferred variants may comprise additional amino acid insertions, substitutions or deletions relative to the parent amylase. For example, a variant amylase of the invention may further comprise the substitutions of a cysteine at either of positions 349, 428 or both in SEQ ID NO:3. Such variants are more thermostable than the parent *B. stearothermophilus* amylase, and are therefore useful, *e.g.*, for starch liquefaction during the production of biofuels, such as ethanol, from corn.

Variant amylases of the invention can be tested for amylase activity by a standard method using a a cross-linked insoluble blue-colored starch polymer as substrate. A tablet of the colored starch polymer substrate is suspended in a tube containing 5 ml of 50 mM Britton-Robinson buffer (50 mM acetic acid, 50 mM phosphoric acid, 50 mM boric acid, 0.1 mM CaCl2) pH adjusted to the value of interest with NaOH. The amylase enzyme to be tested is diluted to an appropriate concentration in 50 mM Britton-Robinson bufer, and 1 ml of the diluted amylase is added to the 5 ml suspension. The enzyme starch suspension should be incubated for 15 minutes in a water bath at the temperature of interest. As the starch is hydrolyzed by the amylase, soluble blue fragments are released. The absorbance of the resulting blue solution, measured spectrophotometrically at 620 nm, is directly proportional to the enzyme activity. The assay can be performed using commercially available kits, such as the Phadebas® Amylase Test available from Pharmacia Diagnostics.

Amylase variants of the invention are well suited for use in a variety of industrial processes, including processing operating at high levels of temperature and/or pH. In particular, they are useful as components in laundery, dishwashing and hard surface cleaning detergent compositions. Amylase enzymes of the invention are also useful in starch liquefaction, *e.g.*, during the production of ethanol from starch. Conditions for conventional starch-converting processes and liquefaction are described in the art, for instance in U.S. 3,912,590 and EP 252,730.

WHAT IS CLAIMED IS:

1. A variant of a parent amylase, said parent having an amino acid sequence set forth in any of SEQ ID NOS:1-4 or having at least 80% identity to one or more of the amino acid sequences set forth in SEQ ID NOS:1-4, in which variant:

(a) at least one amino acid residue of the parent amylase has been deleted;

(b) at least one amino acid residue of the parent amylase has been replaced by a different amino acid residue; or

(c) at least one amino acid residue has been inserted relative to the parent amylase,

and further wherein the variant amylase has amylase activity and exhibits at least one of the following properties relative to the parent: increased thermostability, increased stability towards oxidation, reduced Ca2+ dependency, increased activity at an acidic pH, and increased activity at an alkaline pH.

2. The variant of claim 1, wherein the parent amylase has an amino acid sequence that is at least 80% identical to SEQ ID NO:3.

3. The variant of claim 2, wherein the variant comprises a deletion of amino acids 179 and 180 when aligned with SEQ ID NO:3.

4. The variant of claim 3, further comprise a substitution of a cysteine at amino acids 349 and 428 when aligned with SEQ ID NO:3.

Application No. 15/666,666

**OFFICE ACTION**

Claims 1-4 are currently pending and under consideration.

Claims 1-4 are rejected under 35 U.S.C. § 112(a), as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

Claim 1 is directed to a genus of variants of parent amylases, in which the parents have at least 80% identity to any of SEQ ID NOS:1-4 and having at least one amino acid deletion, substitution or insertion relative to the parent. The claimed variant amylases also have amylase activity while, at the same time, exhibiting any of the properties recited in the claim relative to the parent, these being: increased thermostability, increased stability towards oxidation, reduced Ca2+ dependency, increased activity at an acidic pH, and increased activity at an alkaline pH.

Claims 2-4 are directed to a genus of variants of a parent sequence that is at least 80% identical to SEQ ID NO:3.

The specification describes only a few specific variants of SEQ ID NO:3 within the scope of the instant claims. No information, beyond the characterization of these specific variants has been provied by applicants which would indicate that they had possession of the claimed genus of modified polypeptides. The specification does not contain any disclosure of the function of all polypeptide sequences derived from SEQ ID NO:3, or any other related amylases (including the amaylses of SEQ ID NOS:1-2 and 4). The specification also does not contain any disclosure of which amino acids are critical for and/or could be inserted, substituted or deleted to provide for increased stability towards oxidation, reduced Ca2+ dependency, increased activity at an acidic pH, or increased activity at an alkaline pH for any parent amylase. With respect to increased thermostability, the specification only describes a limited number of species; namely, the deletion of amino acids 179 and 180 in SEQ ID NO:3, and the substitution of cysteine at amino acids 349 and 428 of that sequence.

Claims 1-4 are also rejected under 35 U.S.C. 112(a) because the specification does not reasonably provide enablement for any variant of any parent amylase having at least 80% identity to any of SEQ ID NOS:1-4 and comprising any amino acid insertion, substitution or deletion that confers one or more of the following properties relative to the parent: increased thermostability, increased stability towards oxidation, reduced Ca2+ dependency, increased activity at an acidic pH, and increased activity at an alkaline pH. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The genera of variants covered by these claims include variants with an enormous number of alterations of the parent enzyme (where the parent enzyme can be selected from an enormously large group of enzymes) and include variants with no common function. Predictability of which changes can be tolerated in a protein’s amino acid sequence and obtain the desired activity requires a knowledge of a guidance with regard to which amino acids are tolerant of modification and which are conserved, and detailed knowledge of the ways in which the protein’s structure relates to its function. Guidance is need to enable a skilled artisan to identify which amino acids in a aprent amylase can and should be modified to provide for properties such as increased thermostability, increased stability towards oxidation, reduced Ca2+ dependency, increased activity at an acidic pH, and increased activity at an alkaline pH. However, in this case, the disclosure is limited to only a few representative species (discussed above) with only a small number of altered amino acids compared to a specific parent amylase. While recombinant and mutagenesis techniques are known and routine, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, with a reasonable expectation of success in obtaining the desired activity.

Thus, applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including an enormous nunmber of amino acid modifications of a large number of parent amylases.

Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over the Suzuki reference cited in applicant’s specification, in view of the cited Bisgaard-Frantzen reference.

Suzuki teaches a mutant of a parent amylase from *B. amyloliquefaciens* with increase thermostability in which amino acid residues 176 and 177 are deleted. Bisgaard-Frantzen teaches that *B. amyloliquefaciens*, *B. stearothermophilus* and *B. licheniformis* alpha amylases are homologous enzymes such that modification of corresponding residues in the three enzymes are expected to have similar effects. Bisgaard-Frantzen also teaches an alignment of the amino acid sequences of these three amylases, which shos that positions 176 and 177 of the *B. amyloliquefaciens* amylase corresponds to residues 179 and 180 of the *B. stereothermophilus* amylase.

The *B.stereothermophilus* amylase shown in the alignment of Bisgaard-Frantzen has the same amino acid sequence as SEQ ID NO:3 of applicant’s specification.

Therefore, it would have been obvious to one of ordinary skill in the art to introduce the mutations disclosed by Suzuki into the corresponding positions of *Bacillus stearothermophilus* amylase in order to produce a homologous amylase which would have been reasonably expected to have similar improved properties – namely, improved thermostability – in view of the known homology between these amylases.

No claim is allowed.

Signed:

Examiner Toug

Mr. John Doe

Patent Agent

810 Seventh Avenue

New York, New York 10019

I have considered the Office Action in my application for “AMYLASE VARIANTS,” which you recently forwarded to me for comments.

I am disappointed to see that the Examiner does not believe that the application adequately describes or enables the amylase variants of this invention. Table I clearly shows that my laboratory manufactured a large number of variants from all of the parent amylases identified in the application, many of which have properties of improved thermostability, decreased Ca2+ dependence, and/or improved pH tolerance (at acidic or alkaline pH). While it is relatively straightforward to make such variants once a person knows which modifications they should make, I do not believe any of these modifications would have been obvious to others working in this field back in July 2008, when I filed my first application in Germany. For that matter, these variants could not have been obvious when I filed my PCT application in July 2009, since I had not published any of this research by then.

The Examiner’s rejections are especially disheartening since I recently learned that my nemesis and business competitor, Dr. Evil, is selling a product called RIPOFF that contains one of the amylase variants I invented. The amylase variant in RIPOFF is identical to the wild-type *Bacillus stearothermophilus* amylase, except for the deletion of the amino acids 179 and 180 in the wild-type *B. stearothermphilus* amylase sequence. This is very similar to the *B. amyloliquefaciens* variant Suzuki describes. However, the *B. stearothermophilus* variant is much more thermostable. This was recently confirmed in my lab. We performed the amylase activity assay described in my application on both the wild-type and variant amylases of *B. steorthermophilus* and *B. amyloliquefaciens*. In each assay, the enzyme was incubated at an elevated temperature (80 °C) for various lengths of time before reacting it with the substrate. A plot of our results is shown below:

As you can see, the *B. stearothermophilus* variant (“BSG DEL”) simply blows the other enzymes away, and has a high level of activity (> 50%) even after it is incubated at high temperature for more than 4,000 minutes (for almost three days). In fact, we estimated that the enzyme’s half-life at that temperature is 5,775 minutes. By contrast, Suzuki’s *B. amyloliquefaciens* variant (“BAN DEL”) had a half-life of only 9.5 minutes. This was less than the half-life we measured for the wild-type *B. stearothermphilus* enzyme (“BSG WT”), which was 92 minutes. The *B. amyloliquefaciens* wild-type (“BAN WT”) had a half-life of only 0.9 minutes. We certainly did not expect to see such a dramatic improvement in the *B. stearothermphilus* variant, and I do not think that others working in this field could have expected it either.

The RIPOFF product is a huge market success. Our own sales are suffering while we try to compete with it. So it is important to have a patent covering least that *B. stearothermophilus* variant.

Sincerely,

Dr. Iam Goode