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(54) Title: PEROXIDE-DRIVEN CYTOCHROME P450 OXYGENASE VARIANTS

(57) Abstract: The invention relates to novel variants of cytochrome P450 oxygenases. These variants have an improved ability to use peroxide as an oxygen donor as compared to the corresponding wild-type enzyme. These variants also have a improved thermostability as compared to the cytochrome P450 BM-3 F87A mutant. Preferred variants include cytochrome P450 BM-3 heme domain mutants having I58V, F87A, H100R, F107L, A135S, M145A/V, N239H, S274T, L324I, I366V, K434E, E442K, and/or V446I amino acid substitutions.



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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/11954

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>																						
IPC(7) : C12N 9/02, 15/00; C07H 21/04																						
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According to International Patent Classification (IPC) or to both national classification and IPC																						
<b>B. FIELDS SEARCHED</b>																						
Minimum documentation searched (classification system followed by classification symbols)																						
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched																						
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)																						
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<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																						
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																				
X	NOBLE. M.A. Roles of key active site residues in flavocytochrome P450 BM3. Biochem J. 1999, Vol 339, pages 371-379.	1-4, 11, 15-16, 20-22, 30, 34-38, 41-44, 47, 50																				
X	OST. T.W. Rational re-design of the substrate binding site of flavocytochrome P450 BM3. FEBS Lett. December 2000, Vol 486, No. 2, abstract.	1-4, 11, 15-16, 20-22, 30, 34-38, 41-44, 47, 50																				
X	YEOM. H. The role of Thr268 in oxygen activation of cytochrome P450BM-3. Biochemistry. November 1995, Vol 34, No. 45, abstract.	1-4, 11, 15-16, 20-22, 30, 34-38, 41-44, 47, 50																				
X	LI. Q.S. Critical role of the residue size at position 87 in H <sub>2</sub> O <sub>2</sub> -dependent substrate hydroxylation activity and h <sub>2</sub> O <sub>2</sub> inactivation of cytochrome P450-BM-3. Biochem Biophys Res Commun. February 2001, Vol 280, No. 5, abstract.	1-61																				
Y	GOVINDARAJ. S. Role of the linker region connecting the reductase and heme domains in cytochrome P450BM-3. Biochemistry. September 1995, Vol 34, No. 35, abstract.	1-61																				
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																						
* Special categories of cited documents: <table border="0"> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>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</td> </tr> <tr> <td>"B"</td> <td>earlier application or patent published on or after the international filing date</td> <td>"X"</td> <td>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</td> </tr> <tr> <td>"L"</td> <td>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)</td> <td>"Y"</td> <td>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</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&amp;"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			"A"	document defining the general state of the art which is not considered to be of particular relevance	"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	"B"	earlier application or patent published on or after the international filing date	"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	"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)	"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	"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family	"P"	document published prior to the international filing date but later than the priority date claimed		
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# INTERNATIONAL SEARCH REPORT

PCT/US02/11954

## Continuation of B. FIELDS SEARCHED Item 3:

US patentes, EST, stremble, swissprot, pir, EAST, STN/Caplus

- p450 bm3, bm-3, bacillus megaterium, p450 cytochrome, monooxygenase, oxygenase, reductase, mutant, variant