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(54) Title: EXPRESSION OF PROTECTIVE ANTIGENS IN TRANSGENIC CHLOROPLASTS AND THE PRODUCTION OF IMPROVED VACCINES

(57) Abstract: Vaccines for conferring immunity in mammals to infective pathogens are provided, as well as vectors and methods for plastid transformation of plants to produce protective antigens and vaccines for oral delivery. The invention further provides transformed plastids having the ability to survive selection in both the light and the dark, at different developmental stages by using genes coding for two different enzymes capable of detoxifying the same selectable marker, driven by regulatory signals that are functional in proplastids as well as in mature chloroplasts. The invention utilizes antibiotic-free selectable markers to provide edible vaccines for conferring immunity to a mammal against Bacillus anthracis, as well as Yersina pestis. The vaccines are operative by parenteral administration as well. The invention also extends to the transformed plants, plant parts, and seeds and progeny thereof. The invention is applicable to monocot and dicot plants.



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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US02/41503

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 15/82, 5/04; A61K 39/02, 39/07; A01H 5/00, 5/10  
 US CL : 435/320.1, 419; 424 /190.1; 800/298

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)  
 U.S. : 435/320.1, 419; 424 /190.1; 800/298

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)  
 Please See Continuation Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/03022 A2 (CALGENE LLC) 20 January 2000 (20.01.2000), see page 25-27 and 43-48.	1, 7, 10-13, 22-25, 34-35 and 41-44
X	WO 01/72959 A2 (AUBURN UNIVERSITY) 04 October 2001 (04.10.2001), see page 12-14, 21-25, 49-60, 71-73, 75-76, 113, 141-142, 149-150, 156-157, 162, 163, 199-201, 207, 215, 254.	1-5, 7-30, 34-35 and 41-44
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Y		6
X	WO 99/10513 A1 (AUBURN UNIVERSITY) 04 March 1999 (04.03.1999), see page 31-33 and 28-29.	1-5, 7-13, 15-20, 22-27, 29, 34-35 and 41-44
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Y		41-44
X	DANIELL et al, Expression of the Native Cholera Toxin B Subunit Gene and Assembly as Functional Oligomers in Transgenic Tobacco Chloroplasts. Journal of Molecular Biology, August 2001, Vol. 311, No. 5, pages 1001-1009; see entire document.	1-5, 7-13, 15-20, 22-30, 34-35 and 41-44
X	DANIELL et al, Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. Trends in Plant Science, May 2001, Vol. 6, No. 5, pages 219-226, see pages 221-223.	1-5, 7-13, 15-20, 22-30, 34-35 and 41-44
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Y		6

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

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

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**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions that are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 6, and claims 1-5, 7-30, 34-35 and 41-44, all in part, drawn to a plastid transformation vector encoding a protective antigen for anthrax, plants and plastids transformed with the vector, and a method of using it to produce the antigen.

Group II, claim(s) 31-33, and claims 1-5, 7-30, 34-35 and 41-44, all in part, drawn to a plastid transformation vector encoding a protective antigen for plague, plants transformed with the vector, and a method of using it to produce the antigen.

Group III, claim(s) 36-38, drawn to a vaccine conferring immunity to Bacillus anthracis and a process for administering the vaccine.

Group IV, claim(s) 39-40, drawn to a vaccine conferring immunity to Yersinia pestis and a process for administering the vaccine.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: They do not share a special technical feature.

The technical feature shared by Groups I-II is a plastid vector encoding a protective antigen.

The technical feature shared by Groups III-IV is an orally administered vaccine to a bacterium.

Thus, the groups share no technical feature.

Furthermore, CALGENE LLC (WO 00/03012) teaches plastid transformation vectors encoding the protective antigen aprotinin and plants transformed with it (pg 23-24 and 43), rendering the invention of claim 1, among others, not novel. Thus, the technical feature of Group I is not special.

**Continuation of Box II Item 4:**

1-30, 34-35 and 41-44 to the extent they read on a vector encoding an anthrax antigen

**Continuation of B. FIELDS SEARCHED Item 3:**

USPAT, EPO, JPO, Derwent, PG-PUB, Agricola, Biosis, CaPlus, CABA

Search terms: Plastid, chloroplast, vaccine, antigen, anthrax