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(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0091709 A1****Song et al.**(43) **Pub. Date: Apr. 28, 2005**(54) **TRANSGENIC ORGANISM EXPRESSING
FUNGAL MRP-LIKE ABC TRANSPORTERS**

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C07H 21/04; C12N 5/04;
C07K 14/415
(52) **U.S. Cl.** **800/278**; 435/69.1; 435/419;
435/468; 530/370; 536/23.6(57) **ABSTRACT**Correspondence Address:
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The present invention relates to an isolated DNA encoding fungal MRP (multidrug resistance associated protein) subfamily of ATP-binding cassette (ABC) transporter protein which confers organisms with resistance to and/or accumulation of toxic materials such as heavy metals and herbicides, vectors containing the isolated DNA, and organisms transformed with the isolated DNA. Organisms transformed with the fungal MRP subfamily of ABC transporters of the present invention can be used for remediation of environment polluted with toxic materials. For example, the transformed plants can be used for cleaning polluted soil or water, and thereby providing an environmentally-friendly way to remediate polluted resources with low cost.

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Oct. 16, 2001 (KR) 2001/68302

FIG.1

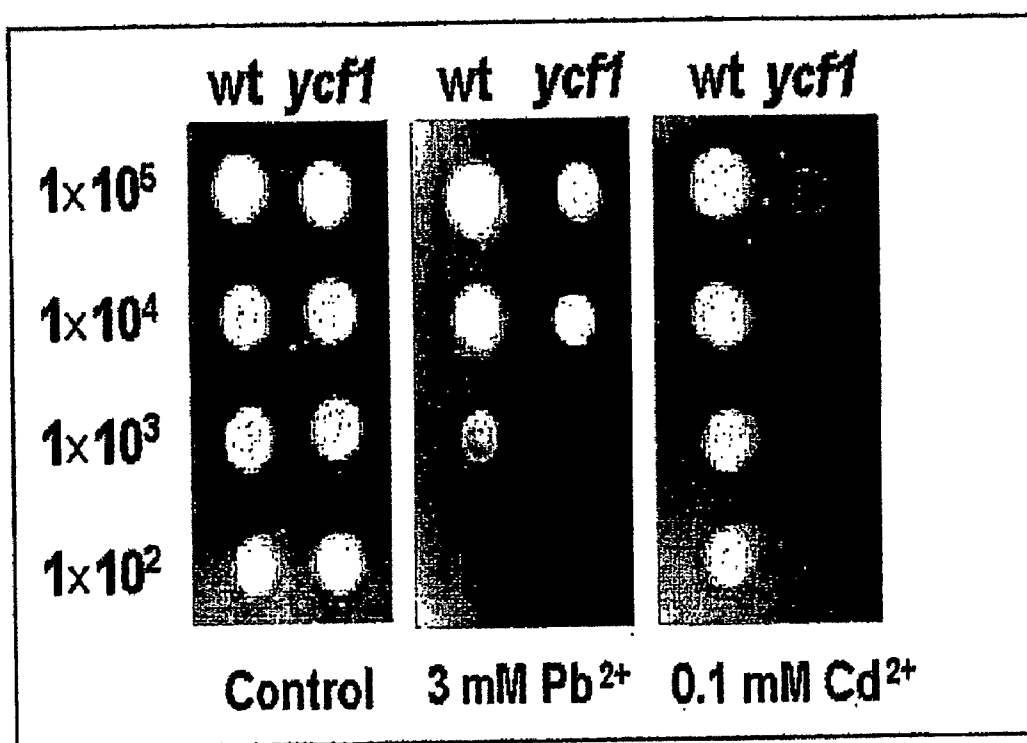


FIG.2

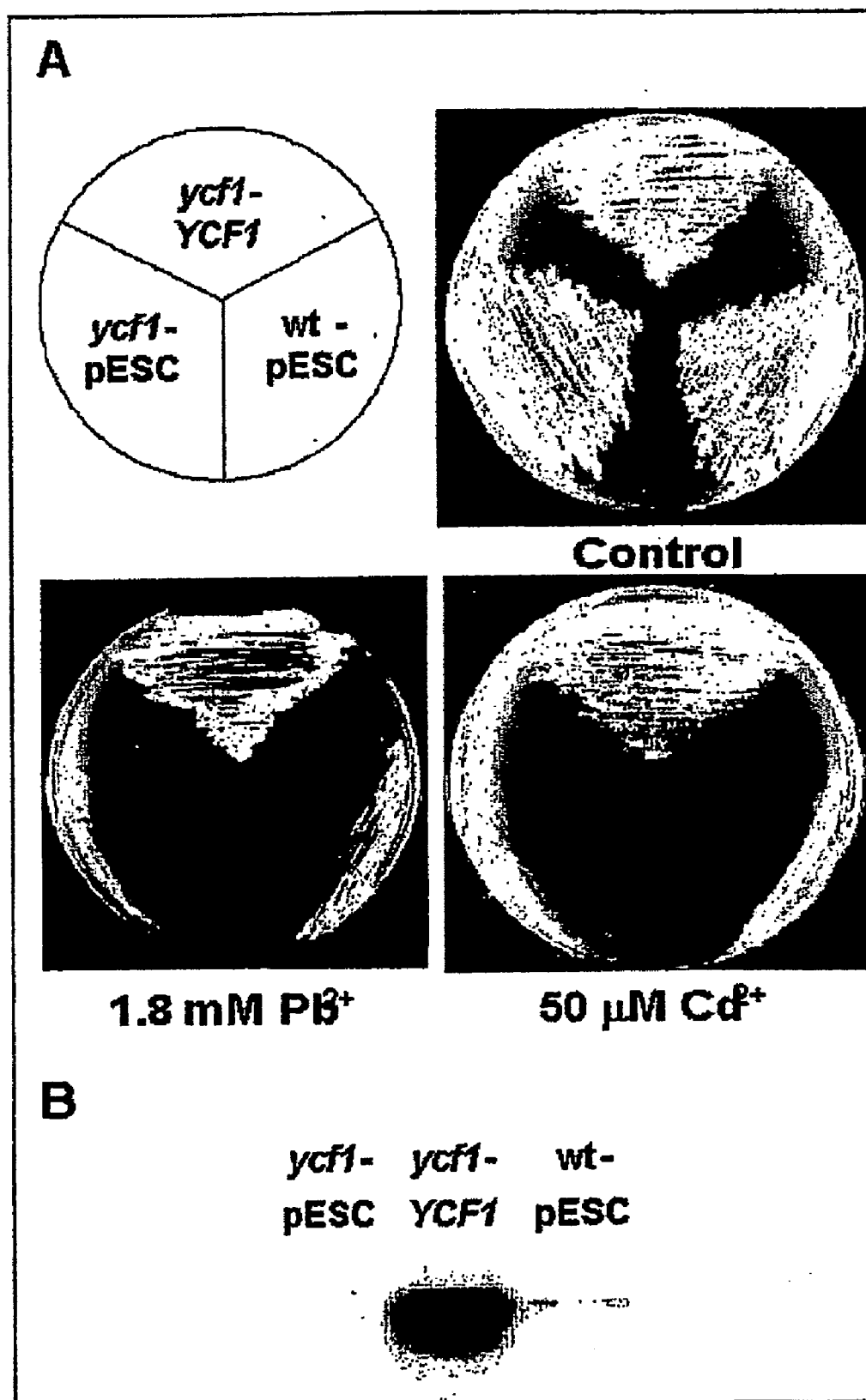


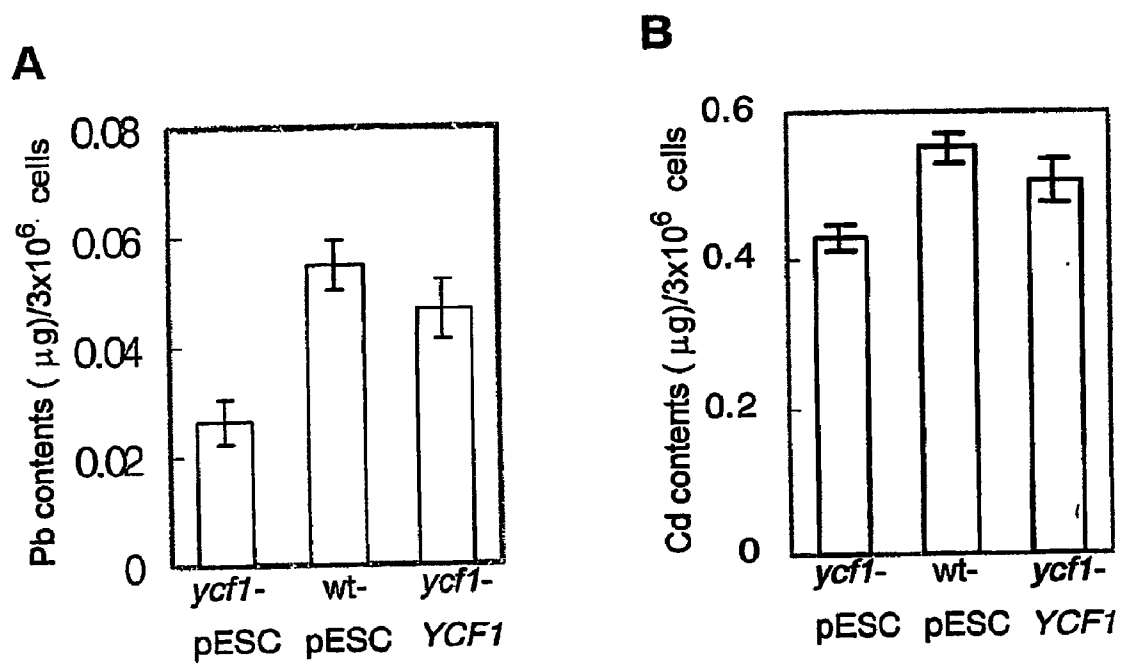
FIG.3

FIG.4

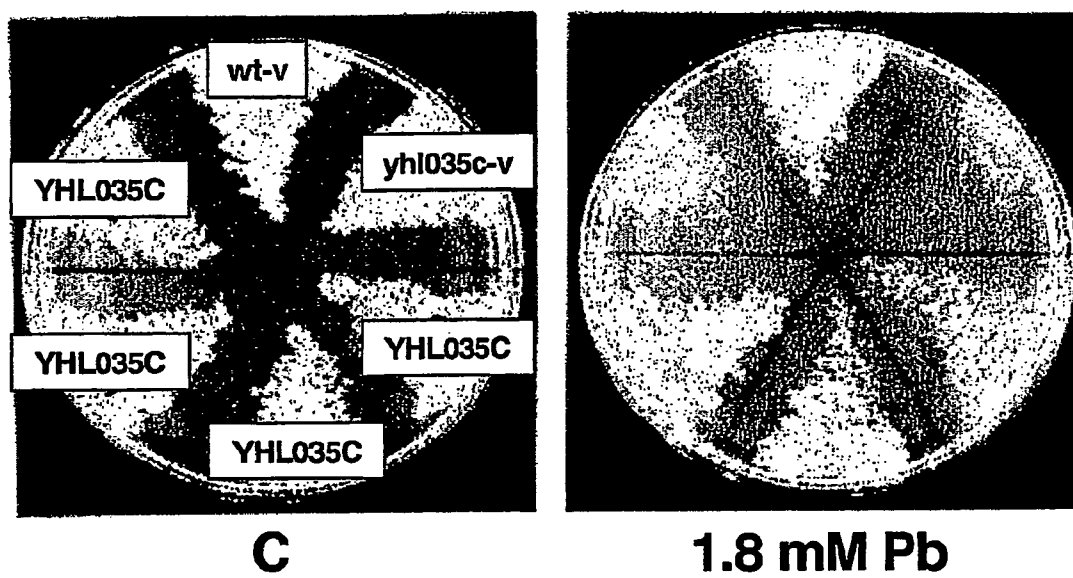


FIG.5

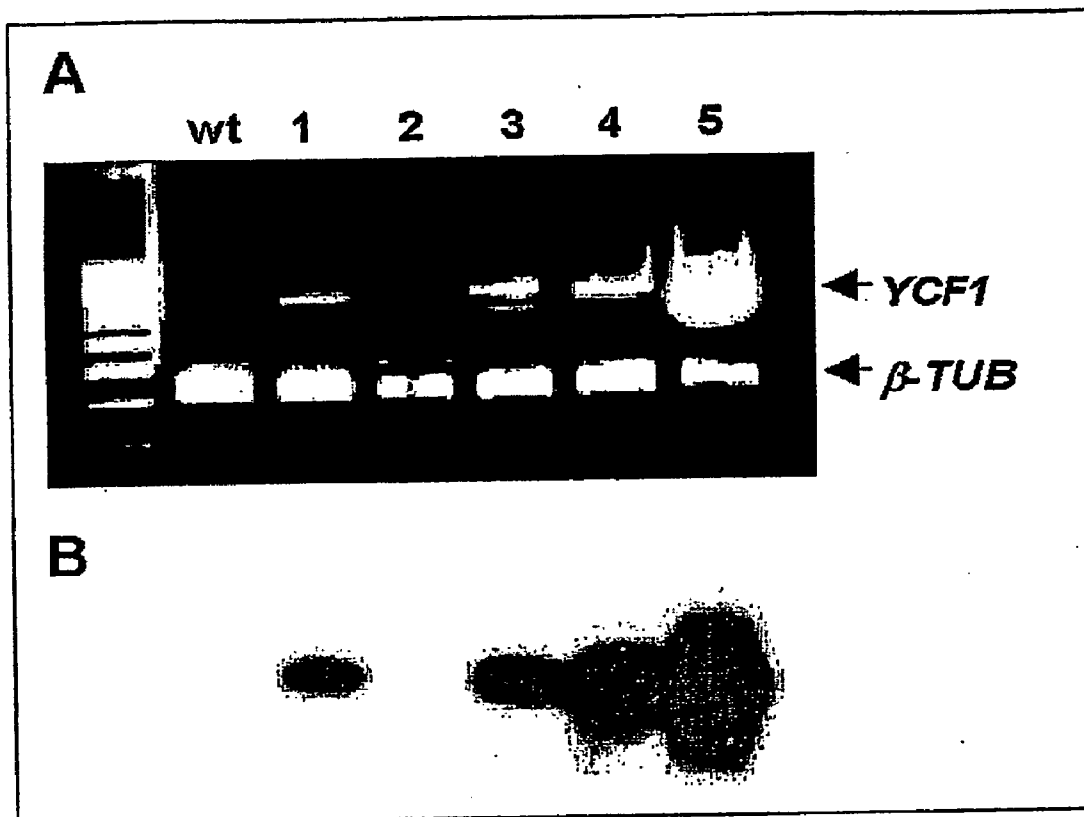


FIG.6

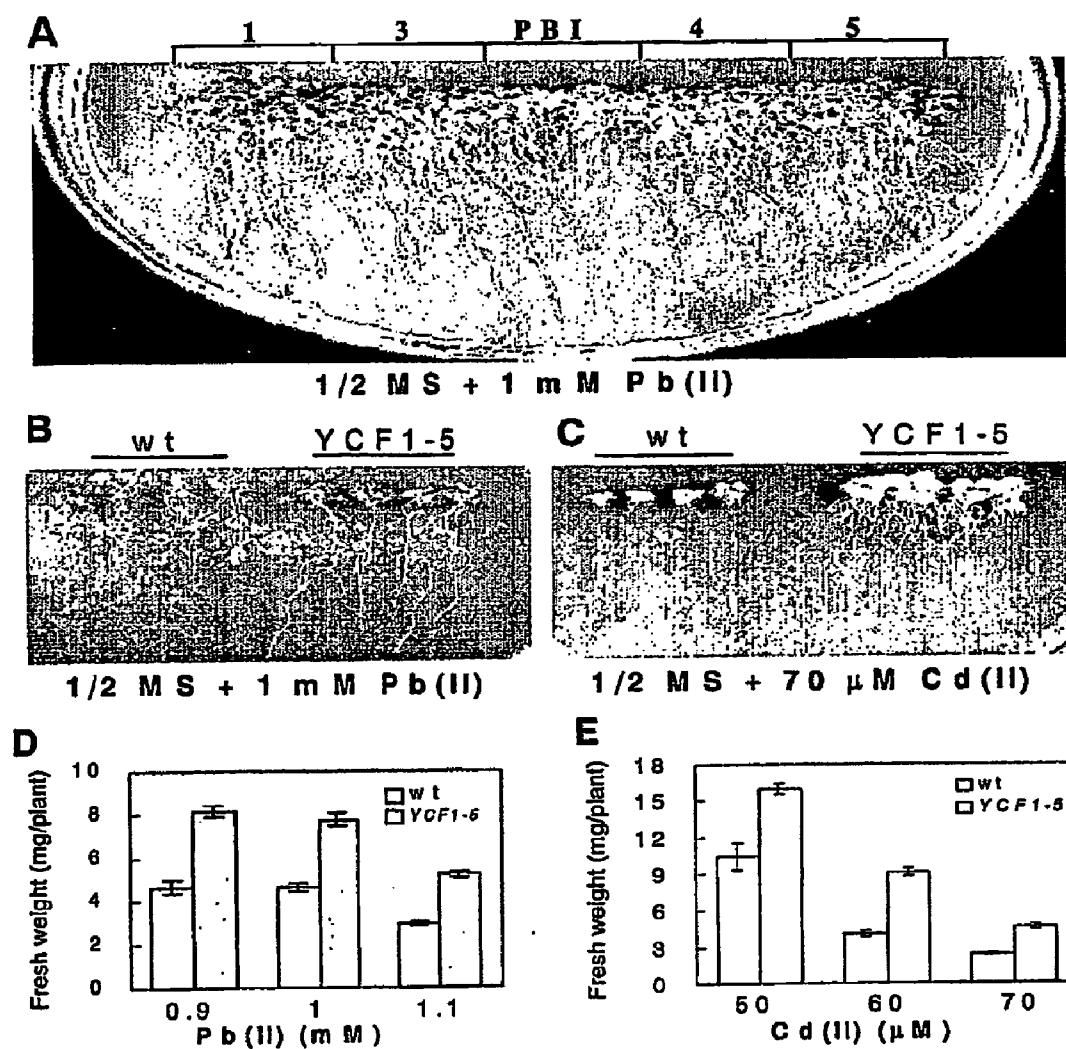


FIG.7

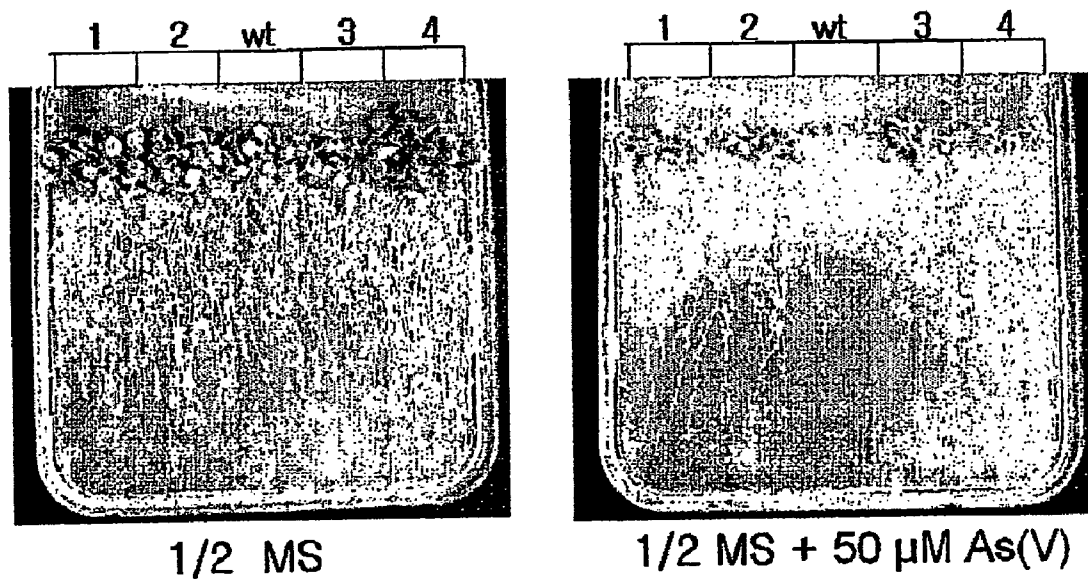


FIG.8

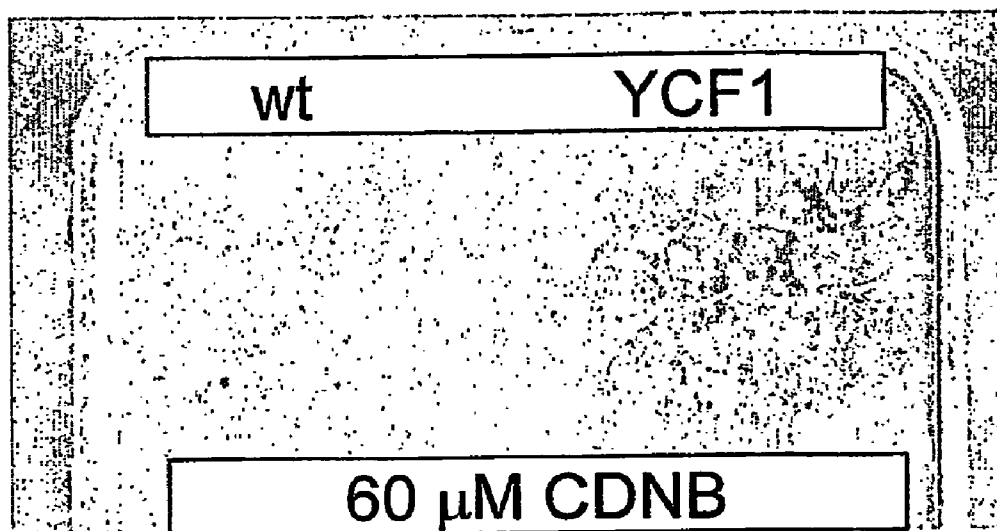
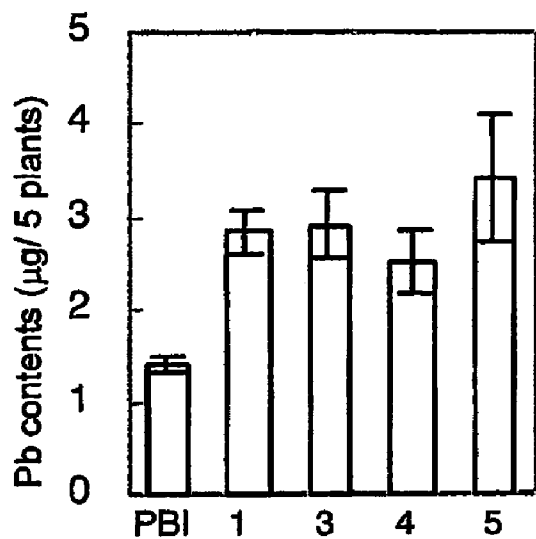


FIG.9

A



B

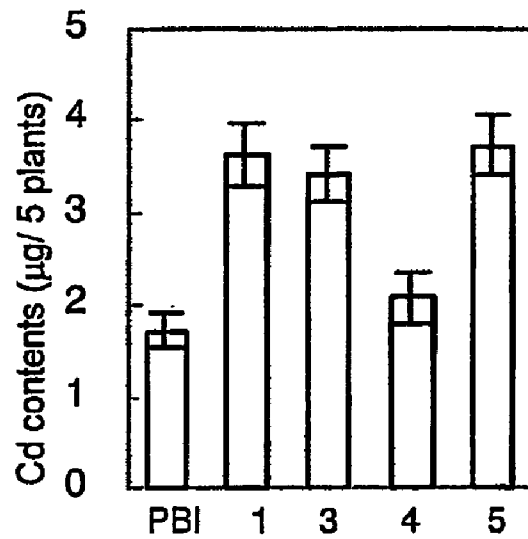
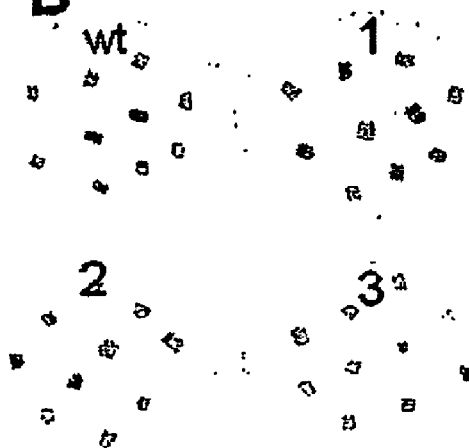


FIG.10

A



B



C

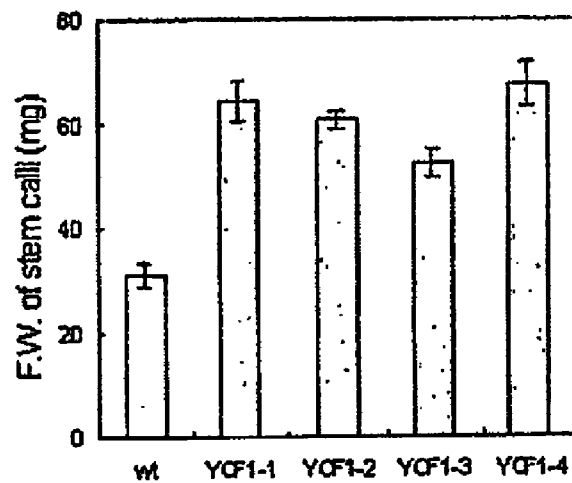
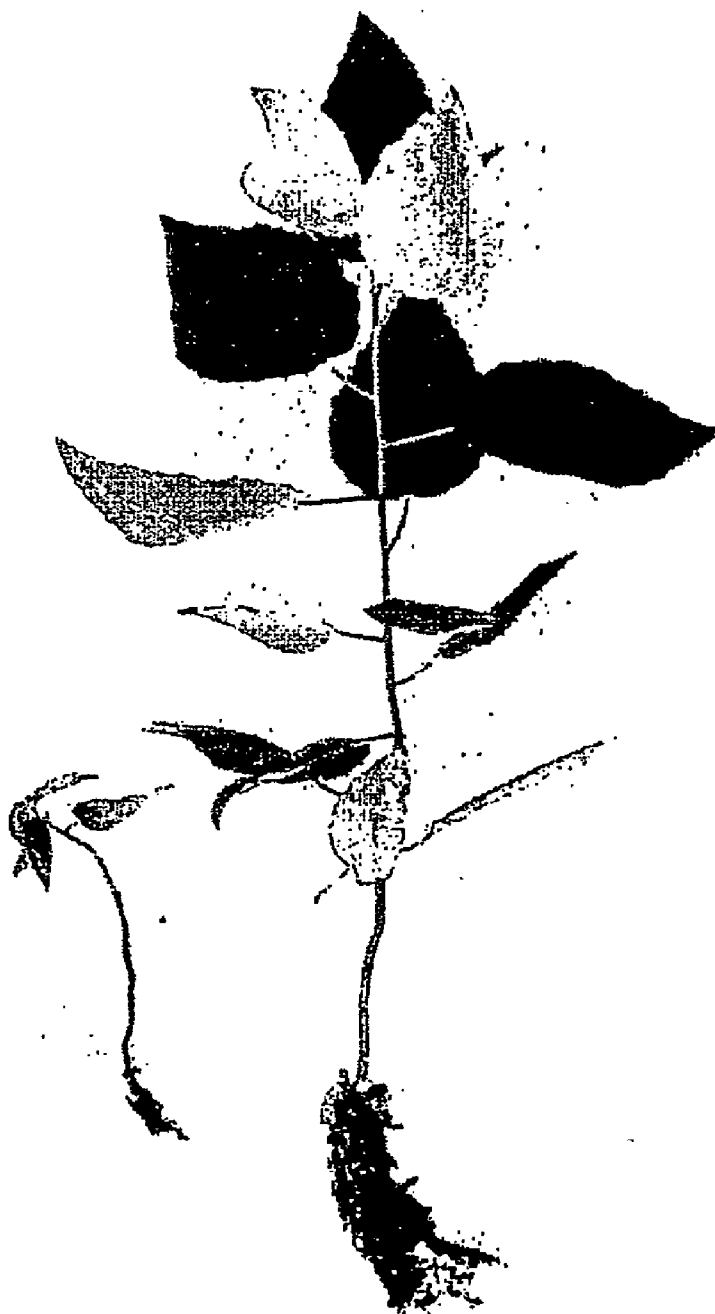


FIG.11



wt

PBI-YHL035C

TRANSGENIC ORGANISM EXPRESSING FUNGAL MRP-LIKE ABC TRANSPORTERS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is based on Korean Patent Application Nos. 2001-0063802 and 2002-0062984 filed with Korean Intellectual Property Office on Oct. 16, 2001 and Oct. 15, 2002, respectively.

BACKGROUND OF THE INVENTION

[0002] (a) Field of the Invention

[0003] The present invention relates to a fungal MRP-like ABC transporter gene and organisms transformed with the gene, and more particularly, to transformed organisms expressing fungal MRP-like ABC transporter genes including YCF1 or YHL035C, and thereby having improved resistance to and accumulation of toxic materials such as lead, cadmium, arsenic, and herbicides.

[0004] (b) Description of the Related Art

[0005] Heavy metals such as lead, cadmium, mercury and so on accumulate in the human body through nature's food chain and cause chronic damage to the brain, nerves, bones, etc., and the polluted environment and damage continues from generation to generation. Typical examples of problems caused by heavy metal toxicity are Minamata disease and itaiitai disease, which have occurred in Japan. As lead is a pollutant that causes the most damage among the heavy metals (Salt, D. E., Smith, R. D., and Raskin, I. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 643-668 (1998)), it is very important to rid lead from the environment. The United States government expends approximately 500 million dollars each year in order to remove lead from environments where children are raised (Lanphear, B. P. The paradox of lead poisoning prevention. *Science*. 281; 1617-1618 (1998)). Additionally, as arsenic pollutes drinking water and causes skin diseases and cancer, it is becoming a serious problem.

[0006] As known genes associated with resistance to or accumulation of toxic materials such as heavy metals and agricultural chemicals, bacterial P-type ATPase has a role in pumping lead to outside of cells at bacterial cell membranes (Rensing, C., Sun, Y., Mitra, B., and Rosen, B. P. Pb(II)-translocating P-type ATPases. *J. Biol. Chem.* 273: 32614-32617 (1998)); genes associated with cadmium resistance include the YCF1 gene of yeast; and genes associated with resistance to arsenic include the YCF1 and ACR genes of yeast, ArsAB of bacteria, etc.

[0007] Living organisms have a mechanism for mitigating toxicity of materials using transporter proteins or biological materials having affinity for noxious materials that invade the body. Use of genes contributing to living organism's resistance against noxious materials would provide an environmentally-friendly way to remediate environments polluted with noxious materials at a very low cost as compared with the physical and/or chemical remediation that is currently widely being employed (Mejare and Bulow, Trends in Biotechnology; 2001, Raskin I. and Ensley B. D. Phytoremediation of Toxic Metals., John Wiley & Sons, New York; 2000). In particular, as plants have many advantages such as their ability to express foreign genes readily and thus

exhibit new phenotypes, they can be produced and maintained at a low cost, they are aesthetically pleasing, etc., research on improvement of plants by inserting useful genes thereinto for use in environmental remediation is being actively conducted. This technique, the use of plants for cleaning up environment, is called "Phytoremediation."

[0008] Under the circumstance where environmental pollution in soil, etc. due to toxic materials such as lead, cadmium, arsenic, and agricultural chemicals is serious, there is a great need for organisms that are transformed by genes that confer resistance to and/or accumulation of these toxic materials.

[0009] Transformed plants that can be used to remove cadmium from the 30 environment have been disclosed in several papers (Zhu et al., (1999) *Plant Physiol.* 119: 73-79, Hirschi et al., (2000) *Plant Physiol.* 124:125-33, Dominguez-Solis et al., (2001) *J. Biol. Chem.* 276: 9297-9302), but there have been no report of transgenic plants that are enhanced in the capacity to remove lead or arsenic from the environment. Further, attempts to develop organisms transformed with YCF1 to improve resistance to not only lead but also to cadmium, arsenic, and herbicides for removal of these toxic materials have not yet been disclosed.

SUMMARY OF THE INVENTION

[0010] The present invention relates to a DNA molecule exhibiting resistance to and accumulation of lead, and encoding fungal MRP-like ABC transporter protein (multi-drug resistance-associated protein ATP-binding cassette transporter protein, MRP-like ABC transporter protein).

[0011] Further, the invention relates to a recombinant vector comprising said DNA molecule encoding MRP-like ABC transporter protein.

[0012] Still further, the invention relates to transformed organisms with improved resistance to and/or accumulation of toxic materials, which are transformed with said DNA molecule encoding fungal MRP-like ABC transporter protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 shows photographs of wild type and YCF1 null (ycf1) yeasts growing on control, lead or cadmium-containing media, and shows that the ycf1 mutant yeast is more sensitive to lead and cadmium than wild type yeast.

[0014] FIGS. 2A and 2B show photographs of wild type yeast transformed with empty vector (wt-pESC), ycf1 yeast transformed with empty vector (ycf1-pESC), and YCF1-transformed ycf1 (ycf1-YCF1) yeast growing on control, lead or cadmium-containing media (A), and the result from Northern blot of YCF1 mRNA from the yeast lines (B).

[0015] FIGS. 3A and 3B show graphs of lead (A) or cadmium (B) content in ycf1-pESC yeast, wt-pESC yeast, and ycf1-YCF1 yeast.

[0016] FIG. 4 shows photographs of wild type, YHL035C mutant (yhl035C-v), and YHL035-transformed yhl035C-v yeasts growing on control or lead-containing media, and shows that yhl035C-v yeast is more sensitive to lead than wild type or yhl035C-v yeast transformed with YHL035C.

[0017] FIGS. 5A and 5B show photographs of RT-PCR results, which show the expression of YCF1 in YCF1-transformed *Arabidopsis thaliana*.

[0018] FIG. 6 shows photographs (A,B,C) and graphs (D,E) showing that YCF1-transformed *Arabidopsis thaliana* are enhanced in resistance to lead and cadmium.

[0019] FIG. 7 shows photographs showing that YCF1-transformed *Arabidopsis thaliana* are enhanced in resistance to arsenic.

[0020] FIG. 8 shows a photograph showing that YCF1-transformed *Arabidopsis thaliana* are enhanced in resistance to herbicide CDNB.

[0021] FIGS. 9A and 9B show graphs of lead (A) and cadmium (B) content in YCF1-transformed *Arabidopsis thaliana*.

[0022] FIGS. 10A-10C show a series of photographs (A,B) and a graph (C) showing that stem calli and leaf segments from YCF1-transformed poplar are enhanced in resistance to lead compared to those from wild type poplar.

[0023] FIG. 11 shows a photograph showing that YHL035C-transformed poplar plant is more resistant to lead than wild type poplar plant.

DETAILED DESCRIPTION AND THE PREFERRED EMBODIMENTS

[0024] The present invention relates to a gene exhibiting resistance to and/or accumulation of toxic materials, a vector comprising the gene, and cells and organisms transformed therewith.

[0025] The gene exhibiting resistance to and/or accumulation of toxic materials in the present invention is a gene encoding fungal multidrug resistance associated protein (MRP)-like ATP-binding cassette transporter protein (hereinafter referred to as "MRP-like ABC transporter protein").

[0026] The fungal MRP-like ABC transporter protein, which is a kind of ABC transporter protein, has a role in transporting several organic materials present in cytoplasm to the outside of the cytoplasm. ABC transporter proteins exist in several organisms ranging from prokaryotic organisms to human liver cells, and they transport a large variety of materials. Organic materials transported by the ABC transporter proteins include heavy metals conjugated with glutathione, bile acids, etc. YCF1 belonging to the fungal MRP-like ABC transporter proteins is known to transport cadmium, arsenic, and agricultural chemicals into vacuoles and thereby confer resistance to these noxious materials (Li et al., (1997) *Proc. Natl. Acad. Sci. USA* 94: 42-47, Ghosh et al., (1999) *Proc. Natl. Acad. Sci. USA* 96: 5001-5006). However, an attempt to develop organisms with improved resistance to all of cadmium, arsenic, and agricultural chemicals using YCF1 for removal of noxious materials has not yet been disclosed. Furthermore, the contribution of YCF1 to resistance to lead has not yet been reported, and there has been no disclosure regarding the function of YHL035C protein and transformants expressing it.

[0027] The fungal MRP-like ABC transporter genes include, as examples, YCF1 (Yeast cadmium factor 1) and YHL035C genes, and MRP-like ABC transporter genes having at least 28% sequence homology in amino acid sequence with YCF1 protein and YHL035C protein. YCF1 and YHL035C genes, or their proteins, have 28% sequence homology with each other. Also, this invention is intended to include DNA molecules encoding fungal MRP-like ABC

transporter proteins and having at least 28% sequence homology, preferably at least 40% homology, and more preferably at least 50% homology in amino acid sequence with YCF1 or YHL035C protein. For example, BPT1, YBT1, and YOR1 genes, which have at least 28% homology in amino acid sequence with YCF1 or YHL035C proteins, can be included from a comparison conducted in the amino acid database from the GenBank using the CLUSTALW program. The BPT1 protein has 40% homology in amino acid sequence with YCF1, YBT1 protein has 51% homology in amino acid sequence with YHL035C, and YOR1 protein has 28% homology in amino acid sequence with YCF1. YBT1 and BPT1 are fungal MRP-like ABC transporter proteins that are known to transport bile acids (Ortiz et al., (1997) *Journal of Biological Chemistry* 272: 15358-15365, Petrovic et al., (2000) *Yeast* 16: 561-571), and YOR1 protein, also a fungal MRP-like ABC transporter protein, has been known to transport several drugs (Decottignies et al., (1998) *Journal of Biological Chemistry* 273: 12612-12622).

[0028] The MRP-like ABC transporter proteins according to the present invention have been known to have a common domain structure, comprising an N-terminal extension domain, which is a considerably lengthy and hydrophobic domain at the N-terminal; a first transmembrane spanning domain; a first nucleotide binding fold domain, which is a cytoplasmic domain; a second transmembrane spanning domain; and a second nucleotide binding fold domain, which is a cytoplasmic domain located at the C-terminal.

[0029] In a preferred embodiment of the invention, the fungal MRP-like ABC transporter proteins have a sequence homology of at least 28%, preferably at least 40%, and more preferably at least 50%, with the amino acid sequence of YCF1 protein of SEQ ID NO:2 or YHL035C protein of SEQ ID NO:4, each of which comprises an N-terminal extension domain, a first transmembrane spanning domain, a first nucleotide binding fold domain, a second transmembrane spanning domain, and a second nucleotide binding fold domain, and each domain of the fungal MRP-like ABC transporter proteins may have at least 28% sequence homology with the amino acid sequence of each corresponding domain of YCF1 protein of SEQ ID NO:2 or YHL035C protein of SEQ ID NO:4.

[0030] The gene conferring both resistance to and accumulation of toxic materials in the present invention is a YCF1 gene comprising a sequence encoding the polypeptide of YCF1 protein, for example, a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 exhibiting resistance to noxious materials and accumulation of noxious materials; preferably a YCF1 gene exhibiting resistance to or accumulation of one or more noxious materials selected from the group consisting of cadmium, arsenic, and herbicides as well as resistance to and accumulation of lead and comprising a nucleotide sequence encoding YCF1 polypeptide of SEQ ID NO:2; and more preferably a YCF1 gene having the nucleotide sequence of SEQ ID NO:1. The YCF1 gene exists at the sixth chromosome in *Saccharomyces cerevisiae*. The YCF1 gene exhibits its function when expressed, and therefore the present invention is intended to encompass proteins having at least 28% homology, preferably at least 40% homology, and more preferably 50% homology with the amino acid sequence of the YCF1 protein and contributing to resistance to and accumulation of noxious materials, and DNA molecules encoding them.

[0031] YCF1 protein is one of the ABC transporter proteins, it exists at the vacuolar membrane in yeast, and it is known to mitigate the toxicity of cadmium by transporting cadmium conjugated with glutathione present within cytoplasm into the inside of vacuoles using MgATP as the energy source (Li, Z. S. et al. A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionato)cadmium. *Proc. Natl. Acad. Sci. USA* 94, 4247 (1997)).

[0032] Another example of a gene exhibiting resistance to and/or accumulation of noxious materials is the YHL035C gene, and a YHL035C gene exhibiting resistance to lead and comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:4, and more preferably the nucleotide sequence of SEQ ID NO:3, is provided. The YHL035C gene exists at the eighth chromosome in *Saccharomyces cerevisiae* and is one of the MRP-like ABC transporter proteins. The YHL035C gene exhibits its function when expressed, and therefore the present invention is intended to encompass proteins having at least 28% homology, preferably at least 40% homology, and more preferably 50% homology with the amino acid sequence of YHL035C protein and contributing to resistance to and accumulation of noxious materials, and DNA molecules encoding them.

[0033] In addition, the present invention provides a recombinant vector comprising said DNA molecule encoding the fungal MRP-like ABC transporter protein, and preferably it provides recombinant vectors comprising the YCF1 gene or YHL035C gene. Specific examples of the recombinant vectors include pESC-YCF1, ENpCambia-YCF1, or PBI121-YCF1 recombinant vectors, or the pESC-YHL035C recombinant vector, and pPBI121-YHL035C. The construction of these recombinant vectors can be conducted according to known processes by a person having ordinary knowledge in the art to which the invention pertains.

[0034] In the present invention, noxious materials can include heavy metals including lead, cadmium, arsenic, etc., or agricultural chemicals and herbicides. The herbicides, which are generally lipophilic compounds having a low molecular weight, have been known to readily pass through plant cell walls and hinder plant-specific processes, for example, photosynthetic electron transport or biosynthetic metabolism of essential amino acids, etc., and for example, chlorosulfuron, axidofluorpen, norflurazon, and chlorodinitrobenzene (CDNB) may be included.

[0035] Hence, transgenic organisms capable of exhibiting resistance to noxious materials as well as accumulating noxious materials can be prepared using the DNA molecules comprising nucleotide sequences encoding the polypeptide of YCF1 protein and YHL035C protein of the present invention, or DNA molecules having at least 28% homology therewith, and the transgenic organisms thus prepared can be employed to remediate sites polluted by noxious materials with ease and low cost.

[0036] In addition, the present invention relates to organisms transformed with said DNA molecules encoding the fungal MRP-like ABC transporter proteins. Also, the invention is directed to transgenic cells, preferably plant cells, which are transformed with said DNA molecules encoding the fungal MRP-like ABC transporter proteins. The ABC

genes include all of the foregoing genes, and as examples, the YCF1 gene and YHL035C gene are preferably employed.

[0037] The transgenic organisms are preferably prokaryotic or eukaryotic organisms, and as examples, plants, animals, yeast, *E. coli*, and fungus may be employed. Transgenic plants comprise heterogeneous DNA sequences according to genetic engineering methods, which are constructed to be properly expressed in plant cells, plant tissues, or plant bodies. Plant transformants can be prepared according to known techniques, and *Agrobacterium tumefaciens*-mediated DNA transfer is typically employed. More preferably, recombinant *agrobacterium* constructed by a method selected from the group consisting of electroporation, micro-particle injection, and use of a gene gun is introduced into plants by a dipping method. In an embodiment of the present invention, transgenic plants can be prepared by constructing an expression cassette comprising the MRP-like ABC transporter protein coding sequence which is operably linked to permit its transcription and translation, constructing a recombinant vector comprising said expression cassette, and introducing said recombinant vector into plant cells or plant tissues.

[0038] The above plants include herbaceous plants such as *Arabidopsis*, rapeseeds, leaf mustards, tobaccos, onions, carrots, cucumbers, sweet potatoes, potatoes, napa cabbages, radishes, lettuces, broccoli, petunias, sunflowers, grass, etc., and trees such as olive, willow, white birch, poplar, and birch, and preferably poplar and *Arabidopsis* are employed.

[0039] In a preferred embodiment of the present invention, the transgenic organisms may include YCF1 *Arabidopsis thaliana* (accession number KCTC10064BP), YCF1 poplar, or YHL035C poplar. YCF1 *Arabidopsis thaliana* of the present invention was deposited with the Korean Collection for Type Cultures at the Korea Research Institute of Bioscience and Biotechnology located at 52, Eoun-dong, Yusong-gu, Daejeon, Korea on Sep. 5, 2001, and assigned Accession Number KCTC10064BP. YCF1 *Arabidopsis thaliana* can be asexually reproduced by tissue culture and grown into a plant according to conventional plant cell culturing methods and differentiation methods. YCF1 *Arabidopsis thaliana* is excellent in resistance to heavy metals and other noxious materials (FIGS. 6, 7, and 8) and accumulation thereof (FIG. 9), as compared with its wild-type counterpart, and in particular, it exhibits resistance to and accumulation of lead, cadmium, arsenic, and agricultural chemicals.

[0040] YCF1 poplar and YHL035C poplar plants of the present invention can be asexually reproduced by tissue culture and grown into plants according to conventional plant cell culturing methods and differentiation methods. YCF1 poplar and YHL035C poplar plants are excellent in resistance to lead as compared with their wild-type counterparts (FIG. 10 and FIG. 11).

[0041] The preferred examples are hereinafter presented for better understanding of the invention. The following examples, however, are provided solely in order for better understanding of the present invention: the present invention should not be construed to be limited thereto.

EXAMPLE 1

Sensitivity to Lead and Cadmium in YCF1 Mutant Yeast

[0042] Wild-type yeast (DTY 165) and ycf1 mutant yeast (DTY 167, MATa ura3 leu2 his3 trp3 lys2 suc2 ycf::hisG) were cultured in YPD liquid media (1% yeast extract, 2% peptone, 2% dextrose) at 30° C. until OD600 reached 1-2, and then yeast cells of the same number (1×10^2 , 1×10^3 , 1×10^4 or 1×10^5) were cultured in half-diluted YPD solid media containing 3 mM lead at 30° C. for three days. Likewise, they were also cultured in half-diluted YPD solid media containing 0.1 mM cadmium. The experimental results are shown in FIG. 1.

[0043] As shown in FIG. 1, the growth of ycf1 mutant yeast was decreased in 3 mM lead-containing media as compared with wild-type yeast, and ycf1 mutant yeast grew little in 0.1 mM cadmium-containing media. Therefore, it was verified that YCF1 is a gene conferring resistance against lead and cadmium.

EXAMPLE 2

Construction of Cloning Vector

[0044] 2-1: Cloning Vector (pESC-YCF1)

[0045] For the isolation of the YCF1 gene, wild-type yeast was incubated in 3 ml YPD liquid media at 30° C. for 12 hours, and then centrifuged (12,000 rpm, 20-60 sec.). Resultant pellets were suspended in 400 μ l of yeast lysis buffer (1 M sorbitol, 0.1 M EDTA, 50 mM DTT (pH 7.5)), and after 40 μ l of zymolase (5 mg/1 ml, 0.9 M sorbitol) was added thereto, they were maintained at 37° C. for 15 to 30 minutes. Subsequently, they were mixed with 400 μ l of a urea buffer solution (7 M urea, 0.3125 M NaCl, 0.05 M Tris-HCl (pH 8.0), 0.02 M EDTA (pH 8.0), 1 % Sarcosine) and then mixed with phenol/chloroform to isolate supernatants. The supernatants were mixed with 1 ml of 100% ethanol and centrifuged (12,000 rpm, 10 min.), and then the precipitated DNA was suspended in a TE buffer solution (10 mM Tris-HCl, 1 mM EDTA pH 8.0). PCR was performed using the isolated DNA as a template, YCFa primer (SEQ ID NO:3), YCFb primer (SEQ ID NO:4), and an LA taq polymerase kit (Takara) to isolate the YCF1 gene.

[0046] To prove that the YCF1 gene in yeast is associated with resistance to lead, the YCF1 gene isolated above (1) was cloned into a pESC-URA (yeast shuttle vector, Stratagene) vector. That is, YCF1 PCR products were cleaved with restriction enzymes Xho I and Sca I to prepare YCF1 (Xho I/ Sca I), and a pESC-URA vector was digested with Hind III, treated with Klenow fragments and dNTPs to create a vector with blunt ends, and cleaved with restriction enzyme Xho I. The above YCF1 (Xho I/Sca I) and digested pESC-URA vector (Xho I/blunt-end) were ligated using T4 DNA ligase to construct the recombinant vector pESC-YCF1.

[0047] 2-2: Cloning Vector (pESC-YHL035C)

[0048] The procedures were carried out in a manner substantially identical to Example 2-1 except that PCR was performed using yeast genomic DNA used in Example 2-1 as a template, YHL035Ca primer (SEQ ID NO:9),

YHL035Cb primer (SEQ ID NO:10), and an LA taq polymerase kit (Takara) to isolate the YHL035C gene from yeast

YHL035Ca:
5'-cgacgcggccgcgatgggaacggatcccccttattatc-3'
YHL035Cb:
5'-cgacgcggccgcgatcatcttacttgattgcttgg-3'

[0049] To express the YHL035C gene in yeast, a YHL035C gene was cloned into pESC-URA (yeast shuttle vector, stratagene). YHL035C PCR products were cleaved with Not I and ligated into pESC-URA using T4 DNA ligase to construct the recombinant vector pESC-YHL035C.

EXAMPLE 3

Resistance to Lead and Cadmium in YCF1 Recombinant Yeast

[0050] Recombinant yeast (ycf1-pESC yeast) where an empty vector was introduced into ycf1 mutant yeast, recombinant yeast (wt-pESC yeast) where an empty vector was introduced into wild-type yeast, and a recombinant yeast (ycf1-YCF1 yeast) where YCF1 was overexpressed in ycf1 mutant yeast were each constructed and tested for resistance to lead or cadmium.

[0051] 3-1: Construction of YCF1 Recombinant Yeast

[0052] Yeast was inoculated into a 3 ml liquid YPC media and cultured at 30° C. for 12 hours, and then 0.5 ml of the culture was put into 10 ml liquid YPD media and cultured at 30° C. for 6 to 8 hours until OD600 reached 0.5 to 0.8. The resultant culture were centrifuged (1,500 rpm, 5 min.) to collect yeasts, which was re-suspended in 5 ml of buffer (0.1 M LiOAc, TE, pH 7.5) and centrifuged. The centrifuged yeast was resuspended in buffer (0.1 M LiOAc, TE (pH 7.5)) and cultured in an agitating incubator at 30° C. for 1 hour, and after plasmid pESC, pESC-YCF1 or pESC-YCF1, and salmon testis DNA were added thereto, it was cultured at 30° C. for 30 minutes. The cultured yeast was mixed with 0.7 ml of buffer (40% PEG 3300, 0.1M LiOAc, TE (pH 7.5)) and incubated while shaking at 30° C. for 1 hour. Thereafter, the above mixture was placed at 42 to 45° C. for 5 minutes to be subjected to heat shock, and centrifuged (2,500 rpm, 5 min.) to collect yeast. The yeast was washed with 1 ml of TE buffer solution (pH 7.5), suspended in 0.2 ml of water or TE buffer solution (pH 7.5), and then cultured on selectable media (CM Ura7⁻) for 2 to 3 days to select transformed yeasts (ycf1-pESC yeast, wt-pESC yeast, ycf1-YCF1 yeast).

[0053] 3-2: Resistance to Lead and Cadmium in Recombinant Yeast

[0054] The ycf1-pESC yeast, wt-pESC yeast, and ycf1-YCF1 yeast 25 constructed above were each cultured in galactose media (2% galactose, 1% of 0.17% YNB, 0.13% dropout powder, 0.5% ammonium sulfate) to which 1.8 mM lead was added, or in galactose media to which 50 μ M of cadmium was added. Control group was cultured in galactose media without heavy metals. Growth degree of each of the recombinant ycf1-pESC yeast, wt-pESC yeast, and ycf1-YCF1 yeast in lead or cadmium-containing media is shown in the photographs of FIG. 2.

[0055] It can be seen from FIG. 2 that ycf1-YCF1 yeast where YCF1 was overexpressed in ycf1 mutant yeast grew

better than ycf1-pESC yeast or wt-pESC yeast in media containing lead or cadmium, which thus supported the previous results that the YCF1 gene has an important role in conferring cadmium resistance. Additionally, it newly revealed that this gene is important to lead resistance.

[0056] 3-3: YCF1 Expression in Recombinant Yeast

[0057] To investigate the expression of YCF1 in ycf1-pESC yeast, wt-pESC yeast, and ycf1-YCF1 yeast constructed in Example 3, Northern Blotting was performed.

[0058] Each recombinant yeast was ground with liquid nitrogen and total RNA extraction buffer solution (0.25 M Tris HCl pH 9.0, 0.25 M NaCl, 0.05 M EDTA, 0.345 M p-Aminosalicylic acid, 0.027 M triisopropyl naphthalene sulfonic acid, 0.02% β -mercaptoethanol, 0.024% phenol) was mixed with phenol/chloroform in a 1:1 ratio. The supernatants obtained from centrifugation at 12,000 rpm for 10 minutes were transferred into a new tube, to which 400 μ l of isopropanol was added. Centrifugation was performed again at 12,000 rpm for 10 minutes to precipitate RNA, which was then lysed in DEPC-treated water and stored in a freezer.

[0059] To perform Northern Blot, 30 μ g of RNA was electrophoresed on agarose gel for RNA and transferred onto a nylon membrane. The nylon membrane was incubated while stirring in a hybridization reaction solution (6 $\times$ SSPE, 0.5% SDS, 10% PEG, 1% nonfat milk, 50% formamide) at 42° C. for 2 hours. Then, YCF1 labeled with ³²P dCTP was added thereto and the reaction was performed at 42° C. for 12 hours. After the hybridization reaction, the nylon membrane was washed twice with a buffer (2 $\times$ SSPE and 0.5% SDS), washed with another buffer (0.2 $\times$ SSPE, 0.5% SDS), dried, and then autoradiographed on X-ray film. The experimental results are shown in FIG. 2B.

[0060] From FIG. 2B, Northern Blot photographs of three kinds of recombinant yeasts, it can be seen that ycf1-YCF1 yeast overexpressed YCF1 mRNA. That is, it was proven that lead and cadmium resistance in ycf1-YCF1 yeast, which was exhibited in Example 3-2, is due to the overexpression of YCF1.

[0061] 3-4: Lead and Cadmium Resistance Mechanism

[0062] To investigate whether lead and cadmium resistance conferred by the YCF1 gene is due to the intracellular accumulation of heavy metals or due to extracellular discharge, experiments were conducted.

[0063] Three kinds of yeasts (ycf1-pESC yeast, wt-pESC yeast, and ycf1-YCF1 yeast) were each cultured in 1/2 galactose solid media containing 1.5 mM lead or 15 μ M cadmium for one day, and the cultured yeasts were scraped for harvest. The harvested yeasts were put into 1 ml of concentrated nitric acid, digested for 200° C. for about 6 hours, and then diluted with 10 ml of 0.5 N nitric acid, and the amount of heavy metals contained in the yeasts was measured using an atomic absorption spectrometer (AAS). The measurement results for ycf1-pESC yeast, wt-pESC yeast, and ycf1-YCF1 yeast are represented by graph in FIG. 3.

[0064] Consequently, wt-pESC yeast and ycf1-YCF1 yeast showed a high accumulation of lead and cadmium as compared with ycf1-pESC yeast, and in particular, wt-pESC yeast and ycf1-YCF1 yeast exhibited about a 2-fold higher

accumulation of lead than ycf1-pESC yeast. Therefore, it was verified that lead and cadmium resistance of the YCF1 gene is due to Intracellular accumulation of these heavy metals.

EXAMPLE 4

Lead Resistance in YHL035C Recombinant Yeast

[0065] 4-1: Construction of Recombinant Yeast

[0066] Recombinant yeast (yhl035c-v yeast) where an empty vector was introduced into yhl035c mutant yeast, recombinant yeast (wt-v yeast) where an empty vector was introduced into wild-type yeast, and recombinant yeast (YHL035C yeast) where YHL035C was overexpressed in yhl035c mutant yeast were each constructed according to methods substantially similar to the above Example 3-1, and transformed yeasts (wt-v yeast, yhl035c-v yeast, YHL035C yeast) were selected. Resistance to lead was tested using these recombinant yeasts.

[0067] 4-2: Lead Resistance Test in Recombinant Yeast

[0068] Resistance to lead in wt-v yeast, yhl035c-v yeast, and YHL035C yeast was tested according to methods substantially similar to the above Example 3-2. The growth degree of each of the recombinant wt-v yeast, yhl035c-v yeast, and YHL035C yeast in lead-containing media is exhibited by photographs in FIG. 4.

[0069] As can be seen in FIG. 4, yhl035c-v, which is a yhl035c mutant yeast, was more sensitive than wt-v yeast in media containing 1.8 mM lead. However, YHL035C yeast, where YHL035C was expressed in yhl035c mutant yeast, again recovered resistance to lead and exhibited growth similar to that of wt-v yeast. In media containing 1.8 mM lead. Hence, it newly revealed that the YHL035C gene has an important role in conferring lead resistance.

EXAMPLE 5

Preparation of Transgenic Plants

[0070] 5-1: Construction of YCF1 Vector and YHL035C Vector for Plant Transformation

[0071] To introduce YCF1 gene into plants, 4.6 kb of YCF1, which is a BamHI/SnaB I fragment of pESC-YCF1 plasmid, was inserted into PBI121 (BamHI/Sma I) whereby a PBI121-YCF1 vector was constructed. To improve the expression of the YCF1 gene, an EnPCAMBIA1302-YCF1 vector was constructed. A PCAMBIA1302 vector was digested with restriction enzyme SalI and treated with Klenow fragments and dNTPs to 25 create a vector with blunt ends, and 35S enhancer (BamHI/blunt-end) was inserted into the vector, which was digested with BamHI, to construct the EnPCAMBIA1302 vector. The EnPCAMBIA1302-YCF1 vector was constructed by the insertion of a YCF1 gene (BamHI/blunt-end) at site BglII/PmlI of the EnPCAMBIA1302 vector.

[0072] PBI121-YCF1 and EnPCAMBIA1302-YCF1 vectors were introduced into *E. coli* using an electroporator (BIO-RAD) and cultured on LB solid media. A single colony was inoculated in 3 ml LB (Amp) liquid media, cultured for 12 to 16 hours, and centrifuged to harvest the transformed *E. coli*. Then, 100 μ l of solution I (50 mM glucose, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA) was added to the har-

vested *E. coli* to re-suspend it, 200 μ l of solution II (1% SDS, 0.2 N NaOH) was added thereto, the mixture was gently mixed, and then incubated on ice water for 5 minutes. Then, 150 μ l of Solution III (5 M Potassium acetate) was added to the above mixture, which was then slowly mixed 3 to 5 times and centrifuged (12,000 rpm, 10 min.) to collect the supernatant. The supernatant was mixed with 100% ethanol to precipitate DNA, which was then isolated and dried. The PBI121-YCF1 plasmid DNA thus obtained was lysed in TE buffer solution and cut with restriction enzymes BamHI and EcoRI, and the insertion of the YCF1 gene in the correct orientation was confirmed.

[0073] To introduce the YHL035C gene into plants, a pHI121-YHL035C vector was constructed according to methods substantially similar to those used in the construction of the above YCF1 vector for plant transformation, except that the YHL035C gene was cut out from the pESC-YHL035C vector constructed in Example 2 and restriction sites SacI and EcoICR1 were created at both termini thereof, which were then put into a pBI121 vector digested with SacI and SmaI to construct the pBI121-YHL035C vector.

[0074] 5-2: Preparation of Transgenic *Arabidopsis thaliana*

[0075] The vectors PBI121-YCF1 and EnPCAMBIA1302-YCF1 constructed in the above Example 5-1 were introduced into *Agrobacterium* (LBA4404). The transformed *Agrobacterium* was screened on MS (Murashige-Skoog) media containing kanamycin, and transfected into the flower of *Arabidopsis thaliana* by a dipping method (Clough, S. J., and Bent, A. F., Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16, 735-743 (1988)) to introduce the YCF1 gene into the plants. After 4 to 5 weeks, the seeds of the *Arabidopsis* plants were harvested and selected for YCF1 *Arabidopsis* plants using kanamycin for plants transformed with the PBI121-YCF1 vector and hygromycin for plants transformed with the EnPCAMBIA1302-YCF1 vector.

[0076] A total of five lines of YCF1 *Arabidopsis* plants were obtained, and they were tested for expression levels of YCF1 mRNA, and whether the expressed mRNA included the C-terminal 700 bp of YCF1 mRNA to ensure that the full length mRNA was expressed. First, mRNA was extracted from the five lines of YCF1 *Arabidopsis* plants and subjected to RT-PCR using the primers of SEQ ID NO:5/SEQ ID NO:6, and the RT-PCR results are exhibited in FIG. 5A. As shown in FIG. 5A, 700 bp of YCF1 are expressed in plants 1, 3, 4, and 5, and in plants 4 and 5, YCF1 was highly expressed.

[0077] Southern Blotting was carried out for the above RT-PCR products, and the results are exhibited in FIG. 5B. It can be seen from FIG. 5B that the gene expressed in plants 1, 3, 4, and 5 in FIG. 5A is YCF1, and the complete transcription of wild-type YCF1 mRNA occurred, confirming the conclusion from FIG. 5A. This YCF1 *Arabidopsis thaliana* showing high YCF1 expression was deposited to the Korean Collection for Type Cultures at the Korea Research Institute of Bioscience and Biotechnology located at 52, Eoun-dong, Yusung-gu, Daejeon, Korea on Sep. 5, 2001, and assigned Accession Number KCTC10064BP.

[0078] 5-3: Preparation of Transgenic Poplar

[0079] As plants for transformation, Bong-hwa 1, a clone of hybrid poplar (*Populus alba* x *P. glandulosa*), which does

not bloom outdoors, was used after proliferation. To ensure in vitro aseptic materials of poplar, a stalk was obtained from clone bank which was being conserved in nursery at the Korea Forest Research Institute, and surface-sterilized with ethanol (5 minutes) and 2% NaCl (20 min.), and then the stalk, which was developed after 4-weeks of cultivation on MS media, was employed as a specimen for transformation.

[0080] For transformation using *Agrobacterium* containing a gene of Interest, Induction of a callus, and induction of the stalk, etc., the method by Noh et al. (Genetic Engineering of Poplar (2002), written by Noh, Eun-un et al. ISBN#89-8176-098-5 93520) was used. *Agrobacterium tumefaciens* constructed in Example 5-2, into which PBI121-YCF1 and EnPCAMBIA1302-YCF1 vectors were introduced, was inoculated in LB media and cultured overnight at 30° C., and after centrifugation at 1,000 g for 10 minutes, the media were discarded and the pellet was re-suspended in a 0.85% NaCl solution. The suspension was poured into a petri dish, and then the internode tissue of poplar cultivated in vitro was dipped thereinto for 20 minutes. After that, it was placed between two disinfected absorption filtering papers and gently pressed to eliminate excessive *Agrobacterium*, and then they were co-cultivated in callus-inducible media containing no antibiotic (MS+2.4-D 1.0 mg/L, BA 0.1mg/L, NM 0.01 mg/L) (Murashige and Skoog, 1962) for 2 days followed by the selection of transformed cells by use of selectable media containing 50 mg/L kanamycin and 500 mg/L cefotaxime.

[0081] The callus thus formed was grown in stalk-inducible media (WPM+zeatin 1.0 mg/L, BA 0.1 mg/L, NM 0.01 mg/L) (Lloyd and McCown, 1981) containing 50 mg/L of kanamycin to induce stalks. Once the stalks were induced, they were induced to elongate through subcultures in MS basic media containing 50 mg/L kanamycin, and roots were induced by addition of 0.2 mg/L of IBA to the same media, thus obtaining the whole plant body.

EXAMPLE 6

Growth of Transformed *Arabidopsis* Plants in the Presence of Noxious Materials

[0082] YCF1 *Arabidopsis* plants prepared in Example 5 were cultivated in 1/2 MS media containing lead (0.9, 1, 1.1 mM), cadmium (50, 60, 70 μ M), herbicide chloro-dinitrobenzene (CDNB) (60 μ M), and pentavalent arsenic (50 μ M) to investigate their growth degree. As a control, *Arabidopsis* plants transformed with an empty vector (PBI) and wild-type plants were used. The experimental results are shown in FIG. 6 to FIG. 8.

[0083] FIG. 6 shows photographs and graphs exhibiting growth of YCF1-transformed *Arabidopsis thaliana*. As shown in FIG. 6, when YCF1 *Arabidopsis* and control plants were grown in media containing lead for three weeks, YCF1 *Arabidopsis* (1, 3, 4, and 5) showed less chlorosis in leaves and better growth of roots than PBI empty vector transformed plants and wild-type plants (A, B, and D). Also, when YCF1 and wild-type *Arabidopsis* plants were grown in cadmium media at various concentrations, wild-type *Arabidopsis* plants showed more chlorosis in leaves and their roots grew poorly and were shorter than the YCF1 transformants (C and E).

[0084] FIG. 7 shows photographs exhibiting resistance to arsenic in YCF1-transformed *Arabidopsis thaliana*. When grown in media containing 60 μ M pentavalent arsenic for three weeks, wild-type *Arabidopsis* showed a little growth,

whereas YCF1 transformants (1, 2, 3, and 4) showed much better growth as compared with wild-type.

[0085] FIG. 8 shows a photograph exhibiting resistance to CDNB in YCF1-transformed *Arabidopsis thaliana*. When YCF1 *Arabidopsis* and control plants were grown in media containing 60 μ M CDNB for two months, wild-type plants showed poor germination and almost died, whereas YCF1 *Arabidopsis* plants grew well, almost like *Arabidopsis* plants grown in normal condition.

[0086] Hence, it was verified that YCF1-transformed *Arabidopsis thaliana* prepared in Example 5 has resistance to lead, cadmium, arsenic, and herbicides.

EXAMPLE 7

Accumulation of Heavy Metals in Transformed *Arabidopsis* Plants

[0087] YCF1 *Arabidopsis* plants obtained in Example 5 and wild-type *Arabidopsis* transformed with empty vector, PBI, were cultivated in 1/2 MS media containing lead (0.75 mM) and cadmium (70 μ M) for three weeks to investigate accumulation of heavy metals. The amount of accumulation of lead or cadmium was investigated by experiments substantially similar to Example 6, and the results are shown in FIG. 9, in which FIG. 9A shows the lead content and FIG. 9B shows the cadmium content of the plants.

[0088] As shown in FIG. 9A, YCF1 *Arabidopsis* plants showed 2-fold or 1.4-fold higher accumulation of lead than PBI. Also, as shown in FIG. 9B, YCF1 *Arabidopsis* plants showed 2-fold or 3-fold higher accumulation of cadmium than PBI.

[0089] Hence, it was verified that YCF1 *Arabidopsis* plants have higher accumulation of lead and cadmium than wild-type *Arabidopsis*.

EXAMPLE 8

Mechanism of Resistance to Noxious Materials in YCF1-Transformed Plants

[0090] YCF1-transformed plants showed resistance to lead, cadmium, arsenic, and herbicides and showed accumulation of lead and cadmium. To investigate that these phenomena occurred due to the fact that YCF1 proteins transport heavy metals into vacuoles, the vacuoles were separated from YCF1 transformed plants and wild-type plants, and experiments of transporting cadmium and herbicides were carried out. The transport experiment results of cadmium (Cd+GSH) and herbicides (DNB-GS) in the vacuoles of the YCF transformed plants and wild-type plants are shown in Table 1 below.

TABLE 1

Accumulation of GS-associated Compounds in the Vacuoles Separated from <i>Arabidopsis</i> (unit: pmol/1 μ l vacuole/20 min)				
Compounds	Wild-type Plants		YCF1 Transformed Plants	
	-MgATP	+MgATP	-MgATP	+MgATP
DNB - GS	21 $\pm$ 1.1	28 $\pm$ 1.3	24 $\pm$ 0.9	33 $\pm$ 1.2
Cd + GSH	75 $\pm$ 3.3	104 $\pm$ 4.9	71 $\pm$ 3.4	177 $\pm$ 9.8
GSH	—	70 $\pm$ 2.2	—	69 $\pm$ 1.1

[0091] As shown in Table 1 above, as YCF1 employs MgATP as its energy source when transporting materials, there was, in the -MgATP group, no difference in cadmium (Cd+GSH) and herbicide (DNB-GS) contents between YCF1 transformants and wild-type plants. However, when MgATP was added, YCF1 transformants' vacuoles showed about a 1.7-fold higher accumulation of cadmium (Cd-GSH) than the wild-type vacuoles. In case of the herbicide (DNB-GS), YCF1 transformants showed a little higher accumulation than wild-type.

[0092] It was verified from FIGS. 6, 7, 8, and 9 that YCF1 transformed *Arabidopsis* plants have higher resistance to lead, cadmium, arsenic, and herbicides than wild-type, and they accumulate more lead and cadmium. It was supported from the results of Table 1 that such phenomenon is due to the fact that YCF1 proteins expressed in the vacuoles of YCF1 transformed *Arabidopsis* plants transport cadmium and herbicides into the vacuoles and stabilize them, thereby conferring resistance to and accumulation of those noxious materials.

EXAMPLE 9

Growth of Transgenic Poplar Plants

[0093] 9-1: YCF1 Transgenic Poplar Plants

[0094] The leaf fragments and stalks of YCF1 poplar plants prepared in Example 5 were mounted on callus-inducible media containing 500 ppm of lead, and cultivated for two weeks to investigate the difference in their growth. As a control, wild-type poplar plants, which were not transformed, were treated in the same manner, and the results of the experiments are shown in FIG. 10. FIG. 10 shows photographs and a graph showing the growth of YCF1 transformed and control poplar plants.

[0095] As shown in FIG. 10, in media containing lead, YCF1 poplar (1, 2, 3, and 4) showed about a 2-fold higher growth in stalk pieces than wild-type (wt) (A and C), and the leaf fragments of YCF1 poplar (1, 2, and 3) showed less browning than those of wild-type (wt) and kept the color of chlorophyll green (B).

[0096] 9-2: YHL035C Transformed Poplar Plants

[0097] YHL035C transformed poplar plants prepared in Example 5 were transferred to a pot and allowed to grow into pot seedlings. These pot seedlings were transferred to soil that was dipped into a solution containing 500 ppm of Pb(NO)₃. The results of the experiment are shown in FIG. 11.

[0098] FIG. 11 shows a photograph showing that wild-type poplar grew poorly when cultivated in soil containing lead for four weeks, whereas the YHL035C transformant poplar grew much better than the wild-type. Hence, it was verified that YCF1 transformed poplar plants and YHL035C poplar plants prepared in Example 5 have resistance to lead.

[0099] As described above, the YCF1 gene of the present invention improves resistance to and accumulation of heavy metals and other noxious materials, and the YHL035C gene improves resistance to lead, and accordingly, the transformants capable of expressing these genes can be used for the purpose of remediating environments polluted with noxious materials. Thus, the transformants of the invention provide an environmentally-friendly way to remediate the environment at a low cost.

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Glu Ala Glu Asn Phe Thr Ile Val Ser Gln Tyr Ala Ser Thr Met Leu
          100         105         110
Ser Leu Phe Val Ala Leu Ala Leu His Trp Ile Glu Tyr Asp Arg Ser
          115         120         125
Val Val Ala Asn Thr Val Leu Leu Phe Tyr Trp Leu Phe Glu Thr Phe
          130         135         140
Gly Asn Phe Ala Lys Leu Ile Asn Ile Leu Ile Arg His Thr Tyr Glu
          145         150         155         160
Gly Ile Trp Tyr Ser Gly Gln Thr Gly Phe Ile Leu Thr Leu Phe Gln
          165         170         175
Val Ile Thr Cys Ala Ser Ile Leu Leu Leu Glu Ala Leu Pro Lys Lys
          180         185         190
Pro Leu Met Pro His Gln His Ile His Gln Thr Leu Thr Arg Arg Lys
          195         200         205
Pro Asn Pro Tyr Asp Ser Ala Asn Ile Phe Ser Arg Ile Thr Phe Ser
          210         215         220
Trp Met Ser Gly Leu Met Lys Thr Gly Tyr Glu Lys Tyr Leu Val Glu
          225         230         235         240
Ala Asp Leu Tyr Lys Leu Pro Arg Asn Phe Ser Ser Glu Glu Leu Ser
          245         250         255
Gln Lys Leu Glu Lys Asn Trp Glu Asn Glu Leu Lys Gln Lys Ser Asn
          260         265         270
Pro Ser Leu Ser Trp Ala Ile Cys Arg Thr Phe Gly Ser Lys Met Leu

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275	280	285
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Pro Gln Lys Leu Pro Ile Val Arg Gly Phe Leu Ile Ala Phe Ala Met 340 345 350		
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Ser Thr Gly Asp Ile Val Asn Leu Met Ser Val Asp Val Gln Lys Leu 405 410 415		
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Ile Ile Ile Cys Leu Tyr Ser Leu Tyr Lys Leu Leu Gly Asn Ser Met 435 440 445		
Trp Val Gly Val Ile Ile Leu Val Ile Met Met Pro Leu Asn Ser Phe 450 455 460		
Leu Met Arg Ile Gln Lys Lys Leu Gln Lys Ser Gln Met Lys Tyr Lys 465 470 475 480		
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Val Ser Ile Gly Arg Leu Phe Thr Phe Phe Thr Asn Glu Glu Leu Gln 595 600 605		
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Glu Tyr Lys Val Ala Leu Lys Asn Ile Asn Phe Gln Ala Lys Lys Gly 645 650 655		
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Leu Ser Cys Met Leu Gly Asp Leu Phe Arg Val Lys Gly Phe Ala Thr 675 680 685		

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Phe	Tyr	Glu	Lys	Thr	Ile	Lys	Ala	Cys	Ala	Leu	Thr	Ile	Asp	Leu	Ala
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Ile	Leu	Met	Asp	Gly	Asp	Lys	Thr	Leu	Val	Gly	Glu	Lys	Gly	Ile	Ser
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Leu	Ser	Gly	Gly	Gln	Lys	Ala	Arg	Leu	Ser	Leu	Ala	Arg	Ala	Val	Tyr
	755						760					765			
Ala	Arg	Ala	Asp	Thr	Tyr	Leu	Leu	Asp	Asp	Pro	Leu	Ala	Ala	Val	Asp
770						775					780				
Glu	His	Val	Ala	Arg	His	Leu	Ile	Glu	His	Val	Leu	Gly	Pro	Asn	Gly
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Leu	Leu	His	Thr	Lys	Thr	Lys	Val	Leu	Ala	Thr	Asn	Lys	Val	Ser	Ala
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Gln	Gln	Gly	Thr	Tyr	Asp	Glu	Ile	Thr	Lys	Asp	Ala	Asp	Ser	Pro	Leu
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Trp	Lys	Leu	Leu	Asn	Asn	Tyr	Gly	Lys	Lys	Asn	Asn	Gly	Lys	Ser	Asn
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Glu	Phe	Gly	Asp	Ser	Ser	Glu	Ser	Ser	Val	Arg	Glu	Ser	Ser	Ile	Pro
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Val	Glu	Gly	Glu	Leu	Glu	Gln	Leu	Gln	Lys	Leu	Asn	Asp	Leu	Asp	Phe
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Gly	Asn	Ser	Asp	Ala	Ile	Ser	Leu	Arg	Arg	Ala	Ser	Asp	Ala	Thr	Leu
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Gly	Ser	Ile	Asp	Phe	Gly	Asp	Asp	Glu	Asn	Ile	Ala	Lys	Arg	Glu	His
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Arg	Glu	Gln	Gly	Lys	Val	Lys	Trp	Asn	Ile	Tyr	Leu	Glu	Tyr	Ala	Lys
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Ala	Cys	Asn	Pro	Lys	Ser	Val	Cys	Val	Phe	Ile	Leu	Phe	Ile	Val	Ile
945					950					955					960
Ser	Met	Phe	Leu	Ser	Val	Met	Gly	Asn	Val	Trp	Leu	Lys	His	Trp	Ser
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Glu	Val	Asn	Ser	Arg	Tyr	Gly	Ser	Asn	Pro	Asn	Ala	Ala	Arg	Tyr	Leu
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Ala	Ile	Tyr	Phe	Ala	Leu	Gly	Ile	Gly	Ser	Ala	Leu	Ala	Thr	Leu	Ile
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Gln	Thr	Ile	Val	Leu	Trp	Val	Phe	Cys	Thr	Ile	His	Ala	Ser	Lys	Tyr
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Leu	His	Asn	Leu	Met	Thr	Asn	Ser	Val	Leu	Arg	Ala	Pro	Met	Thr	Phe
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Phe	Glu	Thr	Thr	Pro	Ile	Gly	Arg	Ile	Leu	Asn	Arg	Phe	Ser	Asn	Asp
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Val	Asn	Ala	Val	Lys	Val	Thr	Phe	Thr	Ile	Thr	Val	Ile	Cys	Ala	Thr
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Thr	Trp	Gln	Phe	Ile	Phe	Ile	Ile	Ile	Pro	Leu	Ser	Val	Phe	Tyr	Ile
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Asp	Ser	Ile	Thr	Arg	Ser	Pro	Ile	Tyr	Ser	His	Phe	Gln	Glu	Thr	Leu
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Gly	Gly	Leu	Ala	Thr	Val	Arg	Gly	Tyr	Ser	Gln	Gln	Lys	Arg	Phe	Ser
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Ser	Ile	Ile	Ile	Leu	Gly	Ala	Ala	Thr	Leu	Ser	Val	Phe	Arg	Leu	Lys
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Leu	Gln	Ile	Thr	Gln	Thr	Leu	Asn	Trp	Ile	Val	Arg	Met	Thr	Val	Glu
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Val	Glu	Thr	Asn	Ile	Val	Ser	Val	Glu	Arg	Ile	Lys	Glu	Tyr	Ala	Asp
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Tyr	Arg	Pro	Glu	Leu	Asp	Leu	Val	Leu	Lys	His	Ile	Asn	Ile	His	Ile
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Asn	Ile	Val	Ile	Asp	Asn	Ile	Ala	Ile	Asn	Glu	Ile	Gly	Leu	Tyr	Asp
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Asp	Val	Glu	Thr	Asp	Lys	Val	Val	Gln	Glu	Thr	Ile	Arg	Thr	Ala	Phe
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Lys	Asp	Arg	Thr	Ile	Leu	Thr	Ile	Ala	His	Arg	Leu	Asn	Thr	Ile	Met
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Asp	Ser	Asp	Arg	Ile	Ile	Val	Leu	Asp	Asn	Gly	Lys	Val	Ala	Glu	Phe
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<210> SEQ ID NO 4

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<212> TYPE: PRT

<213> ORGANISM: *Saccharomyces cerevisiae*

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Glu Tyr Leu Phe Gly Ala Gln Glu Glu Arg Lys Glu Asp Asn Ser Ile
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Lys His Ile Asn Asn 325	Leu Thr Leu Ala Leu 330	Phe Glu Ser Phe Lys Thr 335	
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Ile Asn Leu Ile Ser 450	Ile Asp Ser Phe Lys 455	Val Ser Glu Leu Ala Asn 460	
Tyr Leu Tyr Val Thr 465	Val Gln Ala Val Ile 470	Met Ile Ile Val Val Val 475	480
Gly Leu Leu Phe Asn 485	Phe Leu Gly Val Ser 490	Ala Phe Ala Gly Ile Ser 495	
Ile Ile Leu Val Met 500	Phe Pro Leu Asn Phe 505	Leu Leu Ala Asn Leu Leu 510	
Gly Lys Phe Gln Lys 515	Gln Thr Leu Lys Cys 520	Thr Asp Gln Arg Ile Ser 525	
Lys Leu Asn Glu Cys 530	Leu Gln Asn Ile Arg 535	Ile Val Lys Tyr Phe Ala 540	
Trp Glu Arg Asn Ile 545	Ile Asn Glu Ile Lys 550	Ser Ile Arg Gln Lys Glu 555	560
Leu Arg Ser Leu Leu 565	Lys Lys Ser Leu Val 570	Trp Ser Val Thr Ser Phe 575	
Leu Trp Phe Val Thr 580	Pro Thr Leu Val Thr 585	Gly Val Thr Phe Ala Ile 590	
Cys Thr Phe Val Gln 595	His Glu Asp Leu Asn 600	Ala Pro Leu Ala Phe Thr 605	
Thr Leu Ser Leu Phe 610	Thr Leu Leu Lys Thr 615	Pro Leu Asp Gln Leu Ser 620	
Asn Met Leu Ser Phe 625	Ile Asn Gln Ser Lys 630	Val Ser Leu Lys Arg Ile 635	640

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Ser	Asp	Phe	Leu	Arg	Met	Asp	Asp	Thr	Glu	Lys	Tyr	Asn	Gln	Leu	Thr	645	650	655
Ile	Ser	Pro	Asp	Lys	Asn	Lys	Ile	Glu	Phe	Lys	Asn	Ala	Thr	Leu	Thr	660	665	670
Trp	Asn	Glu	Asn	Asp	Ser	Asp	Met	Asn	Ala	Phe	Lys	Leu	Cys	Gly	Leu	675	680	685
Asn	Ile	Lys	Phe	Gln	Ile	Gly	Lys	Leu	Asn	Leu	Ile	Leu	Gly	Ser	Thr	690	695	700
Gly	Ser	Gly	Lys	Ser	Ala	Leu	Leu	Leu	Gly	Leu	Leu	Gly	Glu	Leu	Asn	705	710	715
Leu	Ile	Ser	Gly	Ser	Ile	Ile	Val	Pro	Ser	Leu	Glu	Pro	Lys	His	Asp	725	730	735
Leu	Ile	Pro	Asp	Cys	Glu	Gly	Leu	Thr	Asn	Ser	Phe	Ala	Tyr	Cys	Ser	740	745	750
Gln	Ser	Ala	Trp	Leu	Leu	Asn	Asp	Thr	Val	Lys	Asn	Asn	Ile	Ile	Phe	755	760	765
Asp	Asn	Phe	Tyr	Asn	Glu	Asp	Arg	Tyr	Asn	Lys	Val	Ile	Asp	Ala	Cys	770	775	780
Gly	Leu	Lys	Arg	Asp	Leu	Glu	Ile	Leu	Pro	Ala	Gly	Asp	Leu	Thr	Glu	785	790	795
Ile	Gly	Glu	Lys	Gly	Ile	Thr	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	805	810	815
Ser	Leu	Ala	Arg	Ala	Val	Tyr	Ser	Ser	Ala	Lys	His	Val	Leu	Leu	Asp	820	825	830
Asp	Cys	Leu	Ser	Ala	Val	Asp	Ser	His	Thr	Ala	Val	Trp	Ile	Tyr	Glu	835	840	845
Asn	Cys	Ile	Thr	Gly	Pro	Leu	Met	Lys	Asn	Arg	Thr	Cys	Ile	Leu	Val	850	855	860
Thr	His	Asn	Val	Ser	Leu	Thr	Leu	Arg	Asn	Ala	His	Phe	Ala	Ile	Val	865	870	875
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Ser	Lys	Gly	Leu	Phe	Lys	Glu	Lys	Tyr	Val	Gln	Leu	Ser	Ser	Arg	Asp	900	905	910
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Asp	Ser	Gln	Lys	Ile	Glu	Pro	Val	Thr	Glu	Asn	Ile	Asn	Phe	Asp	Ala	930	935	940
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Gly	Ala	Ile	Ser	Pro	Asp	Val	Tyr	Lys	Trp	Tyr	Leu	Lys	Phe	Phe	Gly	965	970	975
Gly	Phe	Lys	Ala	Leu	Thr	Ala	Leu	Phe	Ala	Leu	Tyr	Ile	Thr	Ala	Gln	980	985	990
Ile	Leu	Phe	Ile	Ser	Gln	Ser	Trp	Trp	Ile	Arg	His	Trp	Val	Asn	Asp	995	1000	1005
Thr	Asn	Val	Arg	Ile	Asn	Ala	Pro	Gly	Phe	Ala	Met	Asp	Thr	Leu	Pro	1010	1015	1020
Leu	Lys	Gly	Met	Thr	Asp	Ser	Ser	Lys	Asn	Lys	His	Asn	Ala	Phe	Tyr	1025	1030	1035

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Tyr	Leu	Thr	Val	Tyr	Phe	Leu	Ile	Gly	Ile	Ile	Gln	Ala	Met	Leu	Gly	
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Gly	Phe	Lys	Thr	Met	Met	Thr	Phe	Leu	Ser	Gly	Met	Arg	Ala	Ser	Arg	
			1060					1065					1070			
Lys	Ile	Phe	Asn	Asn	Leu	Leu	Asp	Leu	Val	Leu	His	Ala	Gln	Ile	Arg	
		1075					1080					1085				
Phe	Phe	Asp	Val	Thr	Pro	Val	Gly	Arg	Ile	Met	Asn	Arg	Phe	Ser	Lys	
	1090					1095					1100					
Asp	Ile	Glu	Gly	Val	Asp	Gln	Glu	Leu	Ile	Pro	Tyr	Leu	Glu	Val	Thr	
1105					1110					1115				1120		
Ile	Phe	Cys	Leu	Ile	Gln	Cys	Ala	Ser	Ile	Ile	Phe	Leu	Ile	Thr	Val	
			1125						1130					1135		
Ile	Thr	Pro	Arg	Phe	Leu	Thr	Val	Ala	Val	Ile	Val	Phe	Val	Leu	Tyr	
			1140					1145					1150			
Phe	Phe	Val	Gly	Lys	Trp	Tyr	Leu	Thr	Ala	Ser	Arg	Glu	Leu	Lys	Arg	
	1155					1160						1165				
Leu	Asp	Ser	Ile	Thr	Lys	Ser	Pro	Ile	Phe	Gln	His	Phe	Ser	Glu	Thr	
	1170					1175					1180					
Leu	Val	Gly	Val	Cys	Thr	Ile	Arg	Ala	Phe	Gly	Asp	Glu	Arg	Arg	Phe	
1185					1190					1195					1200	
Ile	Leu	Glu	Asn	Met	Asn	Lys	Ile	Asp	Gln	Asn	Asn	Arg	Ala	Phe	Phe	
			1205						1210					1215		
Tyr	Leu	Ser	Val	Thr	Val	Lys	Trp	Phe	Ser	Phe	Arg	Val	Asp	Met	Ile	
			1220					1225					1230			
Gly	Ala	Phe	Ile	Val	Leu	Ala	Ser	Gly	Ser	Phe	Ile	Leu	Leu	Asn	Ile	
		1235					1240					1245				
Ala	Asn	Ile	Asp	Ser	Gly	Leu	Ala	Gly	Ile	Ser	Leu	Thr	Tyr	Ala	Ile	
	1250					1255					1260					
Leu	Phe	Thr	Asp	Gly	Ala	Leu	Trp	Leu	Val	Arg	Leu	Tyr	Ser	Thr	Phe	
1265					1270					1275				1280		
Glu	Met	Asn	Met	Asn	Ser	Val	Glu	Arg	Leu	Lys	Glu	Tyr	Ser	Ser	Ile	
			1285						1290					1295		
Glu	Gln	Glu	Asn	Tyr	Leu	Gly	His	Asp	Glu	Gly	Arg	Ile	Leu	Leu	Leu	
		1300					1305						1310			
Asn	Glu	Pro	Ser	Trp	Pro	Lys	Asp	Gly	Glu	Ile	Glu	Ile	Glu	Asn	Leu	
		1315				1320						1325				
Ser	Leu	Arg	Tyr	Ala	Pro	Asn	Leu	Pro	Pro	Val	Ile	Arg	Asn	Val	Ser	
	1330					1335					1340					
Phe	Lys	Val	Asp	Pro	Gln	Ser	Lys	Ile	Gly	Ile	Val	Gly	Arg	Thr	Gly	
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Ala	Gly	Lys	Ser	Thr	Ile	Ile	Thr	Ala	Leu	Phe	Arg	Leu	Leu	Glu	Pro	
			1365					1370						1375		
Ile	Thr	Gly	Cys	Ile	Lys	Ile	Asp	Gly	Gln	Asp	Ile	Ser	Lys	Ile	Asp	
		1380						1385					1390			
Leu	Val	Thr	Leu	Arg	Arg	Ser	Ile	Thr	Ile	Ile	Pro	Gln	Asp	Pro	Ile	
	1395						1400					1405				
Leu	Phe	Ala	Gly	Thr	Ile	Lys	Ser	Asn	Val	Asp	Pro	Tyr	Asp	Glu	Tyr	
	1410					1415					1420					
Asp	Glu	Lys	Lys	Ile	Phe	Lys	Ala	Leu	Ser	Gln	Val	Asn	Leu	Ile	Ser	
1425					1430					1435				1440		
Ser	His	Glu	Phe	Glu	Glu	Val	Leu	Asn	Ser	Glu	Glu	Arg	Phe	Asn	Ser	

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1445	1450	1455
Thr His Asn Lys Phe Leu Asn Leu His Thr Glu Ile Ala Glu Gly Gly		
1460	1465	1470
Leu Asn Leu Ser Gln Gly Glu Arg Gln Leu Leu Phe Ile Ala Arg Ser		
1475	1480	1485
Leu Leu Arg Glu Pro Lys Ile Ile Leu Leu Asp Glu Ala Thr Ser Ser		
1490	1495	1500
Ile Asp Tyr Asp Ser Asp His Leu Ile Gln Gly Ile Ile Arg Ser Glu		
1505	1510	1515
Phe Asn Lys Ser Thr Ile Leu Thr Ile Ala His Arg Leu Arg Ser Val		
1525	1530	1535
Ile Asp Tyr Asp Arg Ile Ile Val Met Asp Ala Gly Glu Val Lys Glu		
1540	1545	1550
Tyr Asp Arg Pro Ser Glu Leu Leu Lys Asp Glu Arg Gly Ile Phe Tyr		
1555	1560	1565
Ser Met Cys Arg Asp Ser Gly Gly Leu Glu Leu Leu Lys Gln Ile Ala		
1570	1575	1580
Lys Gln Ser Ser Lys Met Met Lys		
1585	1590	
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36

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35

1. A DNA molecule exhibiting resistance to and enhanced accumulation of lead and encoding a fungal multidrug resistance-associated protein (MRP)-like ATP-binding cassette transporter protein (MRP-like ABC transporter protein).

2. The DNA molecule according to claim 1, wherein said fungal MRP-like ABC transporter protein is selected from the group consisting of YCF1 protein, YHL035C protein, BPT1 protein, YBT1 protein, and YOR1 protein.

3. The DNA molecule according to claim 2, wherein said YCF1 protein has the amino acid sequence of SEQ ID NO:2 and the DNA molecule has a nucleotide sequence encoding said amino acid sequence.

4. The DNA molecule according to claim 3, wherein said DNA molecule encoding YCF1 protein has the nucleotide sequence of SEQ ID NO:1.

5. The DNA molecule according to claim 2, wherein said YCF1 protein further exhibits resistance to and enhanced accumulation of one or more noxious materials selected from the group of cadmium, arsenic, and herbicide, in addition to resistance to and accumulation of lead.

6. The DNA molecule according to claim 5, wherein said herbicide is chloro-dinitrobenzene.

7. The DNA molecule according to claim 2, wherein said YHL035C protein has the amino acid sequence of SEQ ID NO:4 and the DNA molecule has a nucleotide sequence encoding said amino acid sequence.

8. The DNA molecule according to claim 7, wherein said DNA molecule encoding YHL035C protein has the nucleotide sequence of SEQ ID NO:3.

9. The DNA molecule according to claim 1, wherein said fungal MRP-like ABC transporter protein is a fungal MRP-like transporter protein having at least 28% amino acid sequence homology with the amino acid sequence of YCF1 protein of SEQ ID NO:2 or the amino acid sequence of YHL035C protein of SEQ ID NO:4.

10. The DNA molecule according to claim 9, wherein said fungal MRP-like ABC transporter protein has at least 28%

sequence homology with the amino acid sequence of YCF1 protein of SEQ ID NO:2 or YHL035C protein of SEQ ID NO:4, and

wherein said YCF1 protein and YHL035C protein each comprise an N-terminal extension domain, a first transmembrane spanning domain, a first nucleotide binding fold domain, a second transmembrane spanning domain, and a second nucleotide binding fold domain, and each domain of said fungal MRP-like ABC transporter proteins has at least 28% sequence homology with the amino acid sequence of each corresponding domain of YCF1 protein of SEQ ID NO:2 or YHL035C protein of SEQ ID NO:4.

11. A recombinant vector comprising the DNA molecule encoding the fungal MRP-like ABC transporter protein according to claim 1.

12. The recombinant vector according to claim 11, wherein said recombinant vector is pESC-YCF1, ENpCambia-YCF1, or PBI121-YCF1.

13. The recombinant vector according to claim 11, wherein said recombinant vector is a pESC-YHL035C recombinant vector, or pPBI121-YHL035C recombinant vector.

14. A transgenic organism transformed with the DNA molecule encoding the fungal MRP-like ABC transporter protein according to claim 1.

15. The transgenic organism according to claim 14, wherein said transgenic organism is a prokaryotic organism or a eukaryotic organism.

16. The transgenic organism according to claim 15, wherein said eukaryotic organism is animal, plant, or yeast.

17. The transgenic organism according to claim 16, wherein said plant is selected from the group of *Arabidopsis*, rapeseed, leaf mustards, tobaccos, onions, carrots, cucumbers, sweet potatoes, potatoes, napa cabbages, radishes, lettuces, broccoli, petunias, sunflowers, grass, white birch, poplar, olive, willow, and birch.

18. The transgenic organism according to claim 17, wherein said transgenic organism is YCF1 *Arabidopsis thaliana* (accession number KCTC10064BP) or YCF1 poplar.

19. The transgenic organism according to claim 17, wherein said transgenic organism is YHL035C poplar.

20. A plant cell transformed with the DNA molecule encoding the fungal MRP-like ABC transporter protein according to claim 1.

21. The plant cell according to claim 20 wherein said plant is selected from the group of *Arabidopsis*, rapes, leaf mustards, tobaccos, onions, carrots, cucumbers, sweet potatoes, potatoes, napa cabbages, radishes, lettuces, broccoli, petunias, sunflowers, grass, white birch, poplar, olive, willow, and birch.

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