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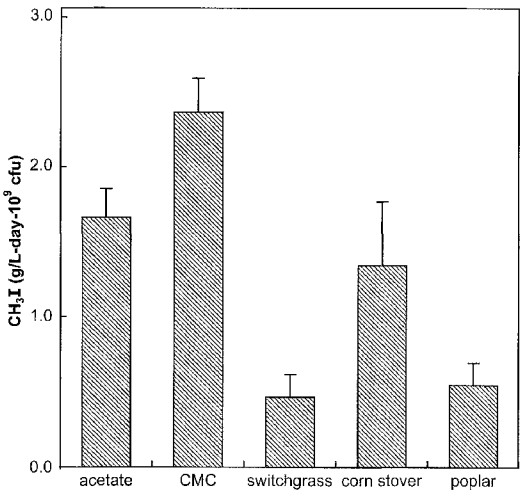
FIG. 9C



(57) Abstract: The invention relates to systems and methods for production of compounds by yeast and other organisms. In one approach yeast engineered for production of a compound of commercial value is cultured together with a cellulosic bacteria, and the yeast uses a metabolic product produced by the bacteria as a carbon source. Methyl halides are an example of compounds that may be produced by this process. The invention also relates to production of organic compounds using genetically engineered organisms expressing a S-adenosylmethionine (SAM)-dependent methyl halide transferase. In one approach the organism, halides and a carbon source are incubated in a cultivation medium under conditions in which methyl halide is produced. The methyl halide may be collected and converted into non-halogenated organic molecules.

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PATENT APPLICATION

BIOLOGICAL SYSTEMS FOR PRODUCTION OF COMMERCIALLY VALUABLE COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of US provisional patent application Nos 60/991,678 (filed November 30, 2007); 61/038,368 (filed March 20, 2008); 61/041,467 (filed April 1, 2008) and 61/098,221 (filed September 18, 2008). Each of these applications is incorporated in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates to production of biofuels and other methyl halide derivatives by cultivation of genetically modified organisms expressing methyl halide transferase.

BACKGROUND

[0003] Methyl halides are reactive one-carbon compounds from which a wide variety of commercially important organic products can be produced. Industrial production of methyl halides has been carried out using chemical methods that often consume high amounts of energy, and involve conditions of high temperature and pressure. For example, a common method for industrial production of methyl halides involves reaction of methanol with gaseous hydrogen chloride in the presence of an aluminum oxide catalyst at elevated temperature and under a pressure of at least 1 bar. See, *e.g.*, McKetta, J., CHEMICAL PROCESSING HANDBOOK, 1993.

[0004] Many plants and fungi produce methyl halides and release them into the environment. These organisms contain methyl halide transferases that combine a chlorine, bromine or iodine ion with a methyl group of the metabolite S-adenosylmethionine ("AdoMet" or "SAM") to form the methyl halide and S-adenosyl homocysteine.

BRIEF SUMMARY OF THE INVENTION

[0005] The invention includes a process comprising combining (i) an organism comprising a S-adenosylmethionine (SAM)-dependent methyl halide transferase (MHT), (ii) a halide selected from the group comprising chlorine, bromine and iodine; and (iii) a carbon source in a cultivation medium, under conditions in which methyl halide is produced. The methyl halide can optionally be collected. The methyl halide can be converted into a non-halogenated organic molecule or a mixture of non-halogenated organic molecules, which can optionally be collected. The process can be carried out on a commercial scale, for example in a reactor. The invention also provides a genetically modified algae, fungus or bacteria, comprising a heterologous S-adenosylmethionine (SAM)-dependent methyl halide transferase gene, that is genetically modified to increase flux through a S-adenosyl-methionine (SAM) biosynthetic pathway; and/or genetically modified to increase the intracellular halide concentration.

[0006] Useful organisms include algae, yeast and bacteria. The recombinant organism can be a gram negative bacterium, *e.g.*, E. coli, Salmonella, Rhodobacter, Synechtocystis, or Erwinia. Other gram negative bacteria include members from the Methylococcaceae and Methylocystaceae families; Thermotoga hypogea, Thermotoga naphthophila, Thermotoga subterranean, Petrotoga halophila, Petrotoga mexicana, Petrotoga miotherma, and Petrotoga mobilis. Alternatively, the recombinant organism can be a gram positive bacterium, *e.g.*, B. subtilis or Clostridium. If desired, the recombinant organism can be a fungus such as Saccharomyces cerevisae, Pichia pastoris, Hansenula polymorpha, Kluyveromyces lactis, Yarrowia lipolytica, Scizosacchromyces pombe or Trichoderma reesei or other yeast species of genus Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma or Scizosacchromyces. The recombinant organism can also be a eukaryote such as an algae. Example of algae include Chlamydomonas.

[0007] The organism optionally comprises a gene encoding a heterologous MHT. The MHT can be a naturally-occurring MHT or a synthetic MHT. If so desired, the expression of the heterologous MHT can be under the control of an inducible promoter. Useful MHTs include, for example and not limitation, MHTs from *Batis maritima, Burkholderia phymatum, Synechococcus elongatus, Brassica rapa, Brassica oleracea, Arabidopsis thaliana, Arabidopsis thaliana, Leptospirillum, Cryptococcus neoformans, Oryza sativa, Ostreococcus tauri, Dechloromonas*

aromatica, Coprinopsis cinerea, Robiginitalea bofirmata, Maricaulis maris, Flavobacteria bacterium, Vitis vinifera or halorhodospira halophila. Other useful MHTs include (but are not limited to) MHTs from *B. xenovorans*, *B. rapa chinensis*, *B. pseudomallei*, *B. thailandensis*, *Marine bacterium HTCC2080*, and *R. picketti.* Also see discussion below and Fig. 10A.

[0008] The organism can be genetically modified to increase flux through a S-adenosyl-methionine (SAM) biosynthetic pathway. For example, the flux through the SAM biosynthetic pathway can be increased by expression or overexpression of a SAM synthetase. The SAM synthetase can be E. coli *metK*, Rickettsia *metK,* S. cerevisae *sam1p*, or S. cerevisae *sam2p*. The SAM synthetase optionally has at least 80% amino acid identity with E. Coli *metK*.

[0009] If desired, the flux through the SAM biosynthetic pathway can be increased by abolishing, inactivating or decreasing the expression and/or activity of at least one gene. In appropriate instances, the gene can be involved in a SAM utilization pathway, *e.g.*, coproporphyrinogen III oxidase, S-adenosylmethionine decarboxylase, cystathionine beta-synthetase, ribulose 5-phosphate 3-epimerase, glucose-6-phosphate dehydrogenase, L-alanine transaminase, 3', 5э–bisphosphate nucleotidase, glycine hydroxymethyltransferase, or glycine hydroxymethyltransferase.

[0010] The flux through the SAM biosynthetic pathway can also be increased by increasing flux through a methionine biosynthetic pathway. For example, the flux through the methionine biosynthetic pathway can be increased by expression or overexpression of the E. coli metL, metA, metB, metC, metE, and/or metH genes. If desired, a gene encoding a repressor of methionine biosynthesis, *e.g.*, E. coli *metJ*, can be inactivated.

[0011] If desired, the flux can be increased by expressing a SAM transporter protein such as the Sam5p yeast mitochondrial gene. In another aspect, methyl halide production can be increased by expressing a gene that increases intracellular concentration and/or availability of ATP, and/or by increasing the intracellular halide concentration, for example through the overexpression of a halide transporter protein gene. The halide transporter can be E. coli clc transporter or a gene that shares at least 80% amino acid sequence identity with the E. coli clc transporter.

[0012] The halide for use in the invention can be provided as a halide salt, *e.g.*, sodium chloride, sodium bromide, and sodium iodide. The halide can be present in the cultivation medium at a concentration of 0.05 to 0.3 M. The cultivation medium optionally comprises methionine. The methyl halide produced can be methyl chloride, methyl bromide, and/or methyl iodide. The conversion of methyl halides into other products can be a result of catalytic condensation. Useful catalysts include a zeolite catalyst, for example ZSM-5 or aluminum bromide ($AlBr_3$). The catalytic condensation step results in the production of a halide which can be recycled back to the cultivation medium. The methods of the invention can be used to produce a composition comprising an alkane, *e.g.*, ethane, propane, butane, pentane, hexane, heptane, octane, or a mixture thereof. Other organic molecules that can be produced include, without limitation, olefins, alcohols, ethers and/or aldehydes.

[0013] The organism can be genetically modified at multiple (*e.g.*, 2, 3, 4, 5, or 6) loci. The effect of each modification individually can be to increase the production of methyl halide.

[0014] In one aspect the invention provides a method including the steps of combining

i) a recombinant yeast comprising a heterologous gene encoding S-denosylmethionine (SAM)-dependent methyl halide transferase (MHT), ii) a halide selected from the group comprising chlorine, bromine and iodine; and iii) a carbon source; in a cultivation medium under conditions in which methyl halide is produced. The method may further include the step of converting the methyl halide into a non-halogenated organic molecule or a mixture of non-halogenated organic molecules. In come embodiments the yeast is from a genus selected from Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces. For example, the yeast may be *Saccharomyces cerevisiae, Pichia pastoris*, *Hansenula polymorpha*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Trichoderma reesei*, or *Scizosacchromyces pombe*. In some embodiments the MHT is from *Batis maritima. In some embodiments the* carbon source is acetate and/or ethanol produced by a metabolism of cellulose by a cellulolytic microorganism. The cellulolytic microorganism may be a bacterium, such as *Actinotalea fermentans.* In some embodiments the cellulose is microcrystalline cellulose. In some embodiments

the cellulose is a chopped or pulverized feedstock (e.g., pulverized switchgrass, bagasse, elephant grass, corn stover, and poplar).

[0015] In an aspect the invention provides a co-culture system comprising yeast and cellulosic bacteria, wherein the yeast express at least one heterologous protein. The co-culture system may contain cellulose. In some embodiments the co-culture system contains one species of yeast and one species of bacteria.

[0016] In some embodiments of the co-culture system, the yeast can be from a genus selected from the group consisting of Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces, for example S. *cerevisiae*.

[0017] In some embodiments the yeast and bacterium of the co-culture have a symbiotic relationship in culture. In some embodiments the bacterium is *Actinotalea fermentans.*

[0018] In an aspect the invention provides a co-culture of two microorganisms adapted to aerobically grow together while maintaining a relatively constant ratio of species populations such that neither microorganism overtakes the other. The co-culture includes (i) a first microorganism component which metabolizes cellulose and produces one or more metabolic products; (ii) a second microorganism component which is recombinantly modified to express a heterologous protein, and which is metabolically incapable of degrading cellulose, where the second microorganism uses the metabolic products of the first microorganism as a carbon source. In one embodiment the first microorganism is a cellulosic bacteria and the second microorganism is a yeast. In one embodiment the yeast expresses a heterologous methyl halide transferase. In some embodiments the yeast is S. *cerevisiae and the bacterium is Actinotalea fermentans.*

[0019] In certain embodiments, the heterologous gene encodes a fusion protein comprising a MHT sequence and a targeting peptide sequence that targets the MHT sequence to the yeast vacuole. The targeting peptide sequence can be the N-terminal peptide domain from carboxypeptidase Y.

[0020] In one aspect the invention provides a method for production of methyhalide comprising culturing a first microorganism which metabolizes cellulose and produces one or more metabolic products together with a second microorganism which does not metabolize cellulose and which is recombinantly modified to express a heterologous methyl halide transferase protein in a medium containing cellulose and

a halide, under conditions in which methyl halide is produced. In some embodiments the halide is chlorine, bromine and iodine.

[0021] In one aspect the invention provides a recombinant yeast cell comprising a heterologous gene encoding S-adenosylmethionine (SAM)-dependent methyl halide transferase (MHT). In certain embodiments the MHT is from *Batis maritima, Burkholderia phymatum, Synechococcus elongatus, Brassica rapa, Brassica oleracea, Arabidopsis thaliana, Arabidopsis thaliana, Leptospirillum, Cryptococcus neoformans, Oryza sativa, Ostreococcus tauri, Dechloromonas aromatica, Coprinopsis cinerea, Robiginitalea bofirmata, Maricaulis maris, Flavobacteria bacterium, Vitis vinifera or halorhodospira halophila.* In certain embodiments the MHT is from *B. xenovorans*, *B. rapa chinensis*, *B. pseudomallei*, *B. thailandensis*, *Marine bacterium HTCC2080*, or *R. picketti* In certain embodiments the recombinant yeast cell is selected from *Saccharomyces cerevisiae, Pichia pastoris*, *Hansenula polymorpha*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Trichoderma reesei*, and *Scizosacchromyces pombe*. For example, the recombinant yeast cell can be a *Saccharomyces cerevisiae* cell expressing a Batis maritima methyl halide transferase protein.

[0022] In some embodiments the MHT is expressed in the yeast cell as a fusion protein comprising a targeting peptide sequence that targets proteins to the yeast vacuole. In one embodiment the targeting peptide sequence is the N-terminal peptide domain from carboxypeptidase Y.

[0023] In another aspect, described herein is a co-culture system comprising a culture medium a cellulosic bacterium component, where the bacteria metabolize cellulose and produce one or more metabolic products, and a yeast component, where the yeast uses at least one metabolic product of the bacteria as a carbon source. In one embodiment the bacteria-yeast co-culture comprises *Actinotalea fermentans* bacteria which metabolize cellulose and produce one or more metabolic products, and S. *cerevisiae* yeast, where the yeast uses at least one metabolic product produced by the bacteria as a carbon source. The culture medium may contain cellulose. In some embodiments the yeast is metabolically incapable of degrading cellulose. In some embodiments the metabolic product(s) is the sole or primary carbon and energy source for the yeast.

[0024] In some embodiments the yeast is recombinantly modified to express a heterologous protein or over-express an endogenous protein. In some embodiments

the yeast is a recombinantly modified to knock out expression of an endogenous protein. In some embodiments the bacteria and yeast grow together while maintaining a relatively constant ratio of species populations such that neither microorganism overtakes the other. The co-culture system may be maintained under substantially aerobic conditions or under substantially anaerobic conditions.

[0025] In various embodiments the yeast is from a genus selected from Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces. In an embodiment the yeast is S. *cerevisiae*. In various embodiments the bacteria is a *Actinotalea* or *cellulomonas* species. In an embodiment the bacterium is *Actinotalea fermentans*. In an embodiment the yeast is S. *cerevisiae* and the bacterium is *Actinotalea fermentans*. In some embodiments the co-culture comprises only one species of yeast and only one species of bacteria. In some embodiments the yeast and bacterium have a symbiotic relationship in culture.

[0026] In some embodiments the carbon source produced by the bacteria is molecule comprising 1-6 carbon atoms, such as, for example, ethanol, acetate, lactate, succinate, citrate, formate or malate.

[0027] In some embodiments the yeast expresses a heterologous protein. For example, the heterologous protein may be a mammalian protein such as, for example a human protein used for treatment of patients. In some embodiments the heterologous protein is an enzyme, such as an enzyme that catalyzes a step in a synthetic pathway in the yeast. In an embodiment the heterologous protein is a methyl halide transferase. In some embodiments the yeast is genetically engineered to produce a commercially valuable small molecule compound. In other embodiments the yeast is a naturally occurring or cultivated strain that is not recombinantly modified.

[0028] In another aspect, described herein is a yeast culture method comprising culturing cellulosic bacteria and yeast together in a culture medium in the presence of cellulose or a cellulose-source, under conditions in which (i) the bacteria metabolize cellulose and produce one or more metabolic products, and, (ii) the yeast component uses at least one metabolic product of the bacteria as a carbon source. Usually the culture medium is a liquid. In one embodiment the cellulose is microcrystalline cellulose. [0029] In some embodiments the cellulose-source is biomass, such as, without limitation, switchgrass, bagasse, elephant grass, corn

stover, poplar (each of which may be pulverized) and mixtures of these and other biomass materials.

[0030] In some embodiments the culture is maintained under aerobic conditions. In some embodiments the culture is maintained under anaerobic conditions. In some embodiments the yeast and bacterium have a symbiotic relationship in culture. In some embodiments the yeast is metabolically incapable of degrading cellulose. In some embodiments the carbon source produced by the bacteria is molecule comprising 1-6 carbon atoms, such as, for example, ethanol, acetate, lactate, succinate, citrate, formate or malate.

[0031] In various embodiments the yeast in the co-culture is from a genus selected from Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces. In an embodiment the yeast is S. *cerevisiae*. In various embodiments the bacteria is a *Actinotalea* or *Cellulomonas* species. In an embodiment the bacterium is *Actinotalea fermentans*. In an embodiment the yeast is S. *cerevisiae* and the bacterium is *Actinotalea fermentans*. In some embodiments the co-culture comprises only one species of yeast and only one species of bacteria. In some embodiments the yeast and bacterium have a symbiotic relationship in culture.

[0032] In some embodiments the yeast is recombinantly modified to express a heterologous protein. For example, the heterologous protein may be a mammalian protein such as, for example a human protein used for treatment of patients. In some embodiments the heterologous protein is an enzyme. In an embodiment the heterologous protein is a methyl halide transferase. In some embodiments the yeast is genetically engineered to produce a commercially valuable small molecule compound. In other embodiments the yeast is a naturally occurring or cultivated strain that is not recombinantly modified.

[0033] In some embodiments the yeast is a recombinantly modified to knock out expression of an endogenous protein. In other embodiments the yeast is a naturally occurring or cultivated strain that is not recombinantly modified.

[0034] In some embodiments the method includes the step of recovering a product from the culture medium which product is produced by the yeast. Examples of products that may be recovered include, but is not limited to, a recombinant protein expressed by the yeast, a small molecule synthesized by the yeast cell, a drug, food product, amino acid, cofactor, hormone, protein, vitamin, lipid, alkane, aromatic,

olefin, alcohol, or biofuel intermediate. In an embodiment the product is a methyl halide. In some embodiments synthesis of the product requires expression of a heterologous protein in the yeast. In some embodiments the synthesis requires expression of an endogenous protein that is overexpressed in the yeast or deletion of one or more endogenous genes of the yeast.

[0035] In one aspect the invention provides a method for production of methyhalide comprising culturing a cellulosic bacteria which metabolizes cellulose and produces one or more metabolic products together with a yeast which does not metabolize cellulose and which is recombinantly modified to express a heterologous methyl halide transferase protein in a medium containing a cellulose source and a halide, under conditions in which methyl halide is produced. The halide may be chlorine, bromine and iodine.

[0036] In an aspect the invention provides a method comprising combining i) a recombinant yeast comprising a heterologous gene encoding S-adenosylmethionine (SAM)-dependent methyl halide transferase (MHT), ii) a halide selected from the group comprising chlorine, bromine and iodine; and iii) a cellulolytic bacteria that produces a carbon source by metabolism of cellulose; in a cultivation medium under conditions in which methyl halide is produced. In some embodiments the carbon source is a molecule comprising 1-6 carbon atoms such as .ethanol, acetate, lactate, succinate, formate, citrate, or malate. In some embodiments the method includes recovering methyl halide from the culture medium and converting the methyl halide into a non-halogenated organic molecule or a mixture of non-halogenated organic molecules. In some embodiments the yeast is S. *cerevisiae* or another yeast described hereinbelow.

[0037] In some embodiments the bacteria is *Actinotalea fermentans* or another cellulosic bacteria described hereinbelow. In some embodiments the MHT is from *Batis maritima* or is another MHT described hereinbelow. In one embodiment the yeast is S. *cerevisiae*, the bacteria is *Actinotalea fermentans* and the MHT is from *Batis maritima.*

BRIEF DESCRIPTION OF THE FIGURES

[0038] Figure 1: Methyl halide production by bacteria containing a recombinant methyl halide transferase (MHT) gene expressed from an IPTG-inducible promoter.

[0039] Figure 2: Time-course of methyl halide production from bacteria containing a recombinant MHT gene expressed from an IPTG-inducible promoter, after addition of IPTG to the medium.

[0040] Figure 3: Effect of bacterial growth phase on methyl halide production.

[0041] Figure 4: Effect of halide salt concentration in the cultivation medium on methyl halide production.

[0042] Figure 5: Effect of different halides on methyl halide production.

[0043] Figure 6: Methyl halide production from bacteria overexpressing genes other than MHTs, *e.g.*, *metK*.

[0044] Figure 7: Methyl halide production achieved by bacteria expressing various heterologous MHTs from various organisms.

[0045] Figure 8: A schematic of a bioreactor system for production of organic compounds.

[0046] Figure 9A-C: CH_3I production from cellulosic feedstocks using a microbial co-culture. Fig. 9A: diagram of co-culture. *A. fermentans* ferments cellulosic feedstocks to acetate and ethanol, which *S. cerevisiae* can respire as a carbon (and energy) source. Fig. 9B, left panel:, growth of yeast in co-culture. Yeast were inoculated on carboxymethylcellulose (CMC) as the sole carbon sources with and without *A. fermentans*. Growth was measured as colony forming units. Fig. 9B, right panel: Growth of bacteria in co-culture. Fig. 9C: CH_3I production from cellulosic feedstocks. Co-cultures were seeded at low density and grown for 36 hours with the indicated feedstock (20 g/L) as the sole carbon source. Sodium iodide was added and CH_3I production was measured by GC-MS as before. CH_3I yields are reported in grams per liter per day, normalized by CFUs per mL of culture. Yields are shown for the *A. fermentans* – *S. cerevisiae* co-culture on acetate, CMC, switchgrass, corn stover, and poplar. Cultures grown without *A. fermentans* showed no methyl iodide activity.

[0047] Figure 10A-B: Screening the MHT library for methyl halide activity. Fig. 10A: methyl halide activity for MHT library in *E. coli*. Organisms that MHT genes are from are shown at left. Bacteria are shown in red font, plants are in green, fungi are blue, and archae are in purple. Production of CH_3I, CH_3Br, and CH_3Cl are shown. Genes are rank ordered by CH_3I activity. Fig. 10B, assay of methyl halide activity for a subset of MHT library. Measurements were performed in triplicate and standard deviations are shown.

[0048] Figure 11A-D: Methyl iodide production in recombinant *S. cerevisiae*. Fig. 11A. CH_3I production pathway. The *B. maritima* MHT is expressed with a N-terminal vacuole targeting tag. The ATP-dependent MHT methylates iodide ions using SAM as a methyl donor. Fig. 11B, CH_3I measured in culture headspace over time. Activity on glucose-grown cells is shown. Fig. 11C, CH_3I yields in grams per liter of culture per day. Values for the culturable red algae *E. muricata* are taken from the literature. Yields from *B. maritima* MHT-expressing *E. coli* and *S. cerevisiae* are calculated by comparison to standard curves. Fig. 11D, CH_3I toxicity in yeast. Exponential phase cultures were diluted to an OD_{600} of 0.05 and commercially available CH_3I was added. OD_{600} was measured at 24 hours of growth. The W303a lab strain is shown in filled boxes, the DNA methylation-sensitive *RAD50Δ* mutant is shown in open boxes.

[0049] Figure 12: Methyl iodide production improvement by targeting the *B. maritima* MHT to the yeast vacuole using a N-terminus fused CPY signal. Methyl iodide counts per hour are shown for each culture. The vacuole targeted (CPY-MHT) and cytoplasmic MHT were expressed in the W303 strain and in a W303 strain harboring a VPS33 deletion, which abolishes vacuole formation.

DETAILED DESCRIPTION

1. INTRODUCTION

[0050] Methyl halides can be converted to commodity chemicals and liquid fuels - including gasoline - using zeolite catalysts prevalent in the petrochemical industry. The methyl halide transferase (MHT) enzyme transfers the methyl group from the ubiquitous metabolite S-adenoyl methionine (SAM) to a halide ion in an ATP-dependent manner. Using bioinformatics and mail-order DNA synthesis, we identified and cloned a library of 89 putative MHT genes from plants, fungi, bacteria, and unidentified organisms. The library was screened in *Escherichia coli* to identify the rates of CH_3Cl, CH_3Br, and CH_3I production, with 56% of the library active on chloride, 85% on bromide, and 69% on iodide. Expression of the highest activity MHT and subsequent engineering in *Saccharomyces cerevisiae* resulted in product yields of 4.5 g/L-day from glucose and sucrose, four orders of magnitude over culturable naturally occurring sources. Using a symbiotic co-culture of the engineered yeast and the cellulolytic bacterium *Actinotalea fermentans*, we were

able to achieve methyl halide production from unprocessed switchgrass (*Panicum virgatum*), corn stover, and poplar. Methyl halides produced from various biorenewable resources can to be used as 1-carbon precursors for the production of alkanes, aromatics, olefins, and alcohols in the chemical industry.

[0051] In one aspect the invention provides methods for production of commodity chemical and fuels. The invention provides methods for production of biofuels and other commercially valuable organic products. In one aspect, recombinant bacteria, fungi or plant cells expressing a methyl halide transferase enzyme (MHT) are cultivated in the presence of a carbon source (*e.g.*, agricultural or waste biomass, cultivation media, petroleum, natural gas application methane) under conditions in which methyl halide gas is produced. In one embodiment the MHT is heterologous. The methyl halide is converted to non-halogenated organic compounds such as long-chain alkanes, olefins, alcohols, ethers, and aldehydes. In one embodiment the organic compounds are suitable for use as biofuel. Conversion of methyl halide to other organic molecules can be achieved by any means and is not limited to a specific mechanism. In one embodiment the MHT-expressing organism also expresses enzymes (endogenous or heterologous) that convert the methyl halide to another organic molecule, such as methanol. In one embodiment the MHT-expressing organism releases methyl halide which is then converted by a different organism (natural or recombinant) to another organic molecule. In one embodiment methyl halide is collected and converted by well-known chemical synthetic methods (*e.g.*, catalytic condensation). Following conversion of the methyl halide into a non-halogenated organic molecule or a mixture of non-halogenated organic molecules, the non-halogenated organic molecule(s) may be collected and/or packaged for subsequent use.

[0052] The invention also includes organisms expressing a heterologous methyl halide transferase enzyme and having at least one other genetic modification that causes the organism to produce more methyl halide than an organism lacking the at least one other genetic modification. An increase in yield of methyl halide in a MHT-expressing cell can be facilitated in various ways, for example by engineered SAM overproduction, increase in concentration and/or availability of ATP, expression of halide ion importers. Manipulation of genes in various metabolic pathways allows creation of organisms able to efficiently convert the carbon from cellulose, sugar, waste materials, or CO_2 to methyl halide gas.

[0053] The invention also provides co-culture systems in which a cellulolytic bacterium and a yeast cell expressing a heterologous protein are cultured together. In this system, the bacterium metabolizes cellulose to produce a product that serves as a carbon source for the yeast. In some examples, accumulation of the product in culture medium is toxic to the bacterial. Consumption of the product by the yeast cells serves to remove the product, so that the bacteria and yeast have a symbiotic relationship.

2. METHYL HALIDE TRANSFERASE-EXPRESSING CELLS

[0054] A variety of types of cells or organisms can be used in the practice of the invention, including cells that express an endogenous methyl-halide transferase (MHT), and cells modified to express an heterologous MHT. Preferably the organism is capable of producing about 1-1000 mg/L of methyl halide per day, often about 10-100 mg/L, such as about 20-60 mg/L, for example about 30-50 mg/L, or about 40 mg/L per day. As used herein, the term “heterologous” refers to a gene not normally in the cell genome, such as a gene from a different species or not found in nature, or a protein encoded by the heterologous gene. A gene found in the wild-type cell genome, or protein normally expressed in the cell, can be referred to as “endogenous.” Additional copies of an endogenous gene (under the control of a constitutive or inducible promoter) can be introduced into a host organisms to increase levels of an endogenous enzyme.

[0055] In principal almost any cell type can be modified for use in the methods of the invention, although in practice, the cells or organism should be suitable for commercial scale bioproduction, *e.g.*, typically unicellular and/or fast-growing. For simplicity, the term "cells" is used herein to encompass both MHT-expressing unicellular organisms, and MHT-expressing cells of multicellular organisms. Suitable cells may be eukaryotic or prokaryotic. Examples include bacterial, fungi, algae and higher plant cells.

[0056] Cells expressing endogenous MHT may be used. In such cases the cell is usually selected or modified to express endogenous MHT at high levels and/or is selected or modified at other loci that affect methyl halide production, as is discussed below. Although selection, with or without antecedent mutagenesis, may be used, recombinant techniques are usually preferred because they allow greater control over the final cell phenotype.

[0057] When recombinant cells are used, they may express a heterologous MHT, express a modified endogenous MHT, express an endogenous MHT at levels higher than wild-type cells, be modified at one or more loci other than the MHT gene (discussed below), or combinations of these modifications. Most preferably the cell expresses a heterologous MHT and is modified at at least one other locus that affects methyl halide production.

[0058] In one aspect, the recombinant organism is not E. Coli. In another aspect, the heterologous enzyme is not Batis MHT. In another aspect, the recombinant organism is not E. coli containing a Batis MHT.

2.1 CELLS EXPRESSING ENDOGENOUS MHT

[0059] A wide variety of plants, fungi and bacteria express endogenous MHT and can be used according to the method of the invention. In addition, MHT-expressing cells are a source of MHT genes that can be transferred to a heterologous host, such as *E. coli*. Organisms expressing MHTs include prokaryotes, *e.g.*, bacteria or achaea. Examples of bacteria that can be used to produce MHT according to the invention include soil bacteria, and *Proteobacteria*, *Methylobacterium chloromethanicu*m, and *Hyphomicrobium chloromethanicum*). The *Proteobacteria* phylum include genuses such as *Pseudomonas* and *Burkholderia*. Examples of *Burkholderia* include *Burkholderia xenovorans* (previously named Pseudomonas cepacia then B. cepacia and B. fungorum), known for the ability to degrade chlororganic pesticides and polychlorinated biphenyls (PCBs). Other Burkholderia species include B. *mallei*, B. *pseudomallei* and B. *cepacia*. Besides bacteria, other prokaryotes such as Archaea can be used to produce MHT with or without modification. Examples of Archaea include Sulfolobuses such as S. acidocaldarius, S. islandicus, S. metallicus, S. neozealandicus, S. shibatae, S. solfataricus, or S. sp. AMP12/99.

[0060] Other especially useful types of organisms include marine algae (*e.g.*, phytoplankton, giant kelp and seaweed), higher plants (*e.g.*, halophytic plants, Brassicaceae such as *Brassica oleracea (TM1 or TM2),* and *Arabidopsis Thaliana (TM1 or TM2)*) and fungi (*e.g.*, yeast). Particular species include *Batis maritima*, *Burkholderia phymatum* STM815, *Synechococcus elongatus* PCC 6301, *Brassica rapa subsp. chinensis*; Leptospirillum sp. Group II UBA; *Cryptococcus neoformans* var. neoformans JEC21; *Oryza sativa* (japonica cultivar-group); *Ostreococcus tauri*;

Dechloromonas aromatica RCB; *Coprinopsis cinerea okayama*; *Robiginitalea bofirmata* HTCC2501; *Maricaulis maris* MCS10; Flavobacteria bacterium BBFL7; *Vitis vinifera*; *halorhodospira halophila* SL1; *Phellinus pomaceus* (a white rot fungus), *Endocladia muricata* (a marine red algae), *Mesembryanthemum crystallium*, Pavlova species such as *P. pinguis* and *P. gyrans*, *Papenfusiella kuromo*, *Sargassum horneri*, and *Laminaria digitata*. See, *e.g.*, Wuosmaa et al., 1990, *Science* 249:160-2; Nagatoshi et al., 2007, *Plant Biotechnology* 24, 503–506. Yet other species are disclosed herein.

2.2 CELLS EXPRESSING HETEROLOGOUS MHT

[0061] In some embodiments, cells used in the invention do not express an endogenous MHT, but are modified to express a heterologous MHT. Alternatively, cells may be used that are modified to express a heterologous MHT and also express an endogenous MHT. The use of cells expressing a heterologous MHT has several advantages. First, it is possible, using the methods described herein, to combine desirable properties of an organism (ease of culture, ability to metabolize a particular feedstocks, suitability for recombinant manipulation of other loci) with desirable properties of an MHT gene (*e.g.*, high enzymatic activity).

[0062] Cells that can be genetically modified to express heterologous MHT Include prokaryotes and eukaryotes such as plants, fungi and others. Exemplary prokaryotes include gram-negative bacteria such as *E. Coli* (*e.g.*, MC1061, BL21 and DH10B), Salmonella (*e.g.*, SL1344), Rhodobacter, Synechtocystis, Rickettsia, and Erwinia and gram-positive bacteria such as *B. subtilis* and Clostridium. Exemplary plants include algae (*e.g.*, Chlamydomonas, Chlorella and Prototheca). Exemplary fungi include *Trichoderma reesei*, *Aspergillus* and yeast (*e.g.*, *Saccharomyces cerevisae* and Pichia). Other cell types are disclosed herein and are known in the art. Other exemplary bacteria include *Sulfobolus sulfaticaricus*, and *Caulobacter* species such as *Maricaulis maris*.

[0063] An organism that efficiently metabolizes a particular carbon source can be selected to match an available feedstock. For example, when cellulosic materials are used as carbon sources, organisms such as Erwinia, *E. coli*, Pichia, Clostridium, and *Aspergillus Niger* can be used. *E. coli* and Saccharomyces are examples of organisms that can be used to metabolize starches and sugarcane. Similarly,

photosynthetic organisms such as algae (*e.g.*, Chlorella and Prototheca) can metabolize carbon sources such as CO_2.

2.3 METHYL HALIDE TRANSFERASES

[0064] In the context of this invention, a "methyl halide transferase (MHT)" is a protein that transfers a methyl group from S-adenosylmethionine to a halide. As noted above, methyl halide transferases are ubiquitous in nature. Exemplary naturally occurring methyl halide transferases include, but are not limited to, those disclosed herein. Other naturally occurring methyl halide transferase can be identified by referring to a protein database (for example, the NCBI protein sequence database, at http://www. *followed by* ncbi.nlm.nih.gov/sites/entrez?db=protein) and scientific literature.

[0065] Table 1 below lists some of the organisms known to have MHTs. Also see Figures , Tables 4 and 6 and Examples 8 and 9.

Table 1

Organism
Batis maritima
Burkholderia phymatum STM815
Synechococcus elongatus PCC 6301
Brassica rapa subsp. chinensis
Brassica oleracea TM1
Brassica oleracea TM2
Arabidopsis thaliana TM1
Arabidopsis thaliana TM2
Leptospirillum sp. Group II UBA
Cryptococcus neoformans var. neoformans JEC21
Oryza sativa (japonica cultivar-group)
Ostreococcus tauri
Dechloromonas aromatica RCB
Coprinopsis cinerea okayama
Robiginitalea bofirmata HTCC2501

Maricaulis maris MCS10
Flavobacteria bacterium BBFL7
Vitis vinifera
Halorhodospira halophila SL1

[0066] MHT genes can be cloned and introduced into a host organism under control of a promoter suitable for use in the host. Alternatively, genes encoding a desired MHT sequence can be synthesized, which allows codon usage in the gene to be optimized for the host. The promoter can be inducible or constitutive. The heterologous MHT gene can be integrated into the host chromosome (*e.g.*, stable transfection) or can be maintained episomally.

[0067] Suitable MHTs are not limited to proteins encoded by naturally occurring genes. For example, techniques of directed evolution can be used to produce new or hybrid gene products with methyl transferase activity. In addition, catalytically active fragments and variants of naturally occurring MHTs can be used. Partially or wholly synthetic MHTs, such as enzymes designed *in silico* or produced by using art-known techniques for directed evolution including gene shuffling, family shuffling, staggered extension process (StEP), random chimeragenesis on transient templates (RACHITT), iterative truncation for the creation of hybrid enzymes (ITCHY), recombined extension on truncated templates (RETT), and the like (see Crameri et al., 1998, "DNA shuffling of a family of genes from diverse species accelerates directed evolution" *Nature* 391:288-91; Rubin-Pitel et al., 2006, "Recent advances in biocatalysis by directed enzyme evolution" *Comb Chem High Throughput Screen* 9:247-57; Johannes and Zhao, 2006, "Directed evolution of enzymes and biosynthetic pathways" *Curr Opin Microbiol.* 9:261-7; Bornscheuer and Pohl, 2001, "Improved biocatalysts by directed evolution and rational protein design" *Curr Opin Chem Biol.* 5:137-43).

[0068] It will be clear that a variety of naturally and non-naturally occurring methyl halide transferases can be used in the methods of the invention, provided the MHT can effect the transfer of a methyl group from S-adenosylmethionine to a halide (*i.e.*, chlorine, iodine and/or bromine) in the host organism. MHT enzyme activity can be measured using various assays known in the art. Assays can measure activity of purified or partially purified protein. See, *e.g.*, Ni and Hager, 1999, *Proc. Natl. Acad.*

Sci USA 96:3611-15 and Nagatoshi and Nakamura, 2007, *Plant Biotechnology* 24:503-506. Alternatively, a protein can be expressed a cell that does otherwise express MHT and methyl halide production measured is described in the Examples, *infra*, and other art-know assays. In one assay an expression vector with a sequence encoding the MHT protein is introduced into a bacterial (e.g.,. *E. coli*) host cell and transformants selected. Clones are incubated in growth media in a tube or flask (e.g., LB media containing NaCl, NaI or NaBr and incubated at 37°C for 4-22 hours with shaking. If the MHT encoding sequence is under control of an inducible promoter the inducing agent is included. The tube or flask is sealed (e.g., with parafilm and aluminum foil cinched with a rubber band). At the end of the incubation period the level of MeX in the headspace gas is determined, *e.g.*, by gas chromatography.

[0069] As is demonstrated in Example 8, *infra*, there is considerable variability in MHT sequences that may be used in the practice of the invention. Sequences with as little as 29% sequence identity with each other have been used to produce methyl halide when heterologously expressed in bacterial or fungal cells. Moreover, as shown in Example 8, diverse methyl halide transferases can function in *E. coli*.

[0070] In certain embodiments the invention includes the use of enzymatically active polypeptides with at least about 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or at least 99% identity with a known SAM-dependent methyhalide transferase (such as a MHT described herein) in the invention. As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

3. OTHER GENETIC MODIFICATIONS THAT AFFECT METHYL HALIDE PRODUCTION

[0071] In addition to introduction and manipulation of MHT genes, other genetic modifications can be made to increase the efficiency of methyl halide production, or increase the amount of methyl halide produced. These changes include increasing the intracellular concentration of reaction substrates such as halides and S-adenosylmethionine (also called "SAM" or "AdoMet"). Intracellular levels of SAM can be increased by changing the rate of SAM biosynthesis (e.g., by raising levels of SAM precursors), reducing SAM consumption, and the like. Intracellular levels of halide can be increased by stimulating transport of halides into the cell, adding halides to the extracellular environment, and the like. In general, techniques of metabolic engineering can be used to maximize production of methyl halides.

3.1 SAM METABOLIC PATHWAYS

[0072] Methyl halide production can be increased by manipulating flux though metabolic pathways that affect SAM levels, such as SAM biosynthetic pathways, methionine biosynthetic pathways, SAM utilization or degradation pathways, and SAM recycling pathways. S-adenosylmethionine is a ubiquitous metabolite involved in multiple metabolic pathways that entail methyl transfer. One such pathway is indicated below:

S-adenosylmethionine $\xrightarrow[\text{SAM-dependent methylase EC 2.1.1}]{}$ S-adenosyl homocysteine

S-adenosyl homocysteine $\xrightarrow[\text{S-adenosyl homocysteine hydrolase EC 3.3.1.1}]{}$ homocysteine

homocysteine + 5-methyltetrahydrofolate $\xrightarrow[\text{methionine synthase EC 2.1.1.13 or EC 2.1.1.14}]{}$ methionine

methionine + ATP $\xrightarrow[\text{SAM Synthase}]{}$ S-adenosylmethionine

3.1.1 OVEREXPRESSION OF SAM SYNTHETASE

[0073] SAM is synthesized from ATP and methionine, a reaction catalyzed by the enzyme S-adenosylmethionine synthetase (SAM synthetase, EC 2.5.1.6; Cantano, 1953, *J. Biol. Chem.* 1953, 204:403-16. In one aspect of the invention, a MHT-expressing cell is modified to increase SAM synthase activity by overexpression of endogenous SAM synthetase or introduction of a heterologous SAM synthase. SAM synthetase (SAMS) genes include *metK* in prokaryotes such as *E. Coli* (Acc. No.

NP_289514.1), and *sam1p* (Acc. No. NP_010790.1) or *sam2p* in *S. Cerevisiae*, or MTO3 in Arabidopsis (Acc. No. NP_188365.1). SAMS can be overexpressed in a cell by introducing a heterologous SAMS gene or introducing additional copies of the SAMS genes of the host organisms, under the control of a constitutive or inducible promoter. For example, Yu et al., 2003, *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* 35:127-32, described enhanced production of SAM by overexpression in *Pichia pastoris* of an *S. cerevisiae* SAM synthetase 2 gene. As discussed below (Section 3.8) reference to particular genes is for illustration and not limitation. It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs, orthologs and variants with the same enzymatic activity.

3.1.2 INCREASING SAM RECYCLING

[0074] As shown above, methyl halide transferase catalyses conversion of SAM to S-adenosyl-homocysteine. S-adenosyl-homocysteine is "recycled" back to SAM via SAM biosynthetic pathways. SAM production or levels can thus be increased by increasing the level and/or activity of enzymes in the pathways. Examples of such enzymes include SAM-dependent methylase (EC 2.1.1), methionine synthase (EC 2.1.1.13 or EC 2.1.1.14), and N^5-methyl-tetrahydropteroyltriglutamate-homocysteine methyltransferase (e.g., yeast MET6). S-adenosyl-L-homocysteine hydrolase (SAH1), a key enzyme of methylation metabolism, catabolizes S-adenosyl-L-homocysteine which acts as strong competitive inhibitor of all AdoMet-dependent methyltransferases.

[0075] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.1.3 IMPAIRMENT OF SAM UTILIZATION PATHWAYS

[0076] Various metabolic pathways within the methyl halide producing organisms cause a decrease in intracellular levels of free SAM (SAM utilization pathways). The content and/or the biological activity of one or more enzymes involved in a SAM utilization pathway can be decreased in order to facilitate or increase methyl halide production.

[0077] Examples of genes that can be inhibited to reduce SAM utilization include S-adenosylmethionine decarboxylase (corresponding to *E. coli* gene *speD*). Further examples include cystathionine beta-synthetase, ribulose 5-phosphate 3-epimerase, glucose-6-phosphate dehydrogenase, L-alanine transaminase, 3',5'-bisphosphate nucleotidase, glycine hydroxymethyl transferase (reversible, mitochondrial), glycine hydroxymethyl transferase (reversible), corresponding to *S. cerevisae* genes *CYS4*, *Rpe1*, *Zwf1*, *Alt*, *Met22*, *Shm 1-m*, and *Shm 2.*

[0078] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.1.4 OVEREXPRESSION OF SAM TRANSPORT GENES

[0079] In one approach, a SAM transport protein involved in the transport of SAM into a cell from the extracellular environment is expressed or over expressed in a cell. Examples include the Sam5p protein from yeast and homologs such as GenBank ID Nos. BC037142 (*Mus musculus*), AL355930 (*Neurospora crassa*), AE003753 (*Drosophila melanogaster*), Z68160 (Caenorhabditis elegans) and SLC25A26 (human). See Marrobio et al., 2003, *EMBO J.* 22:5975–82; and Agrimi *et al.*, 2004, *Biochem. J.* 379:183–90.

[0080] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.2. METHIONINE BIOSYNTHETIC PATHWAYS

[0081] SAM biosynthesis, and in turn methyl halide production, can be increased by the use of microorganisms with increased efficiency for methionine synthesis. In general, the basic metabolic pathways leading to methionine synthesis are well known (see, *e.g.* Voet and Voet, 1995, *Biochemistry*, 2nd edition, Jon Wiley &Sons, Inc.; Rückert et al., 2003, *J. of Biotechnology* 104, 2 13-228; and Lee et al., 2003, *Appl. Microbiol. Biotechnol.*, 62:459-67). These pathways are generally under strict regulation by various mechanisms such as feedback control. (*See*, *e.g.*, Neidhardt, 1996, *E. coli and S. lyphimurium*, ASM Press Washington). Accordingly, the expression or repression of relevant genes, or increase in the levels and/or activity of the corresponding gene products), can result in increased methionine production.

3.2.1 METHIONINE BIOSYNTHETIC ENZYMES

[0082] Genes that can be expressed or upregulated include those involved in methionine biosynthesis. PCT Publication WO 02/10209, incorporated by reference in its entirety, describes the over-expression or repression of certain genes in order to increase the amount of methionine produced. Examples of methionine biosynthetic enzymes include O-acetyl-homoserine sulfhydrylase (*metY*) and O-succinyl-homoserine sulfhydrylase (*metZ*). Other genes include methylene tetrahydrofolate reductase (*MetF*); aspartate kinase (*lysC*); homoserine dehydrogenase (*horn*); homoserine acetyltransferase (*metX*); homoserine succinyltransferase (*metA*); cystathionine γ-synthetase (*metB*); cystathionine β-lyase (*metC*); Vitamin B_{12}-dependent methionine synthase (*metH*); Vitamin B_{12}-independent methionine synthase (*metE*); $N^{5,10}$-methylene-tetrahydrofolate reductase (*metF*) and S-adenosylmethionine synthase (*metK*).

[0083] Variants of these enzymes that are resistant to feedback inhibition by methionine can further increase methyl halide production. Some such variants are set forth in WO 07/011939, and Park *et al.*, 2007, *Metab Eng.* 9:327-36, incorporated by reference in its entirety. By way of example, methyl halide production can be increased in prokaryotes such as *E. Coli* and Corynebacterium by overexpressing genes such as *metY, metA, metB, metC, metE,* and/or *metH*, or otherwise increasing the levels or activity of their gene products. Similarly, decreasing the levels or impairing the activity of the repressor proteins genes can increase methyl halide production (*e.g.*, repressor encoded by the *metJ* or *metD* (McbR) genes, which repress methionine synthesis-related genes such as *metB, metL* and *metF*). See Rey et al., 2003, *J. Biotechnol.*, 103:1-65; Nakamori *et al.*, 1999, *Applied Microbiology and Biotechnology* 52:179-85; WO 02/097096; each of which is incorporated by reference in its entirety).

[0084] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.2.2 METHIONINE BIOSYNTHESIS PRECURSORS

[0085] Methionine synthesis can also be increased by modifying the flux through those pathways that provide additional precursors, examples of which include sulfur

atoms in different oxidative states, nitrogen in the reduced state such as ammonia, carbon precursors including Cl-carbon sources such as serine, glycine and formate, precursors of methionine, and metabolites of tetrathydrofolate substituted with carbon at N5 and or N10. In addition energy e.g. in the form of reduction equivalents such as NADH, NADPH or FADH2 can be involved in the pathways leading to methionine.

[0086] For example, methyl halide production can be increased by increasing the level and/or activity of gene products involved in sulfate assimilation, cysteine biosynthesis and conversion of oxaloacetate to aspartate semialdehyde. Examples of genes include L-cysteine synthase (*cysK*), NADPH-dependent sulphite reductase (*cysI*) and alkane sulfonate monooxygenase (*ssuD*).

[0087] Increasing the levels of serine can also result in increased methionine production. Thus, the organism can be modified with respect to proteins involved in serine metabolism or transport. Enzymes involved in serine synthesis include D-3-phosphoglycerate dehydrogenase (*SerA*), phosphoserine phosphatase (*SerB*) and phosphoserine aminotransferase (*SerC*). See WO 07/135188, incorporated by reference in its entirety. Enzymes involved in serine synthesis can be modified to reduce or prevent feedback inhibition by serine.

[0088] Similarly, the levels and/or the biological activity of one or more enzymes involved in the conversion of serine to methyl-tetrahydrofolate can be increased. Such genes include serine hydroxymethyltransferase (SHMT) and methylene tetrahydrofolate reductase (*metF*).

[0089] Similarly, the content and/or the biological activity of one or more enzymes involved in serine degradation to pyruvate (*e.g.*, serine dehydratase, *sdaA*), or in serine export from the cell (*e.g.*, ThrE) can be decreased.

[0090] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.2.3 METHIONINE UPTAKE

[0091] Genes controlling methionine uptake in a cell can be modified to increase methyl halide production. For example, the *MetD* locus in *E. Coli* encodes an ATPase (*metN*), methionine permease (*metI*) and substrate binding protein (*metQ*). Expression of these genes is regulated by L-methionine and *MetJ*, a common

repressor of the methionine regulon. Orthologs are known in many other species such as Salmonella, Yersinia, Vibrio, Haemophilus, Agrobacterium, Rhizobium and Brucella. See, *e.g.*, Merlin et al., 2002, *J. Bacteriology* 184:5513-17.
et al., 2003, *EMBO J.* 22:5975–82; and Agrimi *et al.*, 2004, *Biochem. J.* 379:183–90.
[0092] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.3 INCREASING INTRACELLULAR HALIDE CONCENTRATION

[0093] Methyl halide production can also be increased by increasing the intracellular halide concentration in MHT-expressing cells. This can be accomplished in various ways, *e.g.*, by introducing or increasing the levels and/or activity of one or more halide transporters, and/or increasing halide concentration in the medium. Examples include *Gef1* of Saccharomyces cerevisiae, *EriC* of *E. coli* (P37019), and Synechocystis (P74477).
[0094] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.4 INCREASING ATP LEVELS

[0095] Methyl halide production can also be increased by methyl halide synthesis activity is increased by increasing the intracellular concentration and/or availability of ATP.
[0096] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.5 IMPAIRING METHYL HALIDE UTILIZATION

[0097] The activity and/or level of methyl halide utilizing enzymes can be decreased. These include enzymes in the *cmu* gene cluster such as *cmuC, cmuA, orf146, paaE* and *hutI*. Other enzymes include bacterial 10-formyl-H_4 folate hydrolases, 5,10-methylene-H_4 folate reductase and *purU* and corrinoid enzymes such as halomethane: bisulfide/halide ion methyltransferase.

[0098] It is understood that gene names vary from organism to organism and reference above to a gene name is not intended to be limiting, but is intended to encompass homologs with equivalent activity.

3.6 RECOMBINANT YEAST EXPRESSING MHT

[0099] We have observed that use of yeast as the MHT expressing cell results in particularly high yield of methyl halide. See Example 10. In one aspect, the invention provides a recombinant yeast cell comprising a heterologous gene encoding S-adenosylmethionine (SAM)-dependent methyl halide transferase (MHT). Examples of MHT proteins that can be expressed in yeast include, as discussed elsewhere herein, those from Batis maritima, Burkholderia phymatum, Synechococcus elongatus, Brassica rapa, Brassica oleracea, Arabidopsis thaliana, Arabidopsis thaliana, Leptospirillum, Cryptococcus neoformans, Oryza sativa, Ostreococcus tauri, Dechloromonas aromatica, Coprinopsis cinerea, Robiginitalea bofirmata, Maricaulis maris, Flavobacteria bacterium, Vitis vinifera or halorhodospira halophila. Examples of suitable recombinant yeast cells include, as discussed elsewhere herein, Saccharomyces cerevisiae, Pichia pastoris, Hansenula polymorpha, Kluyveromyces lactis, Yarrowia lipolytica, Trichoderma reesei, Scizosacchromyces pombe, and others. Methods for culture and genetic manipulation of yeast are well known in the art.

3.7 USE OF TARGETING DOMAIN TO INCREASE PRODUCTION IN YEAST

[0100] Expression of heterologous methyl halide transferase (e.g., *Batis maritima* MCT) in *Saccharomyces cerevisiae* results in the production of methyl halide (e.g., methyl iodide). The yield is increased significantly by using a peptide signal to target the enzyme to vacuoles. See discussion below. Without intending to be limited to a particular mechanism, the increased production is believed to result from (i) the sequestration of the majority of the cell's SAM in the vacuole (Farooqui et al., 1983, Studies on compartmentation of S-adenosyl-L-methionine in Saccharomyces cerevisiae and isolated rat hepatocytes. *Biochim Biophys Acta 757:342-51).* and (ii) the sequestration of halide ions in the vacuole (Wada et al., 1994, *Chemiosmotic coupling of ion transport in the yeast vacuole: its role in acidification inside organelles. J Bioenerg Biomembr 26: 631-7).*

[0101] One peptide signal is the N-terminal peptide domain from carboxypeptidase Y known to target pendant proteins to the yeast vacuole, but other targeting peptides may be used. See, e.g., Valls et al., 1990, Yeast carboxypeptidase Y vacuolar targeting signal is defined by four propeptide amino acids. J Cell Biol 111:361-8; and Tague et al.,1987, "The Plant Vacuolar Protein, Phytohemagglutinin, Is Transported to the Vacuole of Transgenic Yeast", *J. Cell Biology*, 105: 1971-1979; Tague et al., 1990, "A Short Domain of the Plant Vacuolar Protein Phytohemagglutinin Targets Invertase to the Yeast Vacuole", *The Plant Cell*, 2:533-546 and US Pat. No. 6054637, all of which are incorporated herein by reference.

[0102] In one approach, for illustration and not limitation, the coding sequence of *B.maritima* methylchloride transferase (MCT) is synthesized and cloned into a high copy vector under the control of a tet-repressible CYC promoter (plasmid pCM190, Gari et al, 1997,*Yeast* 13:837-48.). The MCT coding sequence is fused to a N-terminal peptide domain from carboxypeptidase Y known to target pendant proteins to the yeast vacuole (amino acid sequence: KAISLQRPLGLDKDVL, Valls et al., 1990, *J Cell Biol.* 111:361-8.) This expression system is transformed into *S. cerevisiae* strain W303a. Yeast carrying MCT expression vectors are streaked on uracil dropout plates from freezer stocks (15% glycerol) and grown for 48 hours. Individual colonies are inoculated into 2 mL of synthetic complete uracil dropout media and grown overnight at 30 degrees. Cultures are next inoculated into 100mL fresh synthetic complete uracil dropout media and grown for 24 hours. Cells are spun down and concentrated to high cell density (OD 50) in fresh YP media with 2% glucose and 100 mM sodium iodide salt. 10 mL of this concentrated culture is aliquoted into 14 mL culture tubes and sealed with a rubber stopper. Cultures are grown at 30 degrees with 250 rpm shaking, and methyl iodide production assayed at specified intervals via GC-MS. The GC-MS system consists of a model 6850 Series II Network GC system (Agilent) and model 5973 Network mass selective system (Agilent). Oven temperature is programmed from 50 degrees (1 min) to 60 degrees (10 degrees / min). 100 microliters of culture headspace is withdrawn through the rubber stopper with a syringe and manually injected into the GC-MS and methyl iodide production measured.

[0103] As is discussed below (Example 10), using the methods described above we targeted the *B. maritima* MHT to the *S. cerevisiae* strain W303a vacuole using the carboxypeptidase Y peptide, and assayed methyl iodide production from glucose

(Fig. 9A). Yeast displayed high activity on glucose (Fig. 9B) and normal growth rates (approximately 90min doubling time), compared to doubling times from natural sources of several days. Methyl iodide yield from glucose was measured at 4.5 g/L-day by comparison to standards, which is approximately 10,000 fold over the best natural sources (Fig. 9C).

[0104] It will be appreciated that, more generally, the targeting of other enzymes involved in metabolic processes to the vacuole can be used to increase production. In particular, yield from reactions in which a substrate(s) is SAM and/or a halide can be increased by such targeting. For example, ethylene may be produced by a metabolic pathway using SAM (see, e.g., US Patent No. 5,416,250, incorporated herein by reference). In a yeast (e.g., *S. cerevisiae*) expressing 1-aminocyclopropane-1 carboxylic acid (ACC) synthase (see Wilson et al., 1993, Apple ripening-related cDNA clone pAP4 confers ethylene-forming ability in transformed Saccharomyces cerevisiae. *Plant Physiol.* 102:783-8, incorporated herein by reference) and a ethylene forming enzyme (EFE, see McGarvey et al., 1992, Characterization and kinetic parameters of ethylene-forming enzyme from avocado fruit. *J Biol Chem.* 267(9):5964-7) ethylene production can be increased by targeting the enzymes to the vacuole.

3.7 COMBINATIONS

[0105] Generally, the process of the invention makes use of cells selected or modified at multiple (e.g., at least 2, sometimes at least 3, sometimes at least 4, and sometimes 5 or more than 5) different loci to increase methyl halide production. Cells may have additional genetic modifications to facilitate their growth on specific feedstocks, to provide antibiotic resistance and the like. In some embodiments strains developed for different purposes may be further modified to meet the needs of the current invention. See, for example, He et al., 2006, "A synergistic effect on the production of S-adenosyl-L-methionine in *Pichia pastoris* by knocking in of S-adenosyl-L-methionine synthase and knocking out of cystathionine-beta synthase" *J Biotechnol.* 126:519-27. Park et al., 2007, "Characteristics of methionine production by an engineered Corynebacterium glutamicum strain" *Metab Eng.* 9:327-36 described genetic manipulation of a *C. glutamicum* strain to increase methionine production. The strain carries a deregulated *hom* gene to abolish feedback inhibition of homoserine dehydrogenase by threonine and a deletion of the *thrB* gene to

abolish threonine synthesis. As also discussed, modified strains can be obtained by selection processes instead of recombinant technology, where organisms can be mutagenized and screened for methionine overproduction. High-producing strains have been isolated in many organisms including *E. coli* and yeast. *See, e.g.*, Alvarez-Jacobs *et al.*, 2005, *Biotechnology Letters*, 12:425-30; Dunyak *et al.*, 1985, 21:182-85; Nakamori et al., 1999, *Applied Microbiology and Biotechnology* 52:179-85.

[0106] For illustration and not limitation, the following exemplary combinations may be used. Specifying specific modifications does not preclude the presence of additional modifications:

[0107] a) Expression of a heterologous MHT and a genetic modification to increase flux through a S-adenosyl-methionine (SAM) biosynthetic pathway. In one embodiment flux through a SAM biosynthetic pathway is increased by increasing expression of a SAM synthetase (which may be heterologous or endogenous). In one embodiment, the *metK* gene or a homolog is over expressed. In one embodiment, the *sam1p* and/or *sam2p* gene or a homolog is over expressed. See Section 3.1.1 above.

[0108] b) Expression of a heterologous MHT and a genetic modification to increase flux through a SAM "recycling" pathway. In one embodiment activity of SAM-dependent methylase, methionine synthase, S-adenosyl-L-homocysteine hydrolase (e.g., *SAH1*) and N^5-methyltetrahydropteroyl-triglutamate-homocysteine methyl transferase (e.g., *MET6*) is increased. See Section 3.1.2 above.

[0109] c) Expression of a heterologous MHT and a genetic modification to inhibit flux through a SAM utilization pathway. In one embodiment a coproporphyrinogen III oxidase, coproporphyrinogen III oxidase, S-adenosylmethionine decarboxylase, cystathionine beta-synthetase, ribulose 5-phosphate 3-epimerase, glucose-6-phosphate dehydrogenase, L-alanine transaminase, 3',5'-bisphosphate nucleotidase, glycine hydroxymethyltransferase or glycine hydroxymethyl-transferase is inhibited. In one embodiment, the *CYS4*, *Rpe1*, *Zwf1*, *Alt*, *Met22*, *Shm 1-m*, *Shm 2*, *HEM 13, or hemF*gene is inhibited. See Section 3.1.3 above.

[0110] d) Expression of a heterologous MHT and a genetic modification to increase methionine biosynthesis. See Section 3.2.1 above.

[0111] e) Expression of a heterologous MHT and a genetic modification to increase activity of gene products involved in sulfate assimilation, cysteine biosynthesis and/or conversion of oxaloacetate to aspartate semialdehyde. In some embodiments, L-cysteine synthase (e.g., *cysK*), NADPH-dependent sulphite reductase (e.g., *cysI*) or alkane sulfonate monooxygenase (e.g., *ssuD*) is over expressed. See Section 3.2.2 above.

[0112] f) Expression of a heterologous MHT and a genetic modification to increase intracellular ATP levels. See Section 3.4 above.

[0113] g) Expression of a heterologous MHT and a genetic modification to increase levels of intracellular serine. See Section 3.2.2 above.

[0114] h) Expression of a heterologous MHT and a genetic modification to increase methionine uptake. See Section 3.2.3 above.

[0115] i) Expression of a heterologous MHT and a genetic modification to increase intracellular halide concentration. See Section 3.3 above.

[0116] j) Expression of a heterologous MHT and a genetic modification that reduces halide utilization other than for the synthesis of methyl halide. See Section 3.5 above.

[0117] k) Combinations of (a)-(j) such as a+b, a+c, a+d, a+e, a+f, a+g, a+h, a+i, a+j, b+c, b+d, b+e, b+f, b+g, b+h, b+i, b+j, c+d, c+e, c+f, c+g, c+h, c+i, c+j, d+e, d+f, d+g, d+h, d+i, d+j, e+f, e+g, e+h, e+i, e+j, f+g, f+h, f+i, f+j, g+h, g+i, g+j, h+i, or h+j.

[0118] l) Modifications presented in (a) - (k) above, except that the cell expresses or overexpresses an endogenous MHT rather than a heterologous MHT.

3.8 HOMOLOGS, ORTHOLOGS AND VARIANTS

[0119] It is understood that gene names vary from organism to organism and reference above to a gene name above is not intended to be limiting, but is intended to encompass homologs with equivalent activity. Moreover, where the method requires overexpression of an activity the encoded protein need not be identical to the naturally occurring version, so long as the overexpressed protein has the appropriate activity and can be expressed in the host. In certain embodiments the invention includes the use of enzymatically active polypeptides with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identity with a known protein described hereinabove.

4. RECOMBINANT TECHNIQUES

[0120] Genetic modification can be achieved by genetic engineering techniques or using classical microbiological techniques, such as chemical or UV mutagenesis and subsequent selection. A combination of recombinant modification and classical selection techniques may be used to produce the organism of interest. Using recombinant technology, nucleic acid molecules can be introduced, deleted, inhibited or modified, in a manner that results in increased yields of methyl halide within the organism or in the culture. Methods for genetic manipulation of procaryotes and eukaryotes are very well known in the art. Accordingly, methods are only very briefly described. Some culture and genetic engineering techniques are generally disclosed, for example, in Sambrook et al., 1989, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press; Sambrook and Russell, 2001, MOLECULAR CLONING: A LABORATORY MANUAL Cold Spring Harbor Laboratory Press; Ausubel, et al, 2002, SHORT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons; Tuan, R.S., 1997, RECOMBINANT GENE EXPRESSION PROTOCOLS Humana Press; Ball, A.S., 1997, BACTERIAL CELL CULTURE: ESSENTIAL DATA John Wiley & Sons; Richmond, A., 2003, HANDBOOK OF MICROALGAL CULTURE Wiley-Blackwell; Becker, E.W., 1994, MICROALGAE: BIOTECHNOLOGY AND MICROBIOLOGY Cambridge University Press; Guthrie and Fink, 2004, GUIDE TO YEAST GENETICS AND MOLECULAR BIOLOGY, Academic Press; and Walker, G.M., 1998, YEAST PHYSIOLOGY AND BIOTECHNOLOGY John Wiley & Sons, each of which is incorporated herein by reference for all purposes.

[0121] Expression and harvest of recombinant proteins or products produced by recombinant cells, on both laboratory and industrial scales, is well known and widely discussed in the literature. For production on an industrial level large bioreactors may be used (see, e.g., McKetta, J., CHEMICAL PROCESSING HANDBOOK, 1993, Marcel Dekker; Lee, S., ENCYCLOPEDIA OF CHEMICAL PROCESSING, 2006, Taylor and Francis Group; Asenjo, J., BIOREACTOR SYSTEM DESIGN, 1995, Marcel Dekker; Nielsen, J., BIOREACTION ENGINEERING PRINCIPLES, 2003, Kluwer Academics; Crow et al., "Process for manufacturing methyl chloride," US Pat. No. 6111153; Van 't Riet and Tramper, 1991, BASIC BIOREACTOR DESIGN, CRC Press; Asenjo and Merchuk, 1995, BIOREACTOR SYSTEM DESIGN, CRC Press).

4.1 EXPRESSION OF RECOMBINANT GENES

[0122] The expression of genes that contribute to methyl halide production, and/or the presence, levels, and/or activity of the corresponding gene products (mRNA and/or protein), can be achieved or increased. Overexpression can be accomplished by introducing a recombinant construct that directs expression of a gene product in a host cell, or by altering basal levels of expression of an endogenous gene product, for example by inducing or de-repressing its transcription, or enhancing the transport, stability and/or activity of gene products such as mRNA and/or protein. Codon optimization of non-endogenous nucleic acid sequences can also increase translation efficiency.

[0123] Stable introduction of cloned genes can be accomplished for example by maintaining the cloned gene(s) on replicating vectors or by integrating the cloned gene(s) into the genome of the production organism. Examples include multi-copy plasmids, transposons, viral vectors or YACs. The vector can contain an origin of replication such as PSC101, BAC, p15a or ColE1 (in prokaryotes) or ARS (yeast) or the SV40 origin (eukaryotes).

[0124] Expression vectors that can be used to produce a desired protein can comprise an operable linkage of (1) DNA elements coding for an origin for the maintenance of the expression vector in a host cell; (2) DNA elements that control initiation of transcription, such as a promoter; (3) DNA elements that control the processing of transcripts, such as a transcriptional terminator, and (4) optionally, a gene encoding a selectable marker, such as antibiotic resistance.

[0125] The sequence to be expressed can be placed under the control of a promoter that is functional in the desired prokaryotic or eukaryotic organism. An extremely wide variety of promoters are well known, and can be used, depending on the particular application. Inducible and constitutive promoters are both encompassed by the invention. Inducible promoters include those induced by arabinose (PBAD); IPTG (PTRC), halide salts (e.g., sodium chloride), osmolarity, sugar, starch, cellulose, or light.

[0126] As shown in Example 4, methyl halide production using an IPTG-inducible promoter in bacteria increases to peak levels within 1-2.5 hours after induction of expression.

[0127] The expression of genes can be increased by operatively linking the gene(s) to native or heterologous transcriptional control elements. This can be done by the use of synthetic operons, ribosome binding sites, transcription termination sites and the like. Various prokaryotic and eukaryotic expression control sequences are known in the art. *See, e.g.*, WO 06/069220, incorporated by reference in its entirety. An example of a sequence encoding a recombinant ribosome binding site is ATTAAAGAGGAGAA ATTAAGC.

[0128] Recombinant sequences can be optimized for protein expression in a particular host species by changing any codons within a cloned gene that are not preferred by the organism's translation system to preferred codons without changing the amino acid sequence of the synthesized protein. Codon optimization can increase the translation of a recombinant gene. Optionally, the DNA sequence of a gene can be varied so as to maximize the difference with the wild-type DNA sequence, for example to avoid the possibility of regulation of the gene by the host cell's regulatory proteins.

4.2 REPRESSION, INHIBITION OR DELETION OF GENES

[0129] The expression of genes that tend to limit, regulate or decrease methyl halide production, or the presence, levels, and/or activity of the corresponding gene products (mRNA and/or protein), can be abolished or decreased. Genetic modifications that result in a decrease in expression and/or function of the gene and/or gene product can be through complete or partial inactivation, suppression, deletion, interruption, blockage or down-regulation of a gene. This can be accomplished for example by gene "knockout," inactivation, mutation, deletion, or antisense technology. Gene knockout can be accomplished using art-known methods including commercially available kits such as the "TargeTron gene knockout system" (Sigma-Aldrich). *E. coli* strains with individual gene knockouts can be obtained from the E. coli genome project (www.genome.wisc.edu). The invention includes multiple knockouts, *e.g.*, 2-6 genes in same organism. The invention also includes any combination of gene introductions, deletions or modifications.

5. CULTIVATION/FERMENTATION MEDIA AND CONDITIONS

[0130] The terms "cultivation" and "fermentation" are used interchangeably herein to refer to the culture of MHT-expressing cells in liquid media under conditions (either

aerobic or anaerobic) in which methyl halides are produced. The growth medium used for production of methyl halides will depend largely on the host organism. Suitable growth conditions many procaryotes and eukaryotes commonly used in the laboratory or industrial settings are known and described in the scientific literature. See, e.g., Ball, A.S., 1997, BACTERIAL CELL CULTURE: ESSENTIAL DATA John Wiley & Sons; Richmond, A., 2003, HANDBOOK OF MICROALGAL CULTURE Wiley-Blackwell; Becker, E.W., 1994, MICROALGAE: BIOTECHNOLOGY AND MICROBIOLOGY Cambridge University Press; and Walker, G.M., 1998, YEAST PHYSIOLOGY AND BIOTECHNOLOGY John Wiley & Sons, each of which is incorporated herein by reference for all purposes. Methods of optimizing cultivation conditions may be determined using art known techniques.

[0131] A nutrient or cultivation media will include a carbon source, a halide source, as well as nutrients. The medium should also contain appropriate amounts of nitrogen and sulfur sources, *e.g.*, in the form of one or more sulfates (such as ammonium sulfate) and/or thiosulfates. The medium can also contain vitamins such as vitamin B12. One suitable medium for bacteria such as *E. coli* is Luria-Bertani (LB) broth.

[0132] Carbon-containing substrates are metabolized to supply the methyl portion of methyl halides. Carbon compounds can also be metabolized to provide energy to drive methyl halide production. Substrates include carbon-containing compounds such as petroleum and/or natural gas, carbohydrates, in which carbon is present in a form that can be metabolized by the organism of choice. Examples of carbohydrates include monosaccharides, sugars such as glucose, fructose, or sucrose, oligosaccharides, polysaccharides such as starch or cellulose, and one-carbon substrates or mixtures thereof, for example presented in the form of feedstock. Carbon dioxide can also be used as a carbon source, especially when photosynthetic organisms such as algae are used. Common carbon-containing raw materials that can be used include but are not limited to wood chips, vegetables, biomass, excreta, animal wastes, oat, wheat, corn (e.g., corn stover), barley, milo, millet, rice, rye, sorghum, potato, sugar beets, taro, cassava, fruits, fruit juices, and sugar cane. Particularly useful are switchgrass (Panicum virgatum), elephant grass (Miscanthus giganteus), bagasse, poplar, corn stover and other dedicated energy crops. The optimal choice of substrate will vary according to choice of organism. As

noted above, when cellulosic materials are used as carbon sources, organisms such as Erwinia, *E. coli*, Pichia, Clostridium, and *Aspergillus Niger* can be used. *E. coli* and Saccharomyces are examples of organisms that can be used to metabolize starches and sugarcane. Similarly, photosynthetic organisms such as algae (*e.g.*, Chlorella and Prototheca) can metabolize carbon sources such as CO_2. See, Schmid, R. D., 2003, POCKET GUIDE TO BIOTECHNOLOGY AND GENETIC ENGINEERING John Wiley & Sons. Optionally cellulosic stocks may be blended or pulverized before addition to culture.

[0133] In addition to various genetic modifications, methyl halide production can be increased by optimizing the composition of the growth medium. As noted, the yield of methyl halides can also be increased by increasing the intracellular concentration of one or more reactants or precursors such as halides, methionine, SAM, and intermediates in SAM biosynthesis. Use of media rich in methionine, serine, and/or halide can increase methyl halide production. In certain embodiments the concentration of methionine in the medium is from about 0.5 gm/L to about 10 gm/L. In other embodiments the concentration of serine in the medium is from about 0.5 gm/L to about 10 gm/L.

[0134] Addition of halide salts to the medium can increase intracellular halide concentration. Halide salts include chlorides, iodides or bromides of sodium, potassium, magnesium, and the like. As shown below in Example 5, methyl halide production increases with atomic weight of the halide. Thus under certain circumstances, iodides can give better yield than bromides which in turn tend to given better yield than chlorides. As shown in Example 5, methyl halide production can be increased by adjusting the concentration of halides in the medium. The optimal osmolarity of a medium is often about 0.01 to 1 M, often about 0.05 to 0.3, such as about 0.1 M. The optimal concentration of a chosen halide salt can be determined empirically by one of skill guided by this disclosure. Using NaCl as an example, the invention contemplates the use of NaCl at about 0.01 to 0.1 M, often about 0.05 to 0.5 M, for example about 0.1 M, such as 0.085 M. Media such as Luria-Bertani (LB) broth (0.171 M of NaCl) are suitable. LB broth can also be prepared with various counter-ions made up to about 0.16 M. For example, an LB broth preparation of 5 g/L yeast extract, 10 g/L tryptone and 0.5 g/L NaCl can be supplemented with 16.7 g/L NaBr or 24.4 g/L NaI.

[0135] Increasing the levels of serine, for example by providing a serine-rich nutrient source can also result in increased methionine production. See, e.g., WO 07/135188, incorporated by reference in its entirety).

[0136] The organisms can be maintained or cultivated under conditions that are conducive for methyl halide production. Many parameters such as headspace ratio, growth phase and oxygen levels can affect methyl halide production.

[0137] The invention contemplates culture conditions in which the organisms are in stationary phase or exponential (log) phase. Stationary phase is often suited for methyl halide production. Similarly, the invention also encompasses both aerobic and anaerobic growth of cultures. On occasion, aerobic growth is appropriate. Cell density can sometimes be increased (and nutrient concentrations can be also increased correspondingly) without impairing methyl halide production. Some host cells are maintained at elevated temperature (*e.g.*, 37°C) with agitation. In one approach, solid state fermentation is used (see, Mitchell et al., SOLID-STATE FERMENTATION BIOREACTORS, 2006, Springer). Aerobic or anaerobic conditions may be selected, depending in part on the organism and strain.

[0138] The ratio of headspace gas (air) per liquid culture volume can be optimized according to the invention using Henry's law. It has been determined that the optimum ratio is generally about 0.5:1 to 4:1, for example about 2:1.

[0139] Methyl halides and non-halogenated organic molecules produced using methods of the invention are usually produced at an industrial scale, for example for production of biofuels suitable as petroleum substitutes. Accordingly, organisms comprising a S-adenosylmethionine (SAM)-dependent methyl halide transferase (MHT) may in some embodiments be cultivated in bioreactors having a liquid capacity of at least 10 liters, at least 50 liters, at least 100 liters, or at least 500 liters. Often a bioreactor with a liquid capacity of at least 1000 liters, at least 5,000 liters, or at least 10,000 liters, for example. Often the volume of cultivation medium in cultures of the invention is at least 10 liters, at least 25 liters, at least 50 liters, at least 100 liters, at least 500 liters, at least 1,000 liters, or at least 5,000 liters. Culture may be carried out as a batch fermentation, in a continuous culture bioreactor, or using other methods known in the art.

5.1 Co-culture of yeast and cellulolytic bacteria

[0140] In another aspect, the invention provides a method for production of any of a variety of biological or organic products using cellulosic feedstocks as the sole or primary carbon source. According to the method, a co-culture comprising a mesophyllic cellulolytic bacterium (e.g., *Actinotalea fermentans*) and a recombinant yeast (e.g., *S. cerevisiae*) is prepared. Cellulose (e.g., cellulose, microcrystalline cellulose, Avicel, a cellulosic feedstock) is provided as an energy source to the co-culture. Where reference is made herein to cellulose, it is contemplated that hemicellulose and/or lignin (other biomass components) may be used in addition to or in place of cellulose in certain embodiments. Often, as described herein, raw or partially processed cellulosic feedstock is used. The cellulose is then metabolized by the bacterium to produce products which serve as a carbon source for the yeast. The recombinant yeast is thus able to carry out metabolic processes in a co-culture fed with cellulose. In some embodiments the bacteria-yeast co-culture is maintained under aerobic conditions. In some embodiments the bacteria-yeast co-culture is maintained under anaerobic conditions.

[0141] In some embodiments the co-culture is a symbiotic co-culture. A symbiotic co-culture is one in which the yeast is dependent on the bacterium for carbon (i.e., in the form of compounds that are waste products of bacteria metabolism), and the bacterium is dependent on the yeast for metabolism of toxic waste products. That is, the accumulation of bacterial waste products, in the absence of the yeast symbiant inhibits growth or viability of the bacteria. Thus, for example a cellulolytic bacterium that (a) metabolizes cellulose to produce ethanol and (b) is subject to growth inhibition by ethanol may be used in a symbiotic co-culture with a yeast that metabolizes ethanol. As another example, a cellulolytic bacterium that (a) metabolizes cellulose to produce acetate and (b) is subject to growth inhibition by acetate may be used in a symbiotic co-culture with a yeast that metabolizes acetate. As another example, a cellulolytic bacterium that (a) metabolizes cellulose to produce lactate and (b) is subject to growth inhibition by lactate may be used in a symbiotic co-culture with a yeast that metabolizes lactate. These examples are for illustration and not to limit the invention. Moreover, in this context the term "dependent" does not necessarily imply absolute dependency, but may mean that growth or viability of the organism is higher or more stable in co-culture. A symbiotic

bacteria-yeast co-culture can be described as a mutually obligatory cooperative system, in which each organism is dependent upon the other for viability.

[0142] A large number of cellulolytic bacteria are suitable for use in co-culture. For a discussion of cellulolytic bacteria see, e.g., Lynd et al., 2002, Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev.* 66:506-77. In some embodiments the cellulolytic bacterium is a cellulomonas or actinotalea species. For illustration and not limitation, exemplary celluolytic bacteria include *Trichoderma harzianum*, *Trichoderma reesei*, *Cellulomonas uda*, *Cellulomonas flavigena*, *Cellulomonas cellulolyticum*, *Pseudomonas* species and *Thermomonospora* species. Bacteria capable of aerobic fermentation of cellulose to ethanol, acetate, or lactate are well suited for co-culture. Also well suited for co-culture are bacteria capable of aerobic fermentation of cellulose to succinate, citrate, formate or malate. In some embodiments bacteria capable of anaerobic fermentation of cellulose to ethanol, acetate, lactate succinate, citrate, formate or malate are used. Cellulosic bacteria may be recombinantly modified (e.g., to incorporate drug resistance markers, modify a synthetic pathway in the cell, etc..). In some embodiments cellulolytic bacteria are selected based on growth inhibition by the product of the bacterial metabolism of cellulose (e.g., growth inhibition by ethanol, acetate, lactate succinate, citrate, formate or malate). It will be appreciated that bacteria exhibiting such growth inhibition are particularly useful for symbiotic co-cultures. Cellulolytic bacteria exhibiting such growth inhibition may be identified by reference to the scientific literature or may be identified or selected in the laboratory. In some embodiments, recombinant techniques are used to render a particular type or stain of bacterial susceptible to such inhibition. Other desirable properties include rapid growth, the ability to grow under either aerobic or anaerobic conditions, and the ability to secrete a significant portion of the carbon derived from cellulose (e.g., at least about 20%, preferably at least about 40%, most preferably at least about 50% under one or both of aerobic and anaerobic conditions). In some embodiments the bacteria is not a *Lactobacillus* species. In some embodiments the bacteria is not *Lactobacillus kefiranofaciens*.

[0143] In one embodiment the bacterium is *Actinotalea fermnentans. A. fermentans* is available from the American Type Culture Collection (ATCC 43279) and was previously referred to as Cellulomonas fermentans (see Yi et al., 2007, "Demequina aestuarii gen. nov., sp. nov., a novel actinomycete of the suborder Micrococcineae,

and reclassification of Cellulomonas fermentans Bagnara et al. 1985 as Actinotalea fermentans gen. nov., comb. nov." *Int J Syst Evol Microbiol* 57(Pt 1):151-6; also see Bagnara et al., 1987, Physiological properties of Cellulomonas fermentans, a mesophilic cellulolytic bacterium. *Appl. Microbiol. Biotechnol.* 26:170-176, 1987). *A. fermentans* metabolizes cellulose to produce acetate and ethanol.

[0144] Similarly, a variety of yeast strains and species may be used. In one embodiment the yeast is *S. cerevisiae (*e.g., *S. cerevisiae* W303a). In other embodiments another yeast species is used (e.g., Pichia pastoris, Hansenula polymorpha, Kluyveromyces lactis, Yarrowia lipolytica, Sacharomyces, and Scizosacchromyces pombe).

[0145] The co-culture may comprise any combination of cellulolytic bacteria and yeast so long as the products of bacterial metabolism of cellulose can be used as a energy and carbon source by the yeast. In one embodiment the metabolism of cellulose by the bacterium produces secreted acetate and/or ethanol. Other end products of cellulosic bacteria include secreted lactate, succinate, citrate, malate, formate and other organic molecules (typically having 1-6 carbon atoms).

[0146] In one embodiment the cellulosic bacterium is *A. fermnentans and the yeast is S. cerevisiae*.

[0147] Usually the yeast is recombinantly engineered to produce a product of interest. For example, *S. cerevisiae* may be modified to express Batis Maritima MHT. Co-cultures with yeast engineered to express MHT may be used to produce may be methylhalide, as described in the examples. However, co-culture may be applied in many other applications. That is, given any yeast recombinantly modified to produce a product of interest, the product may be produced using a co-culture of the yeast and cellulosic bacterium in the presence of a cellulose source and any substrates required by the yeast to produce the product. The yeast product may be a drug, food product, amino acid, cofactor, hormone, proteins, vitamin, lipid, industrial enzyme or the like. Examples of products produced by recombinant yeast include small molecule drugs (see, e.g., Ro et al., 2006 "Production of the antimalarial drug precursor artemisinic acid in engineered yeast" *Nature* 440(7086):940-3; petrochemical building blocks (see, e.g., Pirkov et al., 2008, "Ethylene production by metabolic engineering of the yeast Saccharomyces cerevisiae" *Metab Eng.* 10(5):276-80; commercially or medically useful proteins (see, e.g., Gerngross et al., 2004, "Advances in the production of human therapeutic

proteins in yeasts and filamentous fungi" *Nat Biotechnol* ;22(11):1409-14). Exemplary medically useful proteins include insulin, hepatitis B antigen, desirudin, lepidurin, and glucagon. For other examples see Porro et al., 2005, "Recombinant protein production in yeasts" *Mol Biotechnol.* 31(3):245-59. Other examples of commercially valuable compounds that may be produced by the yeast in the co-cultures of the invention include, but are not limited to, 1,4 diacids (succinic, fumaric and malic); 2,5 furan dicarboxylic acid; 3 hydroxy propionic acid; aspartic acid; glucaric acid; glutamic acid; itaconic acid; levulinic acid; 3-hydroxybutyrolactone; Glycerol; Sorbitol; xylitol/arabinitol; gluconic acid; lactic acid; malonic acid; propionic acid; the triacids (citric and aconitic); xylonic acid; acetoin; furfural; levoglucosan; lysine; serine; threonine, valine and S-adenosylmethionine. Still others include 3 Glycerol, 3 hydroxypropionic acid, lactic acid, malonic acid, propionic acid, Serine; 4 Acetoin, aspartic acid, fumaric acid, 3-hydroxybutyrolactone, malic acid, succinic acid, threonine; 5 Arabinitol, furfural, glutamic acid, itaconic acid, levulinic acid, proline, xylitol, xylonic acid; Aconitic acid, citric acid, and 2,5 furan dicarboxylic acid. See Werpy et al., 2004, "TOP VALUE ADDED CHEMICALS FROM BIOMASS VOLUME I — RESULTS OF SCREENING FOR POTENTIAL CANDIDATES FROM SUGARS AND SYNTHESIS GAS" published by the Department of Energy Washington D.C. Also see the Biomass Document Database at http://www1. *followed by* eere.energy.gov/biomass/publications. Html, incorporated herein by reference in its entirety. Methods for genetically modifying yeast so that they produce desired products are known in the art or may be developed.

[0148] In one aspect the invention includes the further step of collecting or harvesting the product of interest produced by the yeast cells. In one embodiment the product of interest is a small molecule compound with a molecular weight less than 1000.

[0149] Typically and most conveniently, the bacteria and yeast components of the co-culture are grown together (comingled) in the liquid cultivation medium. In some embodiments, however, the co-cultured organisms can be, for example, maintained in separate compartments of a bioreactor, separated by a permeable membrane that allows metabolites and other molecules to diffuse between compartments. A wide variety of suitable bioreactors are known in the art.

[0150] In addition to cellulose, hemicellulose, lignin, biomass, feedstock or the like, which may be added, cultivation or growth media for use in coculture will include appropriate amounts of nitrogen and sulfur sources, *e.g.*, in the form of one or more

sulfates (such as ammonium sulfate) and/or thiosulfates. The medium can also contain vitamins such as vitamin B12. YP media may be used (Bacto-yeast extract (Difco) 10 gram, Bacto-peptone (Difco) 20 gram, ddH2O to 900 ml). Methods of optimizing cultivation conditions may be determined using art known techniques. See, e.g., Ball, A.S., 1997, BACTERIAL CELL CULTURE: ESSENTIAL DATA John Wiley & Sons; Richmond, A., 2003, HANDBOOK OF MICROALGAL CULTURE Wiley-Blackwell; Becker, E.W., 1994, MICROALGAE: BIOTECHNOLOGY AND MICROBIOLOGY Cambridge University Press; and Walker, G.M., 1998, YEAST PHYSIOLOGY AND BIOTECHNOLOGY John Wiley & Sons.

[0151] The invention provides a bacteria-yeast co-culture in which the bacteria metabolizes cellulose and produce one or more metabolic products, and the yeast uses the metabolic products of the bacterium as a carbon source. In some embodiments the microorganisms adapted to grow together while maintaining a relatively constant ratio of species populations such that neither microorganism overtakes the other. In bacteria-yeast co-cultures of the type described below in Section 5.1, we typically observed 100-fold excess of bacteria over yeast (approximately 1 million viable yeast cells and 100 million viable bacterial cells per milliliter).

5.1.1 Co-culture of MHT-Expressing *S. cerevisiae* and *Actinotalea fermentans*

[0152] Methyl iodide production in yeast offers several advantages over existing building block molecules, including compatibility with industrial processes. However, the production of biofuels and bio-based building blocks from food crop derived sugars (such as corn and sugarcane) may directly contribute to global food shortages. To mitigate these problems, methyl iodide (and other bio-based molecules) must be derived from cellulosic feedstocks, which include "energy crops" such as switchgrass (*Panicum virgatum*) and elephant grass (*Miscanthus giganteus*) as well as agricultural wastes such as corn stover. The conversion of these real-world biomass sources to fermentable sugars and products is problematic due to the recalcitrance of lignocellulosic materials to microbial digestion.

[0153] We constructed a co-culture of MHT-expressing yeast (as described above) with a mesophyllic cellulolytic bacterium, *Actinotalea fermentans*. *A. fermentans*

ferments cellulose to acetate and ethanol aerobically, which *S. cerevisiae* are able to utilize as a carbon source. Importantly, *A. fermentans* growth is inhibited by accumulation of acetate and ethanol, creating a metabolic interdependence in the community, with *S. cerevisiae* dependent on *A. fermentans* for carbon, and *A. fermentans* dependent on *S. cerevisiae* for metabolism of toxic waste products (Fig. 9A). We inoculated *S. cerevisiae* with *A. fermentans* in media containing carboxymethylcellulose as the sole carbon source and measured the change in yeast and bacterium colony forming units (CFU) over time. Yeast grown in co-culture for 36 hours increase to 10^6 cfu/ml, where yeast without the cellulolytic partner show little growth (Figure 9B, left panel). The presence of yeast also increases the growth rate of the bacterium by consuming toxic components (Figure 9B, right panel). This interaction demonstrates a symbiotic relationship.

[0154] We next tested the co-culture conversion of cellulosic feedstocks to methyl iodide. We inoculated the co-culture at low density on media containing pulverized dry switchgrass as the sole carbon source. At 36 hours after inoculation, sodium iodide was added to the medium to induce methyl iodide production. Methyl iodide yields on various cellulosic sources, including switchgrass, corn stover, and poplar are shown in Figure 9C. Acetate is included as a non-fermentable carbon source reference and carboxymethylcellulose (CMC) is included as a cellulose standard. Energy crops such as switchgrass offer several advantages over conventional crops by requiring fewer agricultural inputs and by growing on marginal land, or by exhibiting extraordinary growth or genetic tractability (e.g., poplar). Agricultural residues such as corn (*Zea mays*) stover are another source of cellulosic carbon, with approximately 200 mg of stover produced in the United States each year. The results show that methyl iodide can be produced from a variety of cellulosic carbon sources.

[0155] Thus the invention provides a method for production of methyhalide comprising culturing a first microorganism which metabolizes cellulose and produces one or more metabolic products together with a second microorganism which does not metabolize cellulose and which is recombinantly modified to express a heterologous methyl halide transferase protein in a medium containing cellulose and a halide (e.g., chlorine, bromine and iodine) under conditions in which methyl halide is produced.

6. COLLECTION AND PURIFICATION OF METHYL HALIDE

[0156] Methyl halides are volatile and escape into the vapor above the liquid culture. On a production scale this is advantageous over, for example, other biofuel intermediates because relatively little extra energy is required for purification of methyl halides, if so desired. In one embodiment, the methyl halide can be collected before conversion to one or more non-halogenated organic molecules. In another embodiment, the collection step is omitted, for example when the same organisms that produce methyl halide also convert the methyl halide to organic molecules.

[0157] Cultivation, collection of methyl halide, and/or conversion of methyl halide to organic compounds such as higher-molecular weight compounds (below) can be carried out in a reactor system. Methods for chemical processing and bioreactor systems are known in the art and can be readily adapted to the present invention. For illustration and not limitation, guidance is found in the scientific and engineering literature, e.g., McKetta, J., CHEMICAL PROCESSING HANDBOOK, 1993, Marcel Dekker; Lee, S., ENCYCLOPEDIA OF CHEMICAL PROCESSING, 2006, Taylor and Francis Group; Asenjo, J., BIOREACTOR SYSTEM DESIGN, 1995, Marcel Dekker; Nielsen, J., BIOREACTION ENGINEERING PRINCIPLES, 2003, Kluwer Academics; Crow et al., "Process for manufacturing methyl chloride," US Pat. No. 6111153; Van 't Riet and Tramper, 1991, BASIC BIOREACTOR DESIGN, CRC Press; Asenjo and Merchuk, 1995, BIOREACTOR SYSTEM DESIGN, CRC Press; and Narita et al., "Preparation of methyl chloride," US Pat. No. 5917099, each of which is incorporated herein by reference. For illustration and not limitation, one reactor system is shown in Figure 9. Volatile methyl halide can be collected by any known method from the fermenter by transferring methyl halide that is produced in gaseous form to a condenser. In the condenser, the temperature of the gas comprising methyl halide can be lowered, for example resulting in the liquefaction of methyl halides but not other gaseous components, allowing for easy purification. Catalytic condensation or other reactions can take place in a reactor. Halide salts, generated as a by-product of the condensation reaction, can be recycled, *e.g.*, by introducing back into the fermenter.

[0158] Gas phase production can be easily measured by, for example by gas chromatography mass spectroscopy, which determines the number of methyl halide molecules produced. The total amount of methyl halides produced can be calculated using Henry's Law.

7. PROCESSING OF METHYL HALIDES INTO ORGANIC MOLECULES

[0159] The methyl halides can be converted to organic products such as alcohols, alkanes, (ethane-octane or longer), ethers, aldehydes, alkenes, olefins, and silicone polymers. These products in turn can be used to make a very wide range of petrochemical products, sometimes referred to as "biofuels." The use of alkyl halides, including methyl halides, in the production of more complex organic compounds is known in the conventional petrochemical industry. See, e.g., Osterwalder and Stark, 2007, Direct coupling of bromine-mediated methane activation and carbon-deposit gasification, *Chemphyschem* 8: 297-303; Osterwalder and Stark, 2007, "Production of saturated C2 to C5 hydrocarbons" European patent application EP 1 837 320.

[0160] Conversion can be achieved by a variety of known methods, including biological conversion (*e.g.*, through the use of biological organisms that can convert the methyl halide into non-halogenated organic molecules, for example through the action of one or more enzymes). If so desired, the conversion can be carried out in the same reactor or vessel in which the organism(s) that produce methyl halide are maintained. The conversion can be carried out by the same organisms that produce methyl halide or by different organisms, present within the same reactor or segregated in a different compartment or reactor. An organism can be modified to produce or convert (or both produce and convert) methyl halide to a greater rate or extent than an unmodified organism. When conversion is achieved by the same organisms that produce methyl halide, the collection of methyl halide can optionally be omitted. Both production and conversion can optionally be carried out in the same vessel or reactor.

[0161] The methyl halides can be converted to various organic molecules by the use of chemical catalysts. Depending on the choice of substrates (chemical catalyst used and/or methyl halide) as well as adjustment of different variables such as temperature, (partial) pressure and catalyst pre-treatment, various organic products can be obtained. For example, the use of a metal oxide catalyst can result in the production of higher alkanes. The use of an $AlBr_3$ catalyst can result in the production of propane. If the desired product is an alcohol, an ether or an aldehyde, the methyl halide can be passed over a specific metal oxide that is selected based upon its selectivity to produce the desired functionality (*i.e.* alcohol, ether or

aldehyde). Should the desired product selectivity be affected by the amount of water present in the reaction between the alkyl monohalide and the metal oxide, water can be added to the alkyl monobromide feed to the appropriate level.

[0162] The use of a zeolite catalyst can result in the production of olefins. Examples of zeolites include naturally-occurring zeolites such as Amicite, Analcime, Barrerite, Bellbergite, Bikitaite, Boggsite, Brewsterite, Chabazite, Clinoptilolite, Cowlesite, Dachiardite, Edingtonite, Epistilbite, Erionite, Faujasite, Ferrierite, Garronite, Gismondine, Gmelinite, Gobbinsite, Gonnardite, Goosecreekite, Harmotome, Herschelite, Heulandite, Laumontite, Levyne, Maricopaite, Mazzite, Merlinoite, Mesolite, Montesommaite, Mordenite, Natrolite, Offretite, Paranatrolite, Paulingite, Pentasil, Perlialite, Phillipsite, Pollucite, Scolecite, Sodium Dachiardite, Stellerite, Stilbite, Tetranatrolite, Thomsonite, Tschernichite, Wairakite, Wellsite, Willhendersonite, and Yugawaralite. Synthetic zeolites can also be used. The use of zeolites to generate from methyl halides are well known in the art. *See*, *e.g.*, Svelle *et al.*, 2006, *Journal of Catalysis*, 241:243-54, and Millar et al., 1995, US Pat. No. 5,397,560, both incorporated by reference in its entirety, discussing the use of a zeolite to produce hydrocarbon-type products, including alkenes such as ethene, propene and butenes, as well as ethylbenzenes and higher aromatics.

[0163] In addition to being a useful intermediate in the commercial manufacture of organic molecules, the methyl halide have various other uses, for example as a solvent in the manufacture of butyl rubber and in petroleum refining, as a methylating and/or halidating agent in organic chemistry, as an extractant for greases, oils and resins, as a propellant and blowing agent in polystyrene foam production, as a local anesthetic, as an intermediate in drug manufacturing, as a catalyst carrier in low temperature polymerization, as a fluid for thermometric and thermostatic equipment and as a herbicide.

8. EXAMPLES

[0164] The following examples are for illustrative purposes only and are not intended to be limiting.

Example 1. Expressing Batis Maritima MHT cDNA in E. coli.

[0165] Batis Maritima MHT cDNA (Genbank Acc. No. AF109128 or AF084829) was artificially synthesized and cloned into an expression vector pTRC99a.

[0166] The resulting *E. coli* (strain DH10B) comprising the expression construct encoding *Batis maritima* MHT under the control of an IPTG inducible promoter is referred to as the "*E. coli*-MHT_{Batis}" strain.

Example 2: Measuring Methyl Halide Production

[0167] Methyl halide production can be measured by gas chromatography. In the experiments described below an Agilent gas chromatography/mass spectrometry (GC/MS) system was used. Most often the "AIR.U" tune file, uses an ionization voltage of 1341. In some experiments an ionization voltage of about 1250 was used. A solvent delay of 0 was set and the scan parameters set to 15-100 MW. The injection port and column were preset to 50°C. The sample to be tested was mixed by shaking for a few seconds. 100µL of the headspace gas was extracted with a gas-tight syringe. The sample gas was manually injected into the GCMS injection port. The GCMS program was started with the following settings: 1:00 at 50° C; a ramp of 10° C per min to 70° C (the sample typically came off at ~52° C); 1:00 at 70° C. The column was then cleaned (ramp to 240° C for 2 minutes). The sample peak was identified by extracting the GC peak corresponding to 50MW (-0.3,+0.7). This peak was integrated to produce the "GC 50MW" data.

Example 3: Methyl halide production by recombinant E. coli expressing *Batis Maritima* methyl halide transferase

[0168] *E. coli* (strains DH10B, BL21, or MC1061) and Salmonella (SL 1344) was transformed with a plasmid encoding a codon-optimized methyl chloride transferase gene MCT from *Batis Maritima* as described in Example 1. 10mL of LB media with 1mM IPTG was inoculated with a single colony of plated cells in a 16mL culture tube. The tube was then sealed with parafilm and aluminum foil cinched with a rubber band. The cultures were incubated at 37°C while shaking for 4-22 hours and methyl halide production measured. Each of the strains produced methylchloride.

[0169] In addition, the results were found to be highly reproducible. Repeat tests using 5 different clones of one *Batis maritima* MHT enzyme in *E. coli* (strain DH10B) resulted in methyl chloride production in each with a standard deviation of about 12% of the average methyl halide production.

Example 4: Production of methyl halide follows an induction curve seen with other IPTG-inducible constructs

[0170] *E. coli* (strain DH10B) transformed with a plasmid encoding a codon-optimized methyl chloride transferase gene MCT from *Batis Maritima* as described in Example 1 was incubated in the presence of inducer (IPTG). As shown in Figure 1, increasing IPTG levels resulted in increased methylchloride production

[0171] As shown in Figure 2, methyl halide production increased linearly with time in the inducing media up to about 1 to 2.5 hours after induction.

[0172] As shown in Figure 3, cells at stationary phase produced more methyl halide than cells in growth phase. Artificially doubling the density of the culture did not increase production of methylhalide if the concentration of nutrients was not increased.

[0173] Methyl halide production was compared between aerobic and anaerobic culture conditions. Aerobic conditions resulted in higher levels of methyl halide cultures.

Example 5: Effect of salt concentration in the cultivation medium

[0174] *E. coli*-MHT_{Batis} cells were grown in modified Luria-Bertani (LB) media in which the NaCl concentration was varied. Normal LB medium contains 5 g/L yeast extract, 10 g/L Tryptone, 10 g/L NaCl (0.171 M NaCl), at pH 7. Methyl chloride production in LB and modified LB containing 0.85 or 0.017 M NaCl was tested. Results are summarized in Figure 4. 0.085 M NaCl produced the best results. However, normal LB was near optimal.

[0175] Modified Luria-Bertani media with bromine or iodine counter ions were at 0.16 M were made as shown in Table 3.

Table 3

	LB-NaBr	LB-NaI
Yeast Extract	5 g/L	5 g/L
Tryptone	10 g/L	10 g/L
NaCl	0.5 g/L	0.5 g/L
NaBr	16.7 g/L	0
NaI	0	24.4 g/L

Example 6: Effect of different halides

[0176] To compare methyl halide production using different salts halides, a standardized assay was devised. 20 mL of LB was inoculated with a single colony of plated cells, and was incubated at 37°C while shaking for about 10-14 hours. The cells were pelleted and resuspended in LB. Equal aliquots were added to 10 mL LB, LB-Br or LB-I media with IPTG and incubated for 1.5 hours. 100 µL of headspace gas was taken and the amounts of methyl halide present measured as in Example 2.

[0177] As shown in Figure 5 the higher molecular weight halides had higher methyl halide yield, with iodine ion giving the greatest yield, followed by bromine ion and chlorine ion. Using Henry's Law to calculate the total gas produced (dissolved in culture and present in the headspace), the production rate of methyl iodide was calculated to be about 40 (specifically, 43) mg/L per day.

Example 7: Methyl halide production in *E. coli* cells expressing heterologous MHT and overexpressing *E. coli metK*

[0178] The effect on methyl halide production by over-expression of certain accessory proteins was tested. The *E. coli*-MHT_{Batis} strain was transformed with plasmids encoding *E. coli metK*, *E. coli clcA*, or *E. coli vgb* genes. Cells were cultured and methyl chloride production was measured. As shown in Figure 6, overexpression of *metK* improved yield of methyl chloride. Under the conditions used, the expression of *vgb* and *clcA* caused general toxicity.

Example 8: Effect of Heterologous MHT Expression in E. coli

[0179] Nineteen methyl halide transferase genes from various organisms were codon-optimized and introduced into *E. Coli*. Production of methyl bromide and methyl iodide was determined for each. As shown in Table 5, the genes were from *Batis maritima*, *Burkholderia phymatum* STM815, *Synechococcus elongatus* PCC 6301, *Brassica rapa subsp. chinensis*; *Brassica oleracea* TM1, *Brassica oleracea* TM2; *Arabidopsis thaliana* TM1; *Arabidopsis thaliana* TM2; *Leptospirillum sp.* Group II UBA; *Cryptococcus neoformans var. neoformans* JEC21; *Oryza sativa* (japonica cultivar-group); *Ostreococcus tauri*; *Dechloromonas aromatica* RCB; *Coprinopsis cinerea okayama*; *Robiginitalea bofirmata* HTCC2501; *Maricaulis maris* MCS10;

Flavobacteria bacterium BBFL7; *Vitis vinifera* and; *halorhodospira halophila* SL1. The MHT sequences are shown in Table 4. Table 5 shows the level of amino acid identity with the *Batis maritima* protein.

TABLE 4

BATIS MARITIMA
MSTVANIAPVFTGDCKTIPTPEECATFLYKVVNSGGWEKCWVEEVIPWDLGVPTPLVLHLVKNNALP
NGKGLVPGCGGGYDVVAMANPERFMVGLDISENALKKARETFSTMPNSSCFSFVKEDVFTWRPEQPF
DFIFDYVFFCAIDPKMRPAWGKAMYELLKPDGELITLMYPITNHEGGPPFSVSESEYEKVLVPLGFK
QLSLEDYSDLAVEPRKGKEKLARWKKMNN

BURKHOLDERIA PHYMATUM STM815 (29% IDENTICAL TO BATIS)
MSDKRPSVPPSAPDFENRDPNAPGFWDERFGRGFTPWDQAGVPPAFKAFVERHSPVPVLIPGCGSAY
EARWLAEKGWTVRAIDFAPNAVEAARAQLGSHASLVHEADFFTYRPPFDPGWIYERAFLCALPPARR
SDWVARMAQLLSPGGLLAGFFFIGATEKGPPFGIERAELDALMSPDFTLVEDEPVDDSIAVFAGRER
WLTWRRRGAARG

SYNECHOCOCCUS ELONGATUS PCC 6301
MTNAVNQAQFWEQRYQEGSDRWDLGQAAPVWRSLLAGTNAPAPGRIAVLGCGRGHDARLFAEQGFEV
VGFDFAPSAIAAAQALAQGTTAQFLQRDIFALPQEFAGQFDTVLEHTCFCAIDPDRRAEYVEVVRQI
LKPKGCLLGLFWCHDRPSGPPYGCSLTELRDRFAQGWQEEQLESVTESVEGRRGEEYLGRWRRLD

BRASSICA RAPA SUBSP. CHINENSIS
MAEVQQNSAHINGENIIPPEDVAKFLPKTVEEGGWEKCWEDGVTPWDQGRATPLVVHLVESSSLPLG
RALVPGCGGGHDVVAMASPERYVVGLDISESALEKAAETYGSSPKAKYFTFVKEDFFTWRPNELFDL
IFDYVVFCAIEPETRPAWAKAMYELLKPDGELITLMYPITDHDGGPPYKVAFSTYEDVLVPVGFKAV
SIEENPYSIATRKGKEKLARWKKIN

BRASSICA OLERACEA (TM1)
MAEEQQKAGHSNGENIIPPEEVAKFLPETVEEGGWEKCWEDGITPWDQGRATPLVVHLVDSSSLPLG
RALVPGCGGGHDVVAMASPERFVVGLDISESALEKAAETYGSSPKAKYFTFVKEDFFTWRPNELFDL
IFDYVVFCAIEPEMRPAWAKSMYELLKPDGELITLMYPITDHDGGPPYKVAVSTYEDVLVPVGFKAV
SIEENPYSIATRKGKEKLGRWKKIN

BRASSICA OLERACEA (TM2)
MAEVQQNSGNSNGENIIPPEDVAKFLPKTVDEGGWEKCWEDGVTPWDQGRATPLVVHLVESSSLPLG
RGLVPGCGGGHDVVAMASPERYVVGLDISESALEKAAETYGSSPKAKYFTFVKEDFFTWRPNELFDL
IFDYVVFCAIEPETRPAWAKAMYELLKPDGELITLMYPITDHDGGPPYKVAVSTYEDVLVPVGFKAV
SIEENPYSIATRKGKEKLARWKKIN

ARABIDOPSIS THALIANA TM1
MAEEQQNSSYSIGGNILPTPEEAATFQPQVVAEGGWDKCWEDGVTPWDQGRATPLILHLLDSSALPL
GRTLVPGCGGGHDVVAMASPERFVVGLDISDKALNKANETYGSSPKAEYFSFVKEDVFTWRPNELFD
LIFDYVFFCAIEPEMRPAWGKSMHELLKPDGELITLMYPMTDHEGGAPYKVALSSYEDVLVPVGFKA
VSVEENPDSIPTRKGKEKLARWKKIN

ARABIDOPSIS THALIANA TM2
MAEEQQNSDQSNGGNVIPTPEEVATFLHKTVEEGGWEKCWEEEITPWDQGRATPLIVHLVDTSSLPL
GRALVPGCGGGHDVVAMASPERFVVGLDISESALAKANETYGSSPKAEYFSFVKEDVFTWRPTELFD
LIFDYVFFCAIEPEMRPAWAKSMYELLKPDGELITLMYPITDHVGGPPYKVDVSTFEEVLVPIGFKA
VSVEENPHAIPTRQREAGKVEEDQLIPKKEILLFGKSVICVIYKE

LEPTOSPIRILLUM SP. GROUP II UBA
MPDKIFWNQRYLDKNTGWDLGQPAPPFVRLVEKGEFGPPGRVLIPGAGRSYEGIFLASRGYDVTCVD
FAPQAVREAREAARQAGVKLTVVEEDFFRLDPRTIGVFDYLVEHTCFCAIDPPMRQAYVDQSHALLA
PGGLLIGLFYAHGREGGPPWTTTEEEVRGLFGKKFDLLSLGLTDWSVDSRKGEELLGRLRRKNDRIE

CRYPTOCOCCUS NEOFORMANS VAR. NEOFORMANS JEC21 (HYPOTHETICAL PROTEIN)
MAQASGDDNAWEERWAQGRTAFDQSAAHPVFVKFLKSDIARELGVPKSGKALVPGCGRGYDVHLLAS
TGLDAIGLDLAPTGVEAARRWIGSQPSTSGKADILVQDFFTYDPLEKFDLIYDYTFLCALPPSLRQE
WARQTTHLANIAADTNPILITLMYPLPPSAKSGGPPFALSEEIYQELLKEQGWKMVWSEDIEEPTRM
VGAPGGEKLAVWKRI

ORYZA SATIVA (JAPONICA CULTIVAR-GROUP)
MASAIVDVAGGGRQQALDGSNPAVARLRQLIGGGQESSDGWSRCWEEGVTPWDLGQRTPAVVELVHS
GTLPAGDATTVLVPGCGAGYDVVALSGPGRFVVGLDICDTAIQKAKQLSAAAAAAADGGDGSSSFFA
FVADDFFTWEPPEPFHLIFDYTFFCALHPSMRPAWAKRMADLLRPDGELITLMYLAEGQEAGPPFNT
TVLDYKEVLNPLGLVITSIEDNEVAVEPRKGMEKIARWKRMTKSD

OSTREOCOCCUS TAURI (UNNAMED PROTEIN PRODUCT)
MTTSSAPTRHTSMRVALAAPATVTRRLGTYKRVFDRRAMSTRAIDGAVTSNAGDFARQDGSTDWEGM
WSRGITKGAAFDCSRTEPAFQNALDAKEIAIGSGRALVPGCGRGYALASLARAGFGDVVGLEISETA
KEACEEQLKAESIPETARVEVVVADFFAYDPKEAFDAAYDCTFLCAIDPRRREEWARKHASLIKPGG
TLVCLVFPVGDFEGGPPYALTPEIVRELLAPAGFEEIELRETPAEMYARGRLEYLFTWRRRS

DECHLOROMONAS AROMATICA RCB
MSETIKPPEQRPEHPDFWCKRFGEGVTPWDAGKVPMAFVDFVGAQTTPLNSLIPGCGSAWEAAHLAE
LGWPVTALDFSPLAIEKAREVLGDSPVKLVCADFFTFAPRQPLDLIYERAFLCALPRKLWADWGKQV
AELLPSGARLAGFFFLCDQPKGPPFGILPAQLDELLRPNFELIEDQPVGDSVPVFAGRERWQVWRRR

COPRINOPSIS CINEREA OKAYAMA (HYPOTHETICAL PROTEIN)
MADPNLAPEIRAKMQEIFKPDDRHSWDLLWKENITPWDAGDAQPSLIELIEESGLDFARKGRALVPG
CGTGYDAVYLASALGLQTIGMDISESAVEAANRYRDSSGVQGADRAIFQKADFFTYKVPDEERFDLI
MDHTFFCAIHPSLRPEWGQRMSELIKPGGYLITICFPMIPKVETGPPYYLRPEHYDEVLKETFEKVY
DKVPTKSSENHKDKERMLVWKKK

ROBIGINITALEA BIFORMATA HTCC2501
MTDLDRDFWEDRYRAGTDRWDLGGPSPPLTAYIDGLTDQELRILVPGAGRGYEAEYLYRAGFENLTI
VDLARRPLDDLRRRLPELPAAALQQTDFFSFRGGPFDLILEHTFFCALPPARRPDYVQAMHRLLVPG
GRLAGLFFDFPLTEDGPPFGGSETEYRNRFSSLFHIRKLERARNSIPPRAGTELFFIFEKK

MARICAULIS MARIS MCS10
MTHDENRSAFDWEARFIDGNTPWERGALHPAFEAWQHQSAFAAGDRALIPGCGRSPELLALAQAGLA
VTGADLSGTAMAWQRKLFADAGQQVELITGDVFDWQPQQALDLVYEQTFLCAIHPRLRTRYEEALAR
WLKPGGRLYALFMQKPERGGPPFDCALDAMRALFPAERWTWPAEADIQPWPHPQLNGKAELGAVLIR
R

FLAVOBACTERIA BACTERIUM BBFL7
MPLNKQYWEDRYKNNSTGWDLGIISTPIKEYVNQLENKNSKILIPGAGNAHEATYLVKNGFKNIFIL
DIALSPLKFAKQRSKLPEEHLIQQDFFDHKGSYDLIIEQTFFCALEPRFRESYVKKIHMLLRDQGCL
IGVLFNFENNLSSPPFGGSINEYLNLFEPYFEIVTMEPCNNSVIERQGKEIFIKLKKKK

VITIS VINIFERA
MASPDNTKPKARSSESVTGQRRGRRPSDRHWPCVGEESGSFYNTIADGERQYQHRIELRASKNKPSS
WEEKWQQGLTPWDLGKATPIIEHLHQAGALPNGRTLIPGCGRGYDVVAIACPERFVVGLDISDSAIK
KAKESSSSSWNASHFIFLKADFFTWNPTELFDLIIDYTFFCAIEPDMRPAWASRMQQLLKPDGELLT
LMFPISDHTGGPPYKVSIADYEKVLHPMRFKAVSIVDNEMAIGSRKKKYPLKPDLSLFGFVDRPKRA
YEARSEEFRISDWVCGWMGLCVPSGRISGGVCGLLSGRSLTWAKNLGVSTTQLRMSNNGSSIESNPK
VQKLNQIIGSDSAGGWEKSWQQGHTPWDLGKPTPIIQHLHQTGTLPSGKTLVPGCGCGYDVVTIACP
ERFVVGLDISDSAIKKAKEISDHAGGPPYKVSVADYEEVLHPMGFKAVSIVDNKMAIGPRKGREKLG
RWKRTPSKSLL

HALORHODOSPIRA HALOPHILA SL1
MSGDPDPRRAPWEARWREGRTGWDRGGVSPTLEAWLSAGVIPGRRVLVPGAGRGYEVEALARRGYKV
TAVDIAAEACQQLRDGLDAAGVEARVVQADLLAWQPDTPFDAVYEQTCLCALDPADWPAYEQRLYGW
LRPGGVLLALFMQTGASGGPPFHCALPEMATLFDSERWQWPAEPPRQWPHPSGRWEEAVRLLRR

Table 5

Abbreviation	Name	% aa identity
Batis	Batis maritima	100
BP	Burkholderia phymatum STM815	29
BR	Brassica rapa subsp. chinensis	65

SE	Synechococcus elongatus PCC 6301	30
BO-1	Brassica oleracea TM1	65
BO-2	Brassica oleracea TM2	64
LS	Leptospirillum sp. Group II UBA	34
AT-1	Arabidopsis thaliana TM1	69
CN	Cryptococcus neoformans var. neoformans JEC21	33
OS	Oryza sativa (japonica cultivar-group)	58
OT	Ostreococcus tauri	33
DA	Dechloromonas aromatica RCB	30
CC	Coprinopsis cinerea okayama	36
RB	Robiginitalea bofirmata HTCC2501	32
MM	Maricaulis maris MCS10	30
AT-2	Arabidopsis thaliana TM2	67
FB	Flavobacteria bacterium BBFL7	28
VV	Vitis vinifera	59
HH	Halorhodospira halophila SL1	28

[0180] Cells were cultured as follows:

[0181] For each strain a single colony was picked and grown overnight (10-14hrs) in 20mL of LB miller in a 30mL glass test tube with aeration (a loose cap) at 37C and 250 rpm shaking. The culture was spun down in a swinging bucket centrifuge for 5 min @ 3000xg. The cells were resuspended in 20mL of appropriate media (10g/L Tryptone, 5g/L Yeast Extract, 165mM NaX [where X = Cl, Br, I]) containing 100uM IPTG inducer. The cells were sealed with rubber stoppers and parafilm and grown at 37C with 250 rpm shaking for 1.5 hours. Cultures were taken to the GC/MS and 100uL of headspace gas was sampled and loaded onto the column. The method run was VOIGT.m. The number of counts for the appropriate mass (MeCl, MeBr, MeI) were reported. Cells were always grown in the presence of 30ug/mL chloramphenicol.

[0182] Methyl halide production was measured as described in the previous Examples. The results are summarized in Fig. 7. The *B. maritima* transferase was found to give the best methyl bromide production, while the *B. phymatum* transferase gave the best methyl bromide production in bacteria. *C. neoformans* JEC21 gave the best methyl bromide and methyl iodide production. *Leptospirillum* gave the best

methyl iodide production. Enzymes from *O. sativa*, O. *tauri*; *D. aromatica*, and *C. cinerea* showed significant specificity for methyl iodide production. *B. maritima*, *Brassica rapa* subsp. chinensis and *B. oleracea* show significant specificity for methyl bromide production. The enzymes RB, MM, AT-2, FB, VV and HH in Table 5 showed insignificant activity.

Example 9A: Identifying New Methyl Halide Transferases

[0183] Proteins with MHT activity (including proteins not previously known to have this activity) were identified through a BLAST protein-protein search for proteins having sequence identity with known MHTs such as from the MHT from Batis maritima. A cutoff of ~28% identity was assigned based on a 29% identity between Batis maritima and Burkholderia phymatum MHT sequences. Each identified sequence was BLASTed back to the database and a new list was generated. This was repeated until no additional sequences were found. Table 6 sets forth the sequences (and corresponding GenBank accession numbers) that have been identified as having MHT activity, including proteins that were hitherto not recognized to have MHT activity. Many of the newly identified proteins are thiopurine s-methyltransferases.

TABLE 6

> BATIS SEQ
MSTVANIAPVFTGDCKTIPTPEECATFLYKVVNSGGWEKCWVEEVIPWDLGVPTPLVLH
LVKNNALPNGKGLVPGCGGGYDVVAMANPERFMVGLDISENALKKARETFSTMPNSSCF
SFVKEDVFTWRPEQPFDFIFDYVFFCAIDPKMRPAWGKAMYELLKPDGELITLMYPITN
HEGGPPFSVSESEYEKVLVPLGFKQLSLEDYSDLAVEPRKGKEKLARWKKMNN

>GI|30689545|REF|NP_850403.1| THIOL METHYLTRANSFERASE,
PUTATIVE [ARABIDOPSIS THALIANA]
MENAGKATSLQSSRDLFHRLMSENSSGGWEKSWEAGATPWDLGKPTPVIAHLVETGSLP
NGRALVPGCGTGYDVVAMASPDRHVVGLDISKTAVERSTKKFSTLPNAKYFSFLSEDFF
TWEPAEKFDLIFDYTFFCAFEPGVRPLWAQRMEKLLKPGGELITLMFPIDERSGGPPYE
VSVSEYEKVLIPLGFEAISIVDNELAVGPRKGMEKLGRWKKSSTFHSTL

>GI|157353829|EMB|CAO46361.1| UNNAMED PROTEIN PRODUCT
[VITIS VINIFERA]
MANDSTSIESNSELQKISQVIGSGFNGSWEEKWQQGLTPWDLGKATPIIEHLHQAGALP
NGRTLIPGCGRGYDVVAIACPERFVVGLDISDSAIKKAKESSSSSWNASHFIFLKADFF
TWNPTELFDLIIDYTFFCAIEPDMRPAWASRMQQLLKPDGELLTLMFPISDHTGGPPYK
VSIADYEKVLHPMRFKAVSIVDNEMAIGSRKGREKLGRWKRTDEPLL

>GI|157353828|EMB|CAO46360.1| UNNAMED PROTEIN PRODUCT [VITIS VINIFERA]
MGLCVPSGRISGGVCGLLSGRSLTWAKNLGVSTTQLRMSNNGSSIESNPKVQKLNQIIG
SDSAGGWEKSWQQGHTPWDLGKPTPIIQHLHQTGTLPSGKTLVPGCGCGYDVVTIACPE
RFVVGLDISDSAIKKAKELSSSLWNANHFTFLKEDFFTWNPTELFDLIFDYTFFCAIEP
DMRSVWAKRMRHLLKPDGELLTLMFPISDHAGGPPYKVSVADYEEVLHPMGFKAVSIVD
NKMAIGPRKGREKLGRWKRTPSKSLL

>GI|125554131|GB|EAY99736.1| HYPOTHETICAL PROTEIN OSI_020969 [ORYZA SATIVA (INDICA CULTIVAR-GROUP)]
MDRALPLALSVSLWWLLVGDLGGRWTLEDDGGGGGVSRFGSWYRMCGWWWVWADWIIEL
GASSWGNLFGLVLKRRKNEAVERDSSDGWEKSWEAAVTPWDLGKPTPIIEHLVKSGTLP
KGRALGYDVVALASPERFVVGLGISSTAVEKAKQWSSSLPNADCFTFLADDFFKWKPSE
QFDLIFDYTFFCALDPSLRLAWAETVSGLLKPHGELITLIYLVTEESIYSFVYFSIEDV
MVLIISYCAERISYYRSVTKKEDHHSIIQSPILLRCPFRNHSYQKVLEPLGFKAILMED
NELAIKPRKAISAFRTSEQPSLAAQDVTE

>GI|125546406|GB|EAY92545.1| HYPOTHETICAL PROTEIN OSI_013778 [ORYZA SATIVA (INDICA CULTIVAR-GROUP)]
MASAIVDVAGGGRQQALDGSNPAVARLRQLIGGGQESSDGWSRCWEEGVTPWDLGQPTP
AVVELVHSGTLPAGDATTVLVPGCGAGYDVVALSGPGRFVVGLDICDTAIQKAKQLSAA
AAAAADGGDGSSSFFAFVADDFFTWEPPEPFHLIFDYTFFCALHPSMRPAWAKRMADLL
RPDGELITLMYLAEGQEAGPPFNTTVLDYKEVLNPLGLVITSIEDNEVAVEPRKGMEKI
ARWKRMTKSD

>GI|108712049|GB|ABF99844.1| THIOPURINE S-METHYLTRANSFERASE FAMILY PROTEIN, EXPRESSED [ORYZA SATIVA (JAPONICA CULTIVAR-GROUP)]
MASAIVDVAGGGRQQALDGSNPAVARLRQLIGGGQESSDGWSRCWEEGVTPWDLGQRTP
AVVELVHSGTLPAGDATTVLVPGCGAGYDVVALSGPGRFVVGLDICDTAIQKAKQLSAA
AAAAADGGDGSSSFFAFVADDFFTWEPPEPFHLIFDYTFFCALHPSMRPAWAKRMADLL
RPDGELITLMYLVINRRYQHV

>GI|115466488|REF|NP_001056843.1| OS06G0153900 [ORYZA SATIVA (JAPONICA CULTIVAR-GROUP)]
MSSSAARVGGGGGRDPSNNPAVGRLRELVQRGDAADGWEKSWEAAVTPWDLGKPTPIIE
HLVKSGTLPKGRALVPGCGTGYDVVALASPERFVVGLDISSTAVEKAKQWSSSLPNADC
FTFLADDFFKWKPSEQFDLIFDYTFFCALDPSLRLAWAETVSGLLKPHGELITLIYLIS
DQEGGPPFNNTVTDYQKVLEPLGFKAILMEDNELAIKPRKGQEKLGRWKRFVPGSSL

> COPRINOPSIS CINEREA OKAYAMA (HYPOTHETICAL PROTEIN)
MADPNLAPEIRAKMQEIFKPDDRHSWDLLWKENITPWDAGDAQPSLIELIEESGLDFAR
KGRALVPGCGTGYDAVYLASALGLQTIGMDISESAVEAANRYRDSSGVQGADRAIFQKA
DFFTYKVPDEERFDLIMDHTFFCAIHPSLRPEWGQRMSELIKPGGYLITICFPMIPKVE
TGPPYYLRPEHYDEVLKETFEKVYDKVPTKSSENHKDKERMLVWKKK

>GI|71024813|REF|XP_762636.1| HYPOTHETICAL PROTEIN UM06489.1 [USTILAGO MAYDIS 521]
MTSSLSKDDQIQNLRRLFADSGVPNDPKAWDQAWIDSTTPWDANRPQPALVELLEGAHD
ADAKVPDVDGNLIPVSQAIPKGDGTAVVPGCGRGYDARVFAERGLTSYGVDISSNAVAA
ANKWLGDQDLPTELDDKVNFAEADFFTLGTSKSLVLELSKPGQATLAYDYTFLCAIPPS

LRTTWAETYTRLLAKHGVLIALVFPIHGDRPGGPPFSISPQLVRELLGSQKNADGSAAW
TELVELKPKGPETRPDVERMMVWRRS

>GI|145230089|REF|XP_001389353.1| HYPOTHETICAL PROTEIN
AN01G09330 [ASPERGILLUS NIGER]
MTDQSTLTAAQQSVHNTLAKYPGEKYVDGWAEIWNANPSPPWDKGAPNPALEDTLMQRR
GTIGNALATDAEGNRYRKKALVPGCGRGVDVLLLASFGYDAYGLEYSGAAVQACRQEEK
ESTTSAKYPVRDEEGDFFKDDWLEELGLGLNCFDLIYDYTFFCALSPSMRPDWALRHTQ
LLAPSPHGNLICLEYPRHKDPSLPGPPFGLSSEAYMEHLSHPGEQVSYDAQGRCRGDPL
REPSDRGLERVAYWQPARTHEVGKDANGEVQDRVSIWRRR

>GI|111069917|GB|EAT91037.1| HYPOTHETICAL PROTEIN
SNOG_01388 [PHAEOSPHAERIA NODORUM SN15]
MANPNQDRLRSHFAALDPSTHASGWDSLWAEGTFIPWDRGYANPALIDLLANPSSPPTS
SDANPTPGAPKPNTIDGQGVQLPAPLEGGVRRKALVPGCGKGYDVALLASWGYDTWGLE
VSRHAADAAKEYLKDAGEGALEGEYKIKDAKIGKGREECVVADFFDDAWLKDVGAGEFD
VIYDNTFLCALPPLLRPKWAARMAQLLARDGVLICLEFPTHKPASSGGPPWSLPPTVHQ
ELLKRPGEDISYDEGGVVVATDRAESENALVRVAHWTPKRTHNIAVINGVVRDCVSVWR
HKKQS

>GI|119195301|REF|XP_001248254.1| HYPOTHETICAL PROTEIN
CIMG_02025 [COCCIDIOIDES IMMITIS RS]
MANEILRSAPNLSDRFKNLDGRNQGEVWDDLWKESRTPWDRGSHNPALEDALVEKRGFF
GAPVFEDEPLRRKKALVPGCGRGVDVFLLASFGYDAYGLEYSKTAVDVCLKEMEKYGEG
GKVPPRDEKVGSGKVMFLEGDFFKDDWVKEAGVEDGAFDLIYDYTFFCALNPALRPQWA
LRHRQLLAPSPRGNLICLEFPTTKDPAALGPPFASTPAMYMEHLSHPGEDIPYDDKGHV
KSNPLQQPSDKGLERVAHWQPKRTHTVGMDDKGNVLDWVSIWRR
RD

>GI|145234849|REF|XP_001390073.1| HYPOTHETICAL PROTEIN
AN03G01710 [ASPERGILLUS NIGER]
MSEAPNPPVQGRLISHFADRRAEDQGSGWSALWDSNESVLWDRGSPSIALVDVVEQQQD
VFFPYTRDGRRKKALVPGCGRGYDPVMLALHGFDVYGLDISATGVSEATKYATSEMQSP
QDVKFIAGDFFSSEWESQALQDGDKFDLIYDYTFLCALHPDLRRKWAERMSQLLHPGGL
LVCLEFPMYKDTSLPGPPWGLNGVHWDLLARGGDGITNITKEEEDEDSGIQLSGQFRRA
QYFRPIRSYPSGKGTDMLSIYVRR

>GI|119499868|REF|XP_001266691.1| THIOL METHYLTRANSFERASE,
PUTATIVE [NEOSARTORYA FISCHERI NRRL 181]
MSNDPRLLSSIPEFIARYKENYVEGWAELWNKSEGKPLPFDRGFPNPALEDTLIEKRDI
IGGPIGRDAQGNTYRKKALVPGCGRGVDVLLLASFGYDAYGLEYSDTAVQVCKEEQAKN
GDKYPVRDAEIGQGKITFVQGDFFKDTWLEKLQLPRNSFDLIYDYTFFCALDPSMRPQW
ALRHTQLLADSPRGHLICLEFPRHKDTSLQGPPWASTSEAYMAHLNHPGEEIPYDANRQ
CSIDPSKAPSPQGLERVAYWQPARTHEVGIVEGEVQDRVSIWRRPN

>GI|70993254|REF|XP_751474.1| THIOL METHYLTRANSFERASE,
PUTATIVE [ASPERGILLUS FUMIGATUS AF293]
MSNDPRLVSSIPEFIARYKENYVEGWAELWDKSEGKPLPFDRGFPNPALEDTLIEKRDI
IGDPIGRDAQGNTYRKKALVPGCGRGVDVLLLASFGYDAYGLEYSATAVKVCKEEQAKN
GDKYPVRDAEIGQGKITYVQGDFFKDTWWEKLQLPRNSFDLIYDYTFFCALDPSMRPQW

ALRHTQLLADSPRGHLICLEFPRHKDTSLQGPPWASTSEAYMAHLNHPGEEIPYDANRQ
CSIDPSKAPSPQGLERVAYWQPARTHEVGIVEGEVQDRVSIWRRPN

>GI|46137187|REF|XP_390285.1| HYPOTHETICAL PROTEIN
FG10109.1 [GIBBERELLA ZEAE PH-1]
MATENPLEDRISSVPFAEQGPKWDSCWKDALTPWDRGTASIALHDLLAQRPDLVPPSQH
QDHRGHPLRDATGAIQKKTALVPGCGRGHDVLLLSSWGYDVWGLDYSAAAKEEAIKNQK
QAESEGLYMPVDGLDKGKIHWITGNFFAQDWSKGAGDDGKFDLIYDYTFLCALPPDARP
KWAKRMTELLSHDGRLICLEFPSTKPMSANGPPWGVSPELYEALLAAPGEEIAYNDDGT
VHEDPCSKPWADALHRLSLLKPTRTHKAGMSPEGAVMDFLSVWSR

>GI|145228457|REF|XP_001388537.1| HYPOTHETICAL PROTEIN
AN01G00930 [ASPERGILLUS NIGER]
MTTPTDNKFKDAQAYLAKHQGDSYLKGWDLLWDKGDYLPWDRGFPNPALEDTLVERAGT
IGGPIGPDGKRRKVLVPGCGRGVDVLLFASFGYDAYGLECSAAAVEACKKEEEKVNNIQ
YRVRDEKVGKGKITFVQGDFFDDAWLKEIGVPRNGFDVIYDYTFFCALNPELRPKWALR
HTELLAPFPAGNLICLESPRHRDPLAPGPPFASPSEAYMEHLSHPGEEISYNDKGLVDA
DPLREPSKAGLERVAYWQPERTHTVGKDKNGVIQDRVSIWRRRD

>GI|121708664|REF|XP_001272206.1| THIOL METHYLTRANSFERASE,
PUTATIVE [ASPERGILLUS CLAVATUS NRRL 1]
MSTPSLIPSGVHEVLAKYKDGNYVDGWAELWDKSKGDRLPWDRGFPNPALEDTLIQKRA
IIGGPLGQDAQGKTYRKKALVPGCGRGVDVLLLASFGYDAYGLEYSATAVDVCQEEQAK
NGDQYPVRDAEIGQGKITFVQGDFFEDTWLEKLNLTRNCFDVIYDYTFFCALNPSMRPQ
WALRHTQLLADSPRGHLICLEFPRHKDPSVQGPPWGSASEAYRAHLSHPGEEIPYDASR
QCQFDSSKAPSAQGLERVAYWQPERTHEVGKNEKGEVQDRVSIWQRPPQSSL

>GI|67539848|REF|XP_663698.1| HYPOTHETICAL PROTEIN AN6094.2
[ASPERGILLUS NIDULANS FGSC A4]
MSSPSQQPIKGRLISHFENRPTPSHPKAWSDLWDSGKSSLWDRGMPSPALIDLLESYQD
TLLHPFEIDIEDEEDSSDAGKTRKRKRALVPGCGRGYDVITFALHGFDACGLEVSTTAV
SEARAFAKKELCSPQSGNFGRRFDRERARHIGVGKAQFLQGDFFTDTWIENESTGLDQG
RTENGKFDLVYDYTFLCALHPAQRTRWAERMADLLRPGGLLVCLEFPMYKDPALPGPPW
GVNGIHWELLAGGDTGQGKFTRKAYVQPERTFEVGRGTDMISVYERK

>GI|121529427|REF|ZP_01662039.1| CONSERVED HYPOTHETICAL
PROTEIN [RALSTONIA PICKETTII 12J]
MAQPPVFQSRDAADPAFWDERFTREHTPWDAAGVPAAFRQFCEAQPAPLSTLIPGCGNA
YEAGWLAERGWPVTAIDFAPSAVASARAVLGPHADVVQLADFFRFSPPRPVHWIYERAF
LCAMPRRLWPDYAAQVAKLLPPRGLLAGFFAVVEGREAMPKGPPFETTQPELDALLSPA
FERISDMPIAETDSIPVFAGRERWQVWRRRAD

>GI|17545181|REF|NP_518583.1| HYPOTHETICAL PROTEIN RSC0462
[RALSTONIA SOLANACEARUM GMI1000]
MAQPPVFTTRDAAAPAFWDERFSRDHMPWDAHGVPPAFRQFCEAQPAPLSTLIPGCGSA
YEAGWLAERGWPVAAIDFAPSAVASAQAVLGPHAGVVELADFFRFTPRQPVQWIYERAF
LCAMPRRLWADYATQVARLLPPGGLLAGFFVVVDGRAAAPSGPPFEITAQEQEALLSPA
FERIADALVPENESIPVFAGRERWQVWRRRAD

>GI|83644186|REF|YP_432621.1| SAM-DEPENDENT
METHYLTRANSFERASE [HAHELLA CHEJUENSIS KCTC 2396]

MDANFWHERWAENSIAFHQCEANPLLVAHFNRLDLAKGSRVFVPLCGKTLDISWLLSQG
HRVVGCELSEMAIEQFFKELGVTPAISEIVAGKRYSAENLDIIVGDFFDLTVETLGHVD
ATYDRAALVALPKPMRDSYAKHLMALTNNAPQLMLCYQYDQTQMEGPPFSISAEEVQHH
YADSYALTALATVGVEGGLRELNEVSETVWLLESR

> LEPTOSPIRILLUM SP. GROUP II UBA
MPDKIFWNQRYLDKNTGWDLGQPAPPFVRLVEKGEFGPPGRVLIPGAGRSYEGIFLASR
GYDVTCVDFAPQAVREAREAARQAGVKLTVVEEDFFRLDPRTIGVFDYLVEHTCFCAID
PPMRQAYVDQSHALLAPGGLLIGLFYAHGREGGPPWTTTEEEVRGLFGKKFDLLSLGLT
DWSVDSRKGEELLGRLRRKNDRIE

>GI|37520387|REF|NP_923764.1| SIMILAR TO THIOL
METHYLTRANSFERASE [GLOEOBACTER VIOLACEUS PCC 7421]
MPSEESSGVDQPAFWEYRYRGGQDRWDLGQPAPTFVHLLSGSEAPPLGTVAVPGCGRGH
DALLFAARGYKVCGFDFAADAIADATRLALRAGAAATFLQQDLFNLPRPFAGLFDLVVE
HTCFCAIDPVRREEYVEIVHWLLKPGGELVAIFFAHPRPGGPPYRTDAGEIERLFSPRF
KITALLPAPMSVPSRRGEELFGRFVRA

>GI|86130841|REF|ZP_01049440.1| HYPOTHETICAL PROTEIN
MED134_07976 [CELLULOPHAGA SP. MED134]
MELTSTYWNNRYAEGSTGWDLKEVSPPIKAYLDQLENKELKILIPGGGYSYEAQYCWEQ
GFKNVYYVVDFSQLALENLKQRVPDFPSLQLIQEDFFTYDGQFDVIIEQTFFCALQPDLR
PAYVAHMHTLLKAKGKLVGLLFNFPLTEKGPPYGGSTTEYESLFSEHFDIQKMETAYNS
VAARAGKELFIKMVKK

>GI|159875886|GB|EDP69945.1| HYPOTHETICAL PROTEIN
FBALC1_10447 [FLAVOBACTERIALES BACTERIUM ALC-1]
MISMKKNKLDSDYWEDRYTKNSTSWDIGYPSTPIRTYIDQLKDKSLKILIPGAGNSFEA
EYLWNLGFKNIYILDFAKQPLENFKKRLPDFPENQLLHIDFFKLDIHFDLILEQTFFCA
LNPSLREKYVEQMHQLLKPKGKLVGLFFNFPLTKSGPPFGGSLTEYQFLFDKKFKIKIL
ETSINSIKEREGKELFFIFESP

>GI|149199821|REF|ZP_01876851.1| THIOL METHYLTRANSFERASE 1-
LIKE PROTEIN [LENTISPHAERA ARANEOSA HTCC2155]
MRTKGNEKAESWDKIYREGNPGWDIKKPAPPFEDLFKQNPSWLKAGSLISFGCGGGHDA
NFFAQNDFNVTAVDFASEAVKLARSNYPQLNVIQKNILELSPEYDEQFDYVLEHTCFCA
VPLDHRRAYMESAHAILKAGAYLFGLFYRFDPPDQDGPPYSLSLEDLEDAYSGLFTLEE
NAIPKRSHGRRTQRERFIVLKKI

>GI|71066354|REF|YP_265081.1| HYPOTHETICAL PROTEIN
PSYC_1799 [PSYCHROBACTER ARCTICUS 273-4]
MGNVNQAEFWQQRYEQDSIGWDMGQVSPPLKVYIDQLPEAAKEQAVLVPGAGNAYEVGY
LYEQGFTNITLVDFAPAPIKDFAERYPDFPADKLICADFFDLLPKQHQFDWVLEQTFFC
AINPARRDEYVQQMARLLKPKGQLVGLLFDKDFGRNEPPFGGTKEEYQQRFSTHFDTEI
MEQSYNSHPARQGSELFIKMRVKD

>GI|86135149|REF|ZP_01053731.1| HYPOTHETICAL PROTEIN
MED152_10555 [TENACIBACULUM SP. MED152]
MIFDEQFWDNKYITNKTGWDLGQVSPPLKAYFDQLTNKDLKILIPGGGNSHEAEYLLEN
GFTNVYYVIDISKLALTNLKNRVPGFPSSNLIHQNFFELNQTFDLVIEQTFFCALNPNLR

EEYVSKMHSVLNDNGKLVGLLFDAKLNEDHPPFGGSKKEYTSLFRNLFTIEVLEECYNS
IENRKGMELFCKFVK

>GI|93006905|REF|YP_581342.1| THIOPURINE S-
METHYLTRANSFERASE [PSYCHROBACTER CRYOHALOLENTIS K5]
MENVNQAQFWQQRYEQDSIGWDMGQVSPPLKAYIDQLPEAAKNQAVLVPGAGNAYEVGY
LHEQGFTNVTLVDFAPAPIAAFAERYPNFPAKHLICADFFELSPEQYQFDWVLEQTFFC
AINPSRRDEYVQQMASLVKPNGKLIGLLFDKDFGRDEPPFGGTKDEYQQRFATHFDIDI
MEPSYNSHPARQGSELFIEMHVKD

>GI|114778202|REF|ZP_01453074.1| THIOL METHYLTRANSFERASE 1-
LIKE PROTEIN [MARIPROFUNDUS FERROOXYDANS PV-1]
MTVWEERYQRGETGWDRGGVSPALTQLVDHLHLEARVLIPGCGRGHEVIELARLGFRVT
AIDIAPSAIAHLSQQLEQEDLDAELVNGDLFAYAPDHCFDAVYEQTCLCAIEPEQRADY
EQRLHGWLKPEGVLYALFMQTGIRGGPPFHCDLLMMRELFDASRWQWPEETGAVLVPHK
NGRFELGHMLRRTGR

>GI|83855599|REF|ZP_00949128.1| HYPOTHETICAL PROTEIN
CA2559_00890 [CROCEIBACTER ATLANTICUS HTCC2559]
MTSNFWEQRYANNNTGWDLNTVSPPLKHYIDTLSNKTLFILIPGCGNAYEAEYLHNQGF
ENVFIVDLAEHPLLEFSKRVPDFPKSHILHLDFFNLTQKFDLILEQTFFCALHPEQRLH
YAHHTSKLLNSNGCLVGLFFNKEFDKTGPPFGGNKKEYKNLFKNLFKIKKLENCYNSIK
PRQGSELFFIFEKK

>GI|83858455|REF|ZP_00951977.1| THIOPURINE S-
METHYLTRANSFERASE [OCEANICAULIS ALEXANDRII HTCC2633]
MTQASSDTPRSEDRSGFDWESRFQSDDAPWERQGVHPAAQDWVRNGEIKPGQAILTPGC
GRSQEPAFLASRGFDVTATDIAPTAIAWQKTRFQTLGVMAEAIETDALAWRPETGFDAL
YEQTFLCAIHPKRRQDYEAMAHASLKSGGKLLALFMQKAEMGGPPYGCGLDAMRELFAD
TRWVWPDGEARPYPHPGLNAKAELAMVLIRR

>GI|113866478|REF|YP_724967.1| THIOPURINE S-
METHYLTRANSFERASE (TPMT) [RALSTONIA EUTROPHA H16]
MSDPAKPVPTFATRNAADPAFWDERFEQGFTPWDQGGVPEEFRQFIEGRAPCPTLVPGC
GNGWEAAWLFERGWPVTAIDFSPQAVASARQTLGPAGVVVQQGDFFAFTPQPPCELIYE
RAFLCALPPAMRADYAARVAQLLPPGGLLAGYFYLGENRGGPPFAMPAEALDALLAPAF
ERLEDRPTAAPLPVFQGQERWQVWRRRSG

>GI|150025500|REF|YP_001296326.1| HYPOTHETICAL PROTEIN
FP1441 [FLAVOBACTERIUM PSYCHROPHILUM JIP02/86]
MKKIDQKYWQNRYQTNDIAWDTGKITTPIKAYIDQIEDQSIKILIPGCGNGYEYEYLIK
KGFYNSFVADYAQTPIDNLKKRIPNCNANQLLISDFFELEGSYDLIIEQTFFCALNPEL
RVKYAQKMLSLLSPKGKIIGLLFQFPLTEAGPPFGGSKEEYLKLFSTNFNIKTIETAYN
SIKPREGNELFFIFTKK

>GI|124268594|REF|YP_001022598.1| HYPOTHETICAL PROTEIN
MPE_A3410 [METHYLIBIUM PETROLEIPHILUM PM1]
MSGPDLNFWQQRFDTGQLPWDRGAPSPQLAAWLGDGSLAPGRIAVPGCGSGHEVVALAR
GGFSVTAIDYAPGAVRLTQGRLAAAGLAAEVVQADVLTWQPTAPLDAVYEQTCLCALHP
DHWVAYAARLHAWLRPGGTLALLAMQALREGAGQGLIEGPPYHVDVNALRALLPGDRWD
WPRPPYARVPHPSSTWAELAIVLTRR

>GI|86141349|REF|ZP_01059895.1| HYPOTHETICAL PROTEIN MED217_05007 [FLAVOBACTERIUM SP. MED217]
MKTDLNKLYWEDRYQNQQTGWDIGSVSTPLKEYIDQIDDKNIQILVPGAGYGHEVRYLA
QQGFKNVDVIDLSVSALTQLKKALPDTTAYQLIEGDFFEHHTSYDLILEQTFFCALEPD
KRPDYAAHAASLLKDSGKISGVLFNFPLTEKGPPFGGSSEEYKKLFSEYFNIKTLEACY
NSIKPRLGNELFFIFEKSNQES

>GI|124003356|REF|ZP_01688206.1| THIOPURINE S-METHYLTRANSFERASE (TPMT) SUPERFAMILY [MICROSCILLA MARINA ATCC 23134]
MHTTLDKDFWSNRYQAQDTGWDAGSITTPIKAYVDQLEDKHLKILVPGAGNSHEAEYLH
QQGFTNVTVIDIVQAPLDNLKSRSPDFPEAHLLQGDFFELVGQYDLIIEQTFFCALNPS
LRESYVQKVKSLLKPEGKLVGVLFCNVFLDRTEPPFGATEQQHQEYFLPHFIAKHFASC
YNSIAPRQGAEWFICLIND

>GI|151577463|GB|EDN41864.1| THIOPURINE S-METHYLTRANSFERASE [RALSTONIA PICKETTII 12D]
MAEPPVFQSRDAADPAFWDERFSREHTPWDAAGVPAAFQQFCESQPVPLSTLIPGCGSA
YEAGWLAERGWPVTAIDFAPSAVASARAVLGPHADVVEMADFFGFSPARSVQWIYERAF
LCAMPRRLWPDYAAQVAKLLPPGGLLAGFFAVVEGREAVPKGPPFETTQPELDALLSPA
FERISDIPIAEADSIPVFAGRERWQVWRRRAD

>GI|121303859|GB|EAX44825.1| CONSERVED HYPOTHETICAL PROTEIN [RALSTONIA PICKETTII 12J]
MAQPPVFQSRDAADPAFWDERFTREHTPWDAAGVPAAFRQFCEAQPAPLSTLIPGCGNA
YEAGWLAERGWPVTAIDFAPSAVASARAVLGPHADVVQLADFFRFSPPRPVHWIYERAF
LCAMPRRLWPDYAAQVAKLLPPRGLLAGFFAVVEGREAMPKGPPFETTQPELDALLSPA
FERISDMPIAETDSIPVFAGRERWQVWRRRAD

>GI|121583316|REF|YP_973752.1| THIOPURINE S-METHYLTRANSFERASE [POLAROMONAS NAPHTHALENIVORANS CJ2]
MAGPTTDFWQARFDNKETGWDRGAPGPQLLAWLESGALQPCRIAVPGCGSGWEVAELARRGF
EVVGIDYTPAAVERTRALLAAQGLAAEVVQADVLAYQPHKPFEAIYEQTCLCALHPDHWVAY
ARQLQQWLKPQGSIWALFMQMVRPEATDEGLIQGPPYHCDINAMRALFPAQHWAWPRPPYAK
VPHPNVGHELGLRLMLRQGR

>GI|88802008|REF|ZP_01117536.1| HYPOTHETICAL PROTEIN PI23P_05077 [POLARIBACTER IRGENSII 23-P]
MNLSADAWDERYTNNDIAWDLGEVSSPLKAYFDQLENKEIKILIPGGGNSHEAAYLFENGFK
NIWVVDLSETAIGNIQKRIPEFPPSQLIQGDFFNMDDVFDLIIEQTFFCAINPNLRADYTTK
MHHLLKSKGKLVGVLFNVPLNTNKPPFGGDKSEYLEYFKPFFIIKKMEACYNSFGNRKGREL
FVILRSK

>GI|126661882|REF|ZP_01732881.1| THIOPURINE S-METHYLTRANSFERASE [FLAVOBACTERIA BACTERIUM BAL38]
MNYWEERYKKGETGWDAGTITTPLKEYIDQLTDKNLTILIPGAGNGHEFDYLIDNGFKNVFV
VDIAITPLENIKKRKPKYSSHLINADFFSLTTTFDLILEQTFFCALPPEMRQRYVEKMTSLL
NPNGKLAGLLFDFPLTSEGPPFGGSKSEYITLFSNTFSIKTLERAYNSIKPRENKELFFIFE
TK

>GI|149924142|REF|ZP_01912520.1| HYPOTHETICAL PROTEIN PPSIR1_29093 [PLESIOCYSTIS PACIFICA SIR-1]
MRVIVPGAGVGHDALAWAQAGHEVVALDFAPAAVARLRERAAEAGLTIEAHVADVTNPGPAL
NDGLGGRFDLVWEQTCLCAITPELRGAYLAQARSWLTPDGSMLALLWNTGNEGGPPYDMPPE
LVERLMTGLFVIDKFAPVTGSNPNRREHLYWLRPEPT

>GI|126647682|REF|ZP_01720187.1| HYPOTHETICAL PROTEIN ALPR1_06920 [ALGORIPHAGUS SP. PR1]
MAELDEKYWSERYKSGLTGWDIGFPSTPIVQYLDQIVNKDVEILIPGAGNAYEAYYAFQSGF
SNVHVLDISQEPLRNFKDKFPNFPSSNLHHGDFFEHHGSYNLILEQTFFCALNPSLRPKYVK
KMSELLLKGGKLVGLLFNKEFNSPGPPFGGGIKEYQKLFHNSFEIDVMEECYNSIPARAGSE
AFIRLINSKG

>GI|89900214|REF|YP_522685.1| THIOPURINE S-METHYLTRANSFERASE [RHODOFERAX FERRIREDUCENS T118]
MAGPTTEFWQERFEKKETGWDRGSPSPQLLAWLASGALRPCRIAVPGCGSGWEVAELAQRGF
DVVGLDYTAAATTRTRALCDARGLKAEVLQADVLSYQPEKKFAAIYEQTCLCAIHPDHWIDY
ARQLHQWLEPQGSLWVLFMQMIRPAATEEGLIQGPPYHCDINAMRALFPQKDWVWPKPPYAR
VSHPNLSHELALQLVRR

>GI|17545181|REF|NP_518583.1| HYPOTHETICAL PROTEIN RSC0462 [RALSTONIA SOLANACEARUM GMI1000]
MAQPPVFTTRDAAAPAFWDERFSRDHMPWDAHGVPPAFRQFCEAQPAPLSTLIPGCGSAYEA
GWLAERGWPVAAIDFAPSAVASAQAVLGPHAGVVELADFFRFTPRQPVQWIYERAFLCAMPR
RLWADYATQVARLLPPGGLLAGFFVVVDGRAAAPSGPPFEITAQEQEALLSPAFERIADALV
PENESIPVFAGRERWQVWRRRAD

>GI|120436745|REF|YP_862431.1| THIOPURINE S-METHYLTRANSFERASE [GRAMELLA FORSETII KT0803]
MNKDFWSLRYQKGNTGWDIGNISTPLKEYIDHLHKKELKILIPGAGNSYEAEYLFEKGFKNI
WICDIAKEPIENFKKRLPEFPESQILNRDFFELKDQFDLILEQTFFCALPVNFRENYAKKVF
ELLKVNGKISGVLFDFPLTPDGPPFGGSKEEYLAYFSPYFKINTFERCYNSINPRQGKELFF
NFSKK

>GI|86159623|REF|YP_466408.1| METHYLTRANSFERASE TYPE 12 [ANAEROMYXOBACTER DEHALOGENANS 2CP-C]
MGTSYRLAYLIGFTPWEDQPLPPELSALVEGLRARPPGRALDLGCGRGAHAVYLASHGWKVT
GVDLVPAALAKARQRATDAGVDVQFLDGDVTRLDTLGLSPGYDLLLDAGCFHGLSDPERAAY
ARGVTALRAPRAAMLLFAFKPGWRGPAPRGASAEDLTSAFGPSWRLVRSERARESRLPLPLR
NADPRWHLLEAA

>GI|118468119|REF|YP_886428.1| METHYLTRANSFERASE TYPE 12 [MYCOBACTERIUM SMEGMATIS STR. MC2 155]
MDTTPTRELFDEAYESRTAPWVIGEPQPAVVELERAGLIRSRVLDVGCGAGEHTILLTRLGY
DVLGIDFSPQAIEMARENARGRGVDARFAVGDAMALGDLGDGAYDTILDSALFHIFDDADRQ
TYVASLHAGCRPGGTVHILALSDAGRGFGPEVSEEQIRKAFGDGWDLEALETTTYRGVVGPV
HAEAIGLPVGTQVDEPAWLARARRL

>GI|119504877|REF|ZP_01626954.1| THIOPURINE S-METHYLTRANSFERASE [MARINE GAMMA PROTEOBACTERIUM HTCC2080]
MEKFGASAMEPVLDWEARYQESSVPWERTGLNPAFVAWQSWLRDHQGGTVVVPGCGRSPELQ

AFADMGFNVIGVDLSPSAAQFQETVLAAKGLDGKLVVSNLFDWSPDTPVDFVYEQTCLCALK
PDHWRAYENLLTRWLRPGGTLLALFMQTGESGGPPFHCGKAAMEQLFSEQRWIWDETSVRSE
HPLGVHELGFRLTLR

>GI|161325846|GB|EDP97172.1| HYPOTHETICAL PROTEIN KAOT1_18457
[KORDIA ALGICIDA OT-1]
MNSDATKEYWSQRYKDNSTGWDIGSPSTPLKTYIDQLKDRNLKILIPGAGNAYEAEYLLQQG
FTNIYILDISEIPLQEFKQRNPEFPSDRLLCDDFFTHKNTYDLIIEQTFFCSFPPLPETRAQ
YAKHMADLLNPNGKLVGLWFDFPLTDDLEKRPFGGSKEEYLEYFKPYFDVKTFEKAYNSIAP
RAGNELFGIFIKS

>GI|150389542|REF|YP_001319591.1| METHYLTRANSFERASE TYPE 11
[ALKALIPHILUS METALLIREDIGENS QYMF]
MNDKLDQEVILNQEDLLNMLDSLLEKWDEEWWNEFYSDKGKPIPFFVNAPDENLVTYFDKYF
DDIGRALDVGCGNGRNSRFIASRGYDVEGLDFSKKSIEWAKEESKKTGDIALYVNDSFFNIN
RELSSYDLIYDSGCLHHIKPHRRSQYLEKVHRLLKPGGYFGLVCFNLKGGANLSDHDVYKKS
SMAGGLGYSDIKLKKILGTYFEIVEFREMRECADNALYGKDICWSILMRRLAK

>GI|71024813|REF|XP_762636.1| HYPOTHETICAL PROTEIN UM06489.1
[USTILAGO MAYDIS 521]
MTSSLSKDDQIQNLRRLFADSGVPNDPKAWDQAWIDSTTPWDANRPQPALVELLEGAHDADA
KVPDVDGNLIPVSQAIPKGDGTAVVPGCGRGYDARVFAERGLTSYGVDISSNAVAAANKWLG
DQDLPTELDDKVNFAEADFFTLGTSKSLVLELSKPGQATLAYDYTFLCAIPPSLRTTWAETY
TRLLAKHGVLIALVFPIHGDRPGGPPFSISPQLVRELLGSQKNADGSAAWTELVELKPKGPE
TRPDVERMMVWRRS

>GI|20090980|REF|NP_617055.1| HYPOTHETICAL PROTEIN MA2137
[METHANOSARCINA ACETIVORANS C2A]
MFWDEVYKGTPPWDIDHPQPAFQALIESGEIRPGRALDIGCGRGENAIMLAKNGCDVTGIDL
AKDAISDAKAKAIERHVKVNFIVGNVLEMDQLFTEDEFDIVIDSGLFHVITDEERLLFTRHV
HKVLKEGGKYFMLCFSDKEPGEYELPRRASKAEIESTFSPLFNIIYIKDVIFDSLLNPGRRQ
AYLLSATKS

> HALORHODOSPIRA HALOPHILA
SL1MSGDPDPRRAPWEARWREGRTGWDRGGVSPTLEAWLSAGVIPGRRVLVPGAGRGYEVEA
LARRGYKVTAVDIAAEACQQLRDGLDAAGVEARVVQADLLAWQPDTPFDAVYEQTCLCALDP
ADWPAYEQRLYGWLRPGGVLLALFMQTGASGGPPFHCALPEMATLFDSERWQWPAEPPRQWP
HPSGRWEEAVRLLRR

>GI|54295659|REF|YP_128074.1| THIOPURINE S-METHYLTRANSFERASE
[LEGIONELLA PNEUMOPHILA STR. LENS]
MNKGQYFWNELWCEGRISFHKKEVNPDLIAYVSSLNIPAKGRVLVPLCGKSVDMLWLVRQGY
HVVGIELVEKAILQFVQEHQITVRENTIGQAKQYFTDNLNLWVTDIFALNSALIEPVDAIYD
RAALVALPKKLRPAYVDICLKWLKPGGSILLKTLQYNQEKVQGPPYSVSPEEIALSYQQCAK
IKLLKSQKRIQEPNDHLFNFGISEVNDSVWCIRKG

>GI|116187307|REF|ZP_01477195.1| HYPOTHETICAL PROTEIN
VEX2W_02000031 [VIBRIO SP. EX25]
MKQAPTINQQFWDNLFTQGTMPWDAKTTPQELKAYLENALHSGQSVFIPGCGAAYELSSFIQ
YGHDVIAMDYSEQAVKMAQSTLGKHKDKVVLGDVFNADSTHSFDVIYERAFLAALPRDQWPE

YFAMVDKLLPRGGLLIGYFVIDDDYHSRFPPFCLRSGELEGYLEPVFKLVESSVVANSVEVF
KGRERWMVWQKSCRI

>GI|120402886|REF|YP_952715.1| METHYLTRANSFERASE TYPE 11
[MYCOBACTERIUM VANBAALENII PYR-1]
MDLTPRLSRFDEFYKNQTPPWVIGEPQQAIVELEQAGLIGGRVLDVGCGTGEHTILLARAGY
DVLGIDGAPTAVEQARRNAEAQGVDARFELADALHLGPDPTYDTIVDSALFHIFDDADRATY
VRSLHAATRPGSVVHLLALSDSGRGFGPEVSEHTIRAAFGAGWEVEALTETTYRGVVIDAHT
EALNLPAGTVVDEPAWSARIRRL

>GI|134101246|REF|YP_001106907.1| 6-O-METHYLGUANINE DNA
METHYLTRANSFERASE [SACCHAROPOLYSPORA ERYTHRAEA NRRL 2338]
MDDELAESQRAHWQDTYSAHPGMYGEEPSAPAVHAAGVFRAAGARDVLELGAGHGRDALHFA
REGFTVQALDFSSSGLQQLRDAARAQQVEQRVTTAVHDVRHPLPSADASVDAVFAHMLLCMA
LSTEEIHALVGEIHRVLRPGGVLVYTVRHTGDAHHGTGVAHGDDIFEHDGFAVHFFPRGLVD
SLADGWTLDEVHAFEEGDLPRRLWRVTQTLPR

> BURKHOLDERIA PHYMATUM STM815 (29% IDENTICAL TO
BATIS)MSDKRPSVPPSAPDFENRDPNAPGFWDERFGRGFTPWDQAGVPPAFKAFVERHSPV
PVLIPGCGSAYEARWLAEKGWTVRAIDFAPNAVEAARAQLGSHASLVHEADFFTYRPPFDPG
WIYERAFLCALPPARRSDWVARMAQLLSPGGLLAGFFFIGATEKGPPFGIERAELDALMSPD
FTLVEDEPVDDSIAVFAGRERWLTWRRRGAARG

>GI|91781799|REF|YP_557005.1| HYPOTHETICAL PROTEIN BXE_A4046
[BURKHOLDERIA XENOVORANS LB400]
MSDPTQPAVPDFETRDPNSPAFWDERFERRFTPWDQAGVPAAFQSFAARHSGAAVLIPGCGS
AYEAVWLAGQGNPVRAIDFSPAAVAAAHEQLGAQHAQLVEQADFFTYEPPFTPAWIYERAFL
CALPLARRADYAHRMADLLPGGALLAGFFFLGATPKGPPFGIERAELDALLTPYFDLIEDEA
VHDSIAVFAGRERWLTWRRRA

>GI|118038664|REF|ZP_01510068.1| THIOPURINE S-
METHYLTRANSFERASE [BURKHOLDERIA PHYTOFIRMANS PSJN]
MSDPTQPSAPEFESRDPNSPEFWDERFERGFMPWDQAGVPSAFESFAARHAGAAVLIPGCGS
AYEAVWLAGHGYPVRAIDFSPAAVAAAHEQLGAQHADLVEQADFFTYELPFTPAWIYERAFL
CALPLARRADYARRMADLLPGGALLAGFFFIGATPKGPPFGIERAELDGLLKPYFELIEDEP
VHDSIAVFAGRERWLTWRRRV

>GI|83719252|REF|YP_441114.1| THIOPURINE S-METHYLTRANSFERASE
FAMILY PROTEIN [BURKHOLDERIA THAILANDENSIS E264]
MTSEANKGDAAVQAAGDAQPASPASPPSADVQPARAALAPSSVPPAPSAANFASRDPGDASF
WDERFERGVTPWDSARVPDAFAAFAARHPRCPVLIPGCGSAYEARWLARAGWPVRAIDFSAQ
AVAAARRESGADAALVEQADFFAYVPPFVPQWIYERAFLCAIPTSRRADYARRVAELLPAGG
FLAGFFFIGATPKGPPFGIERAELDALLSPNFELVEDEPVADSLPVFAGRERWLAWRRS

>GI|134296925|REF|YP_001120660.1| THIOPURINE S-
METHYLTRANSFERASE [BURKHOLDERIA VIETNAMIENSIS G4]
MSNPTQPPPPSAADFATRDPANASFWDERFARGVTPWEFGGVPDGFRAFAQRRAPCTVLIPG
CGSAQEAGWLAQAGWPVRAIDFAEQAVVAAKATLGAHADVVEQADFFAYQPPFVVQWVYERA
FLCALPPSLRAGYAARMAELLPAGGLLAGYFFVMKKPKGPPFGIERAELDALLAPSFELIED
LPVTDSLAVFDGHERWLTWRRR

>GI|118707586|REF|ZP_01560172.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA CENOCEPACIA MC0-3]
MSDPKQPAAPSAAEFATRDPGSASFWDERFARGVTPWEFGGVPDGFRAFAQRHEPCAVLIPG
CGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKVQLGAHADVVEQADFFQYRPPFDVQWVYERA
FLCALPPSLRADYAARMAELLPTGGLLAGYFFVVAKPKGPPFGIERAELDALLAPHFELLED
LPVTDSLAVFDGHERWLTWRRR

>GI|53724994|REF|YP_102027.1| THIOPURINE S-METHYLTRANSFERASE FAMILY PROTEIN [BURKHOLDERIA MALLEI ATCC 23344]
MKDRLMSQGDGVTNEANQPEAAGQAAGDAQPASPAGPAHIANPANPANPPALPSFSPPAAAS
SSASSAAPFSSRDPGDASFWDERFEQGVTPWDSARVPDAFAARHARVPVLIPGCGSAYEARW
LARAGWPVRAIDFSAQAVAAARRELGEDAGLVEQADFFTYAPPFVPQWIYERAFLCAIPRSR
RADYARRMAELLPPGGFLAGFFFIGATPKGPPFGIERAELDALLCPHFALVEDEPVADSLPV
FAGRERWLAWRRS

>GI|76808612|REF|YP_332262.1| THIOPURINE S-METHYLTRANSFERASE FAMILY PROTEIN [BURKHOLDERIA PSEUDOMALLEI 1710B]
MKDRLMSQGDGVTNEANQPEAAGQATGDAQPASPAGPAHIANPANPANPANPPALPSLSPPA
AAPSSASSAAHFSSRDPGDASFWDERFEQGVTPWDSARVPDAFAAFAARHARVPVLIPGCGS
AYEARWLARAGWPVRAIDFSAQAVAAARRELGEDAGLVEQADFFTYAPPFVPQWIYERAFLC
AIPRSRRADYARRMAELLPPGGFLAGFFFIGATPKGPPFGIERAELDALLCPHFALVEDEPV
ADSLPVFAGRERWLAWRRS

>GI|107023663|REF|YP_621990.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA CENOCEPACIA AU 1054]
MSDPKQPAAPSAADFATRDPGSASFWDERFARGVTPWEFGGVPDGFRVFAQRREPCAVLIPG
CGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKAQLGAHADVVEQADFFQYRPPFDVQWVYERA
FLCALPPGLRAGYAARMAELLPTGGLLAGYFFVVAKPKGPPFGIERAELDALLAPHFELLED
LPVTDSLAVFDGHERWLTWRRR

>GI|84362923|REF|ZP_00987534.1| COG0500: SAM-DEPENDENT METHYLTRANSFERASES [BURKHOLDERIA DOLOSA AUO158]
MTGRSFAMSDPKQPGTPTAADFATRDPGDASFWDERFARGVTPWEFGGVPDGFRAFAQRLER
CAVLIPGCGSAQEAGWLADAGWPVRAIDFAAQAVATAKAQLGAHADVVELADFFTYRPPFDV
RWIYERAFLCALPPARRADYAAQMAALLPAGGLLAGYFFVTAKPKGPPFGIERAELDALLAP
QFDLIDDWPVTDSLPVFEGHERWLTWRRR

>GI|115352830|REF|YP_774669.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA AMBIFARIA AMMD]
MSEPKQPSTPGAADFATRDPGDASFWDERFARGVTPWEFGGVPEGFRAFAQRLGPCAVLIPG
CGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKAQLGAHADVVEQADFFMYRPPFDVQWVYERA
FLCALPPSLRAGYAARMAELLPAGALLAGYFFVTKKPKGPPFGIERAELDALLAPHFELIDD
LPVTDSLAVFEGHERWLTWRRR

>GI|78067524|REF|YP_370293.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA SP. 383]
MSDPKQPKPNAPAAADFTTRDPGNASFWNERFERGVTPWEFGGVPEGFSVFAHRLELCAVLI
PGCGSAQEAGWLAEAGWPVRAIDFAAQAVAAAKAQLGAHAGVVEQADFFAYRPPFDVQWVYE
RAFLCALPPAMRADYAARMAELLPADGLLAGYFFLMAKPKGPPFGIERAELDALLTPHFELI
EDLPVTDSLAVFEGHERWLTWRRR

>GI|161523751|REF|YP_001578763.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA MULTIVORANS ATCC 17616]
MSDPKHAAAPAAASFETRDPGDASFWDERFARGMTPWEFGGVPAGFRAFASARPPCAVLIPG
CGSAREAGWLAQAGWPVRAIDFSAQAVAAAKAQLGAHADVVEQADFFAYRPPFDVQWIYERA
FLCALPPARRADYAATMAALLPAQGLLAGYFFVADKQKGPPFGITRGELDALLGAHFELIDD
APVSDSLPVFEGHERWLAWRRR

>GI|84355663|REF|ZP_00980538.1| COG0500: SAM-DEPENDENT METHYLTRANSFERASES [BURKHOLDERIA CENOCEPACIA PC184]
MLIPGCGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKAQLGAHADVVEQADFFAYRPPFDVQW
VYERAFLCALPPSLRAGYAARMAELLPTGGLLAGYFFVVAKPKGPPFGIEPAELDALLAPHF
ALLEDLPVTDSLAVFDGHERWLTWRRR

>GI|116187307|REF|ZP_01477195.1| HYPOTHETICAL PROTEIN VEX2W_02000031 [VIBRIO SP. EX25]
MKQAPTINQQFWDNLFTQGTMPWDAKTTPQELKAYLENALHSGQSVFIPGCGAAYELSSFIQ
YGHDVIAMDYSEQAVKMAQSTLGKHKDKVVLGDVFNADSTHSFDVIYERAFLAALPRDQWPE
YFAMVDKLLPRGGLLIGYFVIDDDYHSRFPPFCLRSGELEGYLEPVFKLVESSVVANSVEVF
KGRERWMVWQKSCRI

>GI|28901001|REF|NP_800656.1| HYPOTHETICAL PROTEIN VPA1146 [VIBRIO PARAHAEMOLYTICUS RIMD 2210633]
MKSKDSPIINEQFWDALFFNGTMPWDRSQTPNELKHYLKRIADKTHSVFIPGCGAAYEVSHF
VDCGHDVIAMDYSAEAVNLAKSQLGQHQDKVMLGDVFNADFSREFDVIYERAFLAALPREIW
GDYFAMIERLLPSNGLLVGYFVISDDYRSRFPPFCLRSGEIEQKLEANFHLIESTPVTDSVD
VFKGKEQWMVWQKK

>GI|91224783|REF|ZP_01260043.1| HYPOTHETICAL PROTEIN V12G01_01280 [VIBRIO ALGINOLYTICUS 12G01]
MKQAPMINTQFWDDLFIRGTMPWDAQSTPQELKDYLDNSLHVGQSVFIPGCGAAYELSTFIQ
YGHDVIAMDYSQEAVKMAQSALGNYKDKVVLGDVFNADFSHSFDVIYERAFLAALPRDMWSE
YFSTVDKLLPSGGFLIGFFVIDDDYCSRFPPFCLRSGELASFLEPTFELVKSSVVANSVEVF
KGREQWMVWQKR

> SYNECHOCOCCUS ELONGATUS PCC 6301MTNAVNQAQFWEQRYQEGSDRWDLGQAAPVWRSLLAGTNAPAPGRIAVLGCGRGHDAR
LFAEQGFEVVGFDFAPSAIAAAQALAQGTTAQFLQRDIFALPQEFAGQFDTVLEHTCFCAID
PDRRAEYVEVVRQILKPKGCLLGLFWCHDRPSGPPYGCSLTELRDRFAQGWQEEQLESVTES
VEGRRGEEYLGRWRRLD

>GI|148239221|REF|YP_001224608.1| POSSIBLE THIOPURINE S-METHYLTRANSFERASE [SYNECHOCOCCUS SP. WH 7803]
MTNVHLPQAWDARYQHGTDGWELGKAAPPLQAFLEHHPRAPQPEGTVLVPGCGRGHEAALLA
RLGFEVIGLDFSSEAIREARRLHGEHPRLRWLQADLFDADALSGAGLASGSLSGVLEHTCFC
AIDPSQRAHYRSTVDRLLRAEGWLLGLFFCHPRPGGPPFGSDPEQLAASWAQIGFYPLIWEP
ARGSVAGRSEEWLGFWRKPEQRSA

>GI|87124194|REF|ZP_01080043.1| THIOL METHYLTRANSFERASE 1-LIKE PROTEIN [SYNECHOCOCCUS SP. RS9917]
MQLDGASSAPTLTARDWDARYRQGTDRWELGMAAPPLQAFLEQHPLAPKPTGTVLVPGCGRG
HEAALLARLGFDVVGLDFSVEAIREARRLQGEHENLRWLQADLFNGAALDRAGLGAHSLSGV

VEHTCFCAIDPSQRDHYRSTVDRLLEPGGWLLGVFFCHDRPGGPPYGSDAEQLAASWSQIGF
TGVIWEPAQGSVAQRSDEWLGLWRKPSQADNEAIPAGSR

>GI|87124194|REF|ZP_01080043.1| THIOL METHYLTRANSFERASE 1-LIKE
PROTEIN [SYNECHOCOCCUS SP. RS9917]
MQLDGASSAPTLTARDWDARYRQGTDRWELGMAAPPLQAFLEQHPLAPKPTGTVLVPGCGRG
HEAALLARLGFDVVGLDFSVEAIREARRLQGEHENLRWLQADLFNGAALDRAGLGAHSLSGV
VEHTCFCAIDPSQRDHYRSTVDRLLEPGGWLLGVFFCHDRPGGPPYGSDAEQLAASWSQIGF
TGVIWEPAQGSVAQRSDEWLGLWRKPSQADNEAIPAGSR

>GI|111027025|REF|YP_709003.1| POSSIBLE 3-DEMETHYLUBIQUINONE-9
3-METHYLTRANSFERASE [RHODOCOCCUS SP. RHA1]
MVDAPRFPYPGSPPVHGPDDLYVTPPPWDIGRAQPVFVALAEGGAIRGRVLDCGCGTGEHVL
LAAGLGLDATGVDLAATALRIAEQKARDRGLTARFLHHDARRLAELGERFDTVLDCGLFHIF
DPDDRAAYVDSLRDVLVPGGRYLMLGFSDQQPGDWGPHRLTRDEITTAFDDGWTIDSLESAT
LEVTLDPAGMRAWQLAATRTWPHPIERECSAPC

>GI|118038664|REF|ZP_01510068.1| THIOPURINE S-
METHYLTRANSFERASE [BURKHOLDERIA PHYTOFIRMANS PSJN]
MSDPTQPSAPEFESRDPNSPEFWDERFERGFMPWDQAGVPSAFESFAARHAGAAVLIPGCGS
AYEAVWLAGHGYPVRAIDFSPAAVAAAHEQLGAQHADLVEQADFFTYELPFTPAWIYERAFL
CALPLARRADYARRMADLLPGGALLAGFFFIGATPKGPPFGIERAELDGLLKPYFELIEDEP
VHDSIAVFAGRERWLTWRRRV

>GI|91685753|GB|ABE28953.1| CONSERVED HYPOTHETICAL PROTEIN
[BURKHOLDERIA XENOVORANS LB400]
MSDPTQPAVPDFETRDPNSPAFWDERFERRFTPWDQAGVPAAFQSFAARHSGAAVLIPGCGS
AYEAVWLAGQGNPVRAIDFSPAAVAAAHEQLGAQHAQLVEQADFFTYEPPFTPAWIYERAFL
CALPLARRADYAHRMADLLPGGALLAGFFFLGATPKGPPFGIERAELDALLTPYFDLIEDEA
VHDSIAVFAGRERWLTWRRRA

>GI|118655249|GB|EAV62028.1| THIOPURINE S-METHYLTRANSFERASE
[BURKHOLDERIA CENOCEPACIA MC0-3]
MSDPKQPAAPSAAEFATRDPGSASFWDERFARGVTPWEFGGVPDGFRAFAQRHEPCAVLIPG
CGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKVQLGAHADVVEQADFFQYRPPFDVQWVYERA
FLCALPPSLRADYAARMAELLPTGGLLAGYFFVVAKPKGPPFGIERAELDALLAPHFELLED
LPVTDSLAVFDGHERWLTWRRR

>GI|134140082|GB|ABO55825.1| THIOPURINE S-METHYLTRANSFERASE
[BURKHOLDERIA VIETNAMIENSIS G4]
MSNPTQPPPPSAADFATRDPANASFWDERFARGVTPWEFGGVPDGFRAFAQRRAPCTVLIPG
CGSAQEAGWLAQAGWPVRAIDFAEQAVVAAKATLGAHADVVEQADFFAYQPPFVVQWVYERA
FLCALPPSLRAGYAARMAELLPAGGLLAGYFFVMKKPKGPPFGIERAELDALLAPSFELIED
LPVTDSLAVFDGHERWLTWRRR

>GI|83653077|GB|ABC37140.1| THIOPURINE S-METHYLTRANSFERASE
FAMILY PROTEIN [BURKHOLDERIA THAILANDENSIS E264]
MTSEANKGDAAVQAAGDAQPASPASPPSADVQPARAALAPSSVPPAPSAANFASRDPGDASF
WDERFERGVTPWDSARVPDAFAAFAARHPRCPVLIPGCGSAYEARWLARAGWPVRAIDFSAQ
AVAAARRESGADAALVEQADFFAYVPPFVPQWIYERAFLCAIPTSRRADYARRVAELLPAGG
FLAGFFFIGATPKGPPFGIERAELDALLSPNFELVEDEPVADSLPVFAGRERWLAWRRS

>GI|148029498|GB|EDK87403.1| THIOPURINE S-METHYLTRANSFERASE FAMILY PROTEIN [BURKHOLDERIA MALLEI 2002721280]
MKDRLMSQGDGVTNEANQPEAAGQAAGDAQPASPAGPAHIANPANPANPPALPSFSPPAAAS
SSASSAAPFSSRDPGDASFWDERFEQGVTPWDSARVPDAFAARHARVPVLIPGCGSAYEARW
LARAGWPVRAIDFSAQAVAAARRELGEDAGLVEQADFFTYAPPFVPQWIYERAFLCAIPRSR
RADYARRMAELLPPGGFLAGFFFIGATPKGPPFGIERAELDALLCPHFALVEDEPVADSLPV
FAGRERWLAWRRS

>GI|116648837|GB|ABK09478.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA CENOCEPACIA HI2424]
MSDPKQPAAPSAADFATRDPGSASFWDERFARGVTPWEFGGVPDGFRVFAQRREPCAVLIPG
CGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKAQLGAHADVVEQADFFQYRPPFDVQWVYERA
FLCALPPGLRAGYAARMAELLPTGGLLAGYFFVVAKPKGPPFGIERAELDALLAPHFELLED
LPVTDSLAVFDGHERWLTWRRR

>GI|124292927|GB|ABN02196.1| THIOPURINE S-METHYLTRANSFERASE FAMILY PROTEIN [BURKHOLDERIA MALLEI NCTC 10229]
MSQGDGVTNEANQPEAAGQAAGDAQPASPAGPAHIANPANPANPPALPSFSPPAAASSSASS
AAPFSSRDPGDASFWDERFEQGVTPWDSARVPDAFAARHARVPVLIPGCGSAYEARWLARAG
WPVRAIDFSAQAVAAARRELGEDAGLVEQADFFTYAPPFVPQWIYERAFLCAIPRSRRADYA
RRMAELLPPGGFLAGFFFIGATPKGPPFGIERAELDALLCPHFALVEDEPVADSLPVFAGRE
RWLAWRRS

>GI|84362923|REF|ZP_00987534.1| COG0500: SAM-DEPENDENT METHYLTRANSFERASES [BURKHOLDERIA DOLOSA AUO158]
MTGRSFAMSDPKQPGTPTAADFATRDPGDASFWDERFARGVTPWEFGGVPDGFRAFAQRLER
CAVLIPGCGSAQEAGWLADAGWPVRAIDFAAQAVATAKAQLGAHADVVELADFFTYRPPFDV
RWIYERAFLCALPPARRADYAAQMAALLPAGGLLAGYFFVTAKPKGPPFGIERAELDALLAP
QFDLIDDWPVTDSLPVFEGHERWLTWRRR

>GI|147750562|GB|EDK57631.1| THIOPURINE S-METHYLTRANSFERASE FAMILY PROTEIN [BURKHOLDERIA MALLEI JHU]
MTNEANQPEAAGQAAGDAQPASPAGPAHIANPANPANPPALPSFSPPAAASSSASSAAPFSS
RDPGDASFWDERFEQGVTPWDSARVPDAFAARHARVPVLIPGCGSAYEARWLARAGWPVRAI
DFSAQAVAAARRELGEDAGLVEQADFFTYAPPFVPQWIYERAFLCAIPRSRRADYARRMAEL
LPPGGFLAGFFFIGATPKGPPFGIERAELDALLCPHFALVEDEPVADSLPVFAGRERWLAWR
RS

>GI|126220666|GB|ABN84172.1| PUTATIVE THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA PSEUDOMALLEI 668]
MKDRLMSQGDGVTNEANQPEAAGQAAGDAQPASPAGPAHIANPANPANPANPPALPSLSPPA
AAPSSASSAAHFSSRDPGDASFWDERFEQGVTPWDSARVPDAFAAFAARHARVPVLIPGCGS
AYEARWLARAGWLVRAIDFSAQAVAAARRELGEDARLVEQADFFTYAPPFVPQWIYERAFLC
AIPRSRRADYARRMAELLPPGGFLAGFFFIGATPKGPPFGIERAELDALLCPRFALVEDEPV
ADSLPVFAGRERWLAWRRS

>GI|77968269|GB|ABB09649.1| THIOPURINE S-METHYLTRANSFERASE [BURKHOLDERIA SP. 383]
MSDPKQPKPNAPAAADFTTRDPGNASFWNERFERGVTPWEFGGVPEGFSVFAHRLELCAVLI
PGCGSAQEAGWLAEAGWPVRAIDFAAQAVAAAKAQLGAHAGVVEQADFFAYRPPFDVQWVYE

RAFLCALPPAMRADYAARMAELLPADGLLAGYFFLMAKPKGPPFGIERAELDALLTPHFELI
EDLPVTDSLAVFEGHERWLTWRRR

>GI|115282818|GB|ABI88335.1| THIOPURINE S-METHYLTRANSFERASE
[BURKHOLDERIA AMBIFARIA AMMD]
MSEPKQPSTPGAADFATRDPGDASFWDERFARGVTPWEFGGVPEGFRAFAQRLGPCAVLIPG
CGSAQEAGWLAQAGWPVRAIDFAAQAVAAAKAQLGAHADVVEQADFFMYRPPFDVQWVYERA
FLCALPPSLRAGYAARMAELLPAGALLAGYFFVTKKPKGPPFGIERAELDALLAPHFELIDD
LPVTDSLAVFEGHERWLTWRRR

>GI|118659542|GB|EAV66286.1| THIOPURINE S-METHYLTRANSFERASE
[BURKHOLDERIA MULTIVORANS ATCC 17616]
MSDPKHAAAPAAASFETRDPGDASFWDERFARGMTPWEFGGVPAGFRAFASARPPCAVLIPG
CGSAREAGWLAQAGWPVRAIDFSAQAVAAAKAQLGAHADVVEQADFFAYRPPFDVQWIYERA
FLCALPPARRADYAATMAALLPAQGLLAGYFFVADKQKGPPFGITRGELDALLGAHFELIDD
APVSDSLPVFEGHERWLAWRRR

>GI|113866478|REF|YP_724967.1| THIOPURINE S-METHYLTRANSFERASE
(TPMT) [RALSTONIA EUTROPHA H16]
MSDPAKPVPTFATRNAADPAFWDERFEQGFTPWDQGGVPEEFRQFIEGRAPCPTLVPGCGNG
WEAAWLFERGWPVTAIDFSPQAVASARQTLGPAGVVVQQGDFFAFTPQPPCELIYERAFLCA
LPPAMRADYAARVAQLLPPGGLLAGYFYLGENRGGPPFAMPAEALDALLAPAFERLEDRPTA
APLPVFQGQERWQVWRRRSG

>GI|151577463|GB|EDN41864.1| THIOPURINE S-METHYLTRANSFERASE
[RALSTONIA PICKETTII 12D]
MAEPPVFQSRDAADPAFWDERFSREHTPWDAAGVPAAFQQFCESQPVPLSTLIPGCGSAYEA
GWLAERGWPVTAIDFAPSAVASARAVLGPHADVVEMADFFGFSPARSVQWIYERAFLCAMPR
RLWPDYAAQVAKLLPPGGLLAGFFAVVEGREAVPKGPPFETTQPELDALLSPAFERISDIPI
AEADSIPVFAGRERWQVWRRRAD

>GI|34102667|GB|AAQ59032.1| CONSERVED HYPOTHETICAL PROTEIN
[CHROMOBACTERIUM VIOLACEUM ATCC 12472]
MADSSRADFWEQRYREGVTPWEGGQLPPRARAFFAAQRPLRVLMPGCGSAADLPPLLAMGHD
VLAVDFSEAAIELAARQWPEAAGRLLLADFFQLQMPAFDCLFERAFLCALPVGMRSQYAERV
AALIAPGGALAGVFFVADTERGPPFGMQAEALRELLSPWFELEEDLALDESVAVFRNRERWM
VWRRRGFDLGQVSEHESTGNCGAHRKE

>GI|157353828|EMB|CAO46360.1| UNNAMED PROTEIN PRODUCT [VITIS
VINIFERA]
MGLCVPSGRISGGVCGLLSGRSLTWAKNLGVSTTQLRMSNNGSSIESNPKVQKLNQIIGSDS
AGGWEKSWQQGHTPWDLGKPTPIIQHLHQTGTLPSGKTLVPGCGCGYDVVTIACPERFVVGL
DISDSAIKKAKELSSSLWNANHFTFLKEDFFTWNPTELFDLIFDYTFFCAIEPDMRSVWAKR
MRHLLKPDGELLTLMFPISDHAGGPPYKVSVADYEEVLHPMGFKAVSIVDNKMAIGPRKGRE
KLGRWKRTPSKSLL

>GI|46102042|GB|EAK87275.1| HYPOTHETICAL PROTEIN UM06489.1
[USTILAGO MAYDIS 521]
MTSSLSKDDQIQNLRRLFADSGVPNDPKAWDQAWIDSTTPWDANRPQPALVELLEGAHDADA
KVPDVDGNLIPVSQAIPKGDGTAVVPGCGRGYDARVFAERGLTSYGVDISSNAVAAANKWLG
DQDLPTELDDKVNFAEADFFTLGTSKSLVLELSKPGQATLAYDYTFLCAIPPSLRTTWAETY

TRLLAKHGVLIALVFPIHGDRPGGPPFSISPQLVRELLGSQKNADGSAAWTELVELKPKGPE
TRPDVERMMVWRRS

>GI|134057747|EMB|CAK38144.1| UNNAMED PROTEIN PRODUCT
[ASPERGILLUS NIGER]
MSEAPNPPVQGRLISHFADRRAEDQGSGWSALWDSNESVLWDRGSPSIALVDVVEQQQDVFF
PYTRDGRRKKALVPGCGRGYDPVMLALHGFDVYGLDISATGVSEATKYATSEMQSPQDVKFI
AGDFFSSEWESQALQDGDKFDLIYDYTFLCALHPDLRRKWAERMSQLLHPGGLLVCLEFPMY
KDTSLPGPPWGLNGVHWDLLARGGDGITNITKEEEDEDSGIQLSGQFRRAQYFRPIRSYPSG
KGTDMLSIYVRR

>GI|46137187|REF|XP_390285.1| HYPOTHETICAL PROTEIN FG10109.1
[GIBBERELLA ZEAE PH-1]
MATENPLEDRISSVPFAEQGPKWDSCWKDALTPWDRGTASIALHDLLAQRPDLVPPSQHQDH
RGHPLRDATGAIQKKTALVPGCGRGHDVLLLSSWGYDVWGLDYSAAAKEEAIKNQKQAESEG
LYMPVDGLDKGKIHWITGNFFAQDWSKGAGDDGKFDLIYDYTFLCALPPDARPKWAKRMTEL
LSHDGRLICLEFPSTKPMSANGPPWGVSPELYEALLAAPGEEIAYNDDGTVHEDPCSKPWAD
ALHRLSLLKPTRTHKAGMSPEGAVMDFLSVWSR

>GI|88184126|GB|EAQ91594.1| HYPOTHETICAL PROTEIN CHGG_03529
[CHAETOMIUM GLOBOSUM CBS 148.51]
MAHPKSDPPGRLITHFANRDRQSQKAGWSELWDSDQTDLWDRGMPSPALIDFITTRRDIIGR
LGGGRRRPRALVPGCGRGYDVVMLAFHGFDAIGLEVSQTAVNSARAYAEVELSDPSAYNFAT
EDDEKRRATCQPGTVSFVCGDFFQREWETSCFAPGDDGGFDLIYDYTFLCALLPEMRKDWAQ
QMRELIRPTGVLVCLEFPLYKDVTADGPPWGLQGIYWNLLAEGGNGRMDGPAATDGGRGPFS
RVAYIKPSRSYEMGRGTDMLSVWAPQEPSGDRKRPATAATPIPWCAHYLLNDTPAPFPLAYT
TSIVVNRVCVRPSSQKQLAEARVAVPVAGARSYMKGRLARVVRLPARRSHFQKGLGGWVKLE
LYCALEIRPGCVAGLHLSYRAPLDMRCARNLEPAASPSELD

>GI|119414856|GB|EAW24794.1| THIOL METHYLTRANSFERASE, PUTATIVE
[NEOSARTORYA FISCHERI NRRL 181]
MSNDPRLLSSIPEFIARYKENYVEGWAELWNKSEGKPLPFDRGFPNPALEDTLIEKRDIIGG
PIGRDAQGNTYRKKALVPGCGRGVDVLLLASFGYDAYGLEYSDTAVQVCKEEQAKNGDKYPV
RDAEIGQGKITFVQGDFFKDTWLEKLQLPRNSFDLIYDYTFFCALDPSMRPQWALRHTQLLA
DSPRGHLICLEFPRHKDTSLQGPPWASTSEAYMAHLNHPGEEIPYDANRQCSIDPSKAPSPQ
GLERVAYWQPARTHEVGIVEGEVQDRVSIWRRPN

>GI|90307040|GB|EAS36671.1| HYPOTHETICAL PROTEIN CIMG_02025
[COCCIDIOIDES IMMITIS RS]
MANEILRSAPNLSDRFKNLDGRNQGEVWDDLWKESRTPWDRGSHNPALEDALVEKRGFFGAP
VFEDEPLRRKKALVPGCGRGVDVFLLASFGYDAYGLEYSKTAVDVCLKEMEKYGEGGKVPPR
DEKVGSGKVMFLEGDFFKDDWVKEAGVEDGAFDLIYDYTFFCALNPALRPQWALRHRQLLAP
SPRGNLICLEFPTTKDPAALGPPFASTPAMYMEHLSHPGEDIPYDDKGHVKSNPLQQPSDKG
LERVAHWQPKRTHTVGMDDKGNVLDWVSIWRRRD

>GI|145018369|GB|EDK02648.1| THIOL METHYLTRANSFERASE 1,
PUTATIVE [MAGNAPORTHE GRISEA 70-15]
MGTPEQTNKLSNLFLDQPLSEHGKRWDGLWKEDYTPWDRAGPSMALYDVLTGRPDLVPPPTG
GQKKRALVPGCGRGYDVLLLSRLGYDVWGLDYSEEATKQSIIYEKKVEQGDDGTYAELEREG
VKKGKVTWLTGDFFSDEWVNKAGVQQFDLTYDYTFLCALPISARPAWARRMADLLAHEGRLV

CLQWPTAKPWSGGGPPWGVLPEHYIAQLARPGEKVEYESDGKIPAQAMPKVVEQGGLRRLEL
VVPSRTHNSGIADGVLHDRIAVFAH

>GI|111069917|GB|EAT91037.1| HYPOTHETICAL PROTEIN SNOG_01388
[PHAEOSPHAERIA NODORUM SN15]
MANPNQDRLRSHFAALDPSTHASGWDSLWAEGTFIPWDRGYANPALIDLLANPSSPPTSSDA
NPTPGAPKPNTIDGQGVQLPAPLEGGVRRKALVPGCGKGYDVALLASWGYDTWGLEVSRHAA
DAAKEYLKDAGEGALEGEYKIKDAKIGKGREECVVADFFDDAWLKDVGAGEFDVIYDNTFLC
ALPPLLRPKWAARMAQLLARDGVLICLEFPTHKPASSGGPPWSLPPTVHQELLKRPGEDISY
DEGGVVVATDRAESENALVRVAHWTPKRTHNIAVINGVVRDCVSVWRHKKQS

>GI|39577142|EMB|CAE80965.1| CONSERVED HYPOTHETICAL PROTEIN
[BDELLOVIBRIO BACTERIOVORUS HD100]
MAIPTNFIQIDEEGFALSREVRIQDPIVGQEILQNLKIHEGGTLLSTFGDVPVIVEAFDEPY
VAAQVNLKEDKTWEILLPYGVHYAFELESLSLDEWDRFHGYAANKIPFVMSRKAQATFFNLL
EEFGDDFIEFDGKTYDIPAYWPPHKDVEKETYWSQIYQQEENPGWNLGEPAEALKDMIPRLK
ISRSRVLVLGCGEGHDAALFAAAGHFVTAVDISPLALERAKKLYGHLPTLTFVEADLFKLPQ
DFDQSFDVVFEHTCYCAINPERRQELVKVWNRVLVQGGHLMGVFFTFEKRQGPPYGGTEWEL
RQRLKNHYHPIFWGRWQKSIPRRQGKELFIYTKKK

>GI|35211380|DBJ|BAC88759.1| GLL0818 [GLOEOBACTER VIOLACEUS
PCC 7421]
MPSEESSGVDQPAFWEYRYRGGQDRWDLGQPAPTFVHLLSGSEAPPLGTVAVPGCGRGHDAL
LFAARGYKVCGFDFAADAIADATRLALRAGAAATFLQQDLFNLPRPFAGLFDLVVEHTCFCA
IDPVRREEYVEIVHWLLKPGGELVAIFFAHPRPGGPPYRTDAGEIERLFSPRFKITALLPAP
MSVPSRRGEELFGRFVRA

>GI|85818252|GB|EAQ39412.1| HYPOTHETICAL PROTEIN MED134_07976
[DOKDONIA DONGHAENSIS MED134]
MELTSTYWNNRYAEGSTGWDLKEVSPPIKAYLDQLENKELKILIPGGGYSYEAQYCWEQGFK
NVYVVDFSQLALENLKQRVPDFPSLQLIQEDFFTYDGQFDVIIEQTFFCALQPDLRPAYVAH
MHTLLKAKGKLVGLLFNFPLTEKGPPYGGSTTEYESLFSEHFDIQKMETAYNSVAARAGKEL
FIKMVKK

>GI|151939691|GB|EDN58518.1| THIOPURINE S-METHYLTRANSFERASE
(TPMT) SUPERFAMILY [VIBRIO SP. EX25]
MKQAPTINQQFWDNLFTQGTMPWDAKTTPQELKAYLENALHSGQSVFIPGCGAAYELSSFIQ
YGHDVIAMDYSEQAVKMAQSTLGKHKDKVVLGDVFNADSTHSFDVIYERAFLAALPRDQWPE
YFAMVDKLLPRGGLLIGYFVIDDDYHSRFPPFCLRSGELEGYLEPVFKLVESSVVANSVEVF
KGRERWMVWQKSCRI

>GI|124261369|GB|ABM96363.1| HYPOTHETICAL PROTEIN MPE_A3410
[METHYLIBIUM PETROLEIPHILUM PM1]
MSGPDLNFWQQRFDTGQLPWDRGAPSPQLAAWLGDGSLAPGRIAVPGCGSGHEVVALARGGF
SVTAIDYAPGAVRLTQGRLAAAGLAAEVVQADVLTWQPTAPLDAVYEQTCLCALHPDHWVAY
AARLHAWLRPGGTLALLAMQALREGAGQGLIEGPPYHVDVNALRALLPGDRWDWPRPPYARV
PHPSSTWAELAIVLTRR

>GI|114551449|GB|EAU54004.1| THIOL METHYLTRANSFERASE 1-LIKE
PROTEIN [MARIPROFUNDUS FERROOXYDANS PV-1]
MTVWEERYQRGETGWDRGGVSPALTQLVDHLHLEARVLIPGCGRGHEVIELARLGFRVTAID

IAPSAIAHLSQQLEQEDLDAELVNGDLFAYAPDHCFDAVYEQTCLCAIEPEQRADYEQRLHG
WLKPEGVLYALFMQTGIRGGPPFHCDLLMMRELFDASRWQWPEETGAVLVPHKNGRFELGHM
LRRTGR

>GI|92394583|GB|ABE75858.1| THIOPURINE S-METHYLTRANSFERASE
[PSYCHROBACTER CRYOHALOLENTIS K5]
MENVNQAQFWQQRYEQDSIGWDMGQVSPPLKAYIDQLPEAAKNQAVLVPGAGNAYEVGYLHE
QGFTNVTLVDFAPAPIAAFAERYPNFPAKHLICADFFELSPEQYQFDWVLEQTFFCAINPSR
RDEYVQQMASLVKPNGKLIGLLFDKDFGRDEPPFGGTKDEYQQRFATHFDIDIMEPSYNSHP
ARQGSELFIEMHVKD

>GI|83849399|GB|EAP87267.1| HYPOTHETICAL PROTEIN CA2559_00890
[CROCEIBACTER ATLANTICUS HTCC2559]
MTSNFWEQRYANNNTGWDLNTVSPPLKHYIDTLSNKTLFILIPGCGNAYEAEYLHNQGFENV
FIVDLAEHPLLEFSKRVPDFPKSHILHLDFFNLTQKFDLILEQTFFCALHPEQRLHYAHHTS
KLLNSNGCLVGLFFNKEFDKTGPPFGGNKKEYKNLFKNLFKIKKLENCYNSIKPRQGSELFF
IFEKK

>GI|120596574|GB|ABM40010.1| THIOPURINE S-METHYLTRANSFERASE
[POLAROMONAS NAPHTHALENIVORANS CJ2]
MAGPTTDFWQARFDNKETGWDRGAPGPQLLAWLESGALQPCRIAVPGCGSGWEVAELARRGF
EVVGIDYTPAAVERTRALLAAQGLAAEVVQADVLAYQPHKPFEAIYEQTCLCALHPDHWVAY
ARQLQQWLKPQGSIWALFMQMVRPEATDEGLIQGPPYHCDINAMRALFPAQHWAWPRPPYAK
VPHPNVGHELGLRLMLRQGR

[0184] Codon-optimized nucleic acids encoding the sequences above are synthesized and inserted into expression vectors active in E. coli, S. cervisiae, and other host cells. The cells are cultured in the presence of carbon and halide sources and under conditions in which the methylhalide transferase is expressed un under conditions in which methyl halide is produced. The methyl halide is optionally collected and converted into non-halogenated organic molecules.

Example 9B: Identifying New Methyl Halide Transferases

[0185] As described in Example 9A, to screen for MHTs with high activity in a recombinant host, we synthesized all putative MHTs from the NCBI sequence database and assayed methyl halide production in *E. coli*. We first identified a self-consistent set of 89 genes with similarity to known MHTs (Rhew et al., 2003, "Genetic control of methyl halide production in Arabidopsis," *Curr Biol* 13:1809-13; Attieh et al., 1995, "Purification and characterization of a novel methyltransferase responsible for biosynthesis of halomethanes and methanethiol in Brassica oleracea," *J Biol Chem* 270:9250-7; Ni and Hager, 1999, "Expression of Batis maritima methyl chloride transferase in Escherichia coli". *Proc Natl Acad Sci USA*

96:3611-5) The library contains a remarkable degree of sequence diversity, with an average of 26% amino acid identity between sequences. The library includes putative, hypothetical, and misannotated genes, as well as genes from uncharacterized organisms and environmental samples. These genes were computationally codon optimized for *E. coli* and yeast expression and constructed using automated whole gene DNA synthesis. This is an example of information-based cloning, where genetic data was retrieved from databases, the genes chemically synthesized, and function assayed, without contact with the source organisms.

[0186] Methyl halide activity was assayed on three ions (chloride, bromide, and iodide) by adding the appropriate halide salt to the growth media. Methyl halide production was sampled by analyzing the headspace gas using GC-MS (Supplementary Information). We found a wide distribution of activities on each ion, with 51 % of genes showing activity on chloride, 85% of genes showing activity on bromide, and 69 % of genes showing activity on iodide (Fig. 10A). In particular, the MHT from *Batis maritima*, a halophytic plant, displayed the highest activity of all genes on each ion. Several genes showed unique specificities for given ions (Figure 10B), a phenomenon that has also been observed on the organism level (Rhew et al., 2003, *supra*). The highest yield of methyl iodide is about 10-fold higher than methyl bromide, which is 10-fold higher then methyl chloride. This is consistent with the measured K_M of these enzymes: I^- (8.5 mM), Br^- (18.5 mM), and Cl^- (155 mM) (Attieh et al., 1995, *supra*, Ni and Hager, 1999, *supra*).

Example 10: Expression of B. maritima MHT in *Saccharomyces cerevisia*

[0187] We transferred the *B. maritima* MHT gene to the yeast *Saccharomyces cerevisia* (Fig. 11A). One advantage to metabolic engineering in a eukaryotic host is the ability to target gene products to specific cellular compartments that may be more favorable environments for enzyme function. We hypothesized that targeting the *B. maritima* MHT to the yeast vacuole could increase methyl iodide yield: the majority of SAM is sequestered in the vacuole (Farooqui et al., 1983, "Studies on compartmentation of S-adenosyl-L-methionine in Saccharomyces cerevisiae and isolated rat hepatocytes," *Biochim Biophys Acta* 757:342-51) and halide ions are sequestered there as well (Wada and Anraku, 1994 "Chemiosmotic coupling of ion transport in the yeast vacuole: its role in acidification inside organelles," *J Bioenerg*

Biomembr 26: 631-7). We targeted the *B.maritima* MHT to the yeast vacuole using a sixteen amino acid N-terminal tag from Carboxypeptidase Y as discussed above.

[0188] Yeast displayed high production rate from glucose or sucrose (Figures 11B and 11C) and normal growth rates. Methyl iodide yield from glucose was measured at 4.5 g/L-day, which is 10-fold higher than that obtained from *E. coli* and approximately 12,000-fold over the best natural source (Fig. 11C). In addition to rate, the carbon conversion efficiency of glucose to methyl iodide is an important parameter in determining process viability. For yeast, we determined the maximum theoretical yield of methyl iodide as 0.66 (mole fraction) from the balanced equation:

$$C_6H_{12}O_6 + 4I^- + 4H^+ + 8ATP \longrightarrow 4CH_3I + 2CO_2 + 2H_20$$

The maximum efficiency of carbon liberation from glucose is identical to the maximum efficiency of ethanol from glucose. The measured carbon conversion efficiency of glucose to methyl iodide is 2.5%, indicating room for yield improvement by redirecting carbon flux to SAM.

[0189] The response of the host organism to toxic effects of an overproduced metabolite is important for development of an integrated industrial process. Methyl halides are S_N2 methylating agents known to cause cytotoxic lesions in ssDNA and RNA. We found that yeast were resistant to deleterious methylating effects of methyl iodide up to high levels (> 5 g/L, Fig. 11D). Because the fermentation is aerobic and methyl iodide has a large Henry's constant (see Moore et al., 1995, *Chemosphere* 30 :1183-91), it can be recovered from the off-gas of the fermentor. A mutant strain deficient in a DNA-repair gene (*RAD50*ΔSymington et al., 2002, *Microbiol Mol Biol Rev* 66 :630-70) showed increased sensitivity to methyl iodide, confirming the role of methylation stress in cellular toxicity.

Example 11: Methyl iodide production by vacuole-targeted MHT

[0190] We fused a 16 amino acid vacuolar targeting tag (KAISLQRPLGLDKDVL) from yeast carboxypeptidase Y to the N-terminus of the B. maritima MCT and expressed the enzyme from the vector pCM190. Assays of methyl iodide production indicated that targeting the MCT to the vacuole resulted in a 50% increase in production rate (Figure 12). We next expressed the cytosolic and vacuolar targeted enzymes in a VPS33Δ background, which is unable to form functional vacuoles. The difference in production rate was abolished in the VPS33D strain, indicating that

MCT targeting to fully formed vacuoles is necessary for enhancing the rate of methyl iodide formation.

Example 12: Materials and Methods

[0191] This example describes materials and methods used in the examples discussed above.

STRAINS AND PLASMIDS

[0192] Cloning was performed using standard procedures in *E. coli* TOP10 cells (Invitrogen). Primers are listed below. The MHT coding regions were synthesized by DNA 2.0 (Menlo Park, CA) in the pTRC99a inducible expression vector carrying a gene for chloramphenicol resistance. Constructs were transformed into DH10B strain for methyl halide production assays. For yeast expression, the *B. maritima* MHT coding region was cloned into vector pCM190.

[0193] Cloning was performed using standard procedures in E. coli TOP10 cells (Invitrogen). The *B. maritima* MCT coding region was synthesized by DNA 2.0 (Menlo Park, CA) and amplified using specified primers with PfuUltra II (Stratagene) according to manufacturer's instructions. PCR products were purified using a Zymo Gel Extraction kit according to manufacturer's instructions. Purified expression vector (pCM190) and coding region insert were digested with restriction enzymes NotI and PstI overnight at 37 degrees and gel purified on a 1 % agarose gel and extracted using a Promega Wizard SV Gel kit according to manufacturer's instructions. Vector and insert were quantitated and ligated (10 fmol vector to 30 fmol insert) with T4 ligase (Invitrogen) for 15 minutes at room temperature and transformed into chemically competent *E. coli* TOP10 cells (Invitrogen). Transformants were screened and plasmids were sequenced using specified primers to confirm cloning.

[0194] Constructs were transformed into the *S. cerevisiae* W303a background using standard lithium acetate technique and plated on selective media. Briefly, competent W303a cells were prepared by sequential washes with water and 100mM lithium acetate in Tris-EDTA buffer. 1 □g of plasmid was incubated for 30 minutes at 30 degrees with 50 □L of competent cells along with 300 □L of PEG 4000 and 5 □g of boiled salmon sperm DNA as a carrier. Cells were then heat-shocked at 42 degrees for 20 minutes. Cells were spun down and resuspended in 100 □L water and plated on synthetic complete uracil dropout plates. Plates were incubated at 30 degrees for

48 hours and positive transformants were confirmed by streaking on uracil dropout plates.

MEDIA AND GROWTH CONDITIONS

[0195] Bacteria carrying MHT expression vectors were inoculated from freshly streaked plates and grown overnight. Cells were diluted 100-fold into media containing 1 mM IPTG and 100 mM appropriate sodium halide salt. Culture tubes were sealed with a rubber stopper and grown at 37 degrees for 3 hours. Yeast carrying MHT expression vectors were streaked on uracil dropout plates from freezer stocks (15% glycerol) and grown for 48 hours. Individual colonies were inoculated into 2 mL of synthetic complete uracil dropout media and grown overnight at 30 degrees. Cultures were next inoculated into 100mL fresh synthetic complete uracil dropout media and grown for 24 hours. Cells were spun down and concentrated to high cell density (OD 50) in fresh YP media with 2% glucose and 100 mM sodium iodide salt. 10 mL of this concentrated culture was aliquoted into 14 mL culture tubes and sealed with a rubber stopper. Cultures were grown at 30 degrees with 250 rpm shaking.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

[0196] The GC-MS system consisted of a model 6850 Series II Network GC system (Agilent) and model 5973 Network mass selective system (Agilent). Oven temperature was programmed from 50 degrees (1 min) to 70 degrees (10 degrees / min). 100 □L of culture headspace was withdrawn through the rubber stopper with a syringe and manually injected into the GC-MS. Samples were confirmed as methyl iodide by comparison with commercially obtained methyl iodide (Sigma), which had a retention time of 1.50 minutes and molecular weight of 142. Methyl iodide production was compared to a standard curve of commercially available methyl iodide in YPD. Standards were prepared at 0.1 g/L, 0.5 g/L 1.0 g/L, and 10 g/L in 10mL YP media plus 2% glucose, aliquoted into 14 mL culture tubes and sealed with rubber stoppers. Standards were incubated at 30 degrees for 1 hour and methyl iodide in the headspace was measured as above. A standard curve was fit to the data to relate headspace counts with methyl iodide.

METHYL IODIDE TOXICITY ASSAY

[0197] Individual colonies were inoculated in YP media with 2% glucose and grown overnight. Cultures were diluted to an OD_{600} of 0.05 and methyl iodide was added to the specified amount. Cultures were grown at 30 degrees with 250 rpm shaking for 24 hours. OD_{600} was measured by spectrometry with YP media used as a blank. Each data point was performed in triplicate. The *RAD50*Δ mutant was obtained from the Saccharomyces Genome Deletion Project (Invitrogen).

EFFICIENCY OF GLUCOSE TO METHYL IODIDE CONVERSION

[0198] Efficiency was measured as grams of high energy carbon produced per grams of glucose consumed. Methyl iodide production was measured by GC-MS of the culture headspace and the fraction of methyl iodide in the liquid phase was calculated using a standard curve. Grams of high energy carbon ($-CH_3$) are calculated by subtracting the molecular weight of the halide ion to give a comparison with other hydrocarbon production technologies. Amount of glucose consumed was calculated by measuring glucose in the growth media before and after a defined amount of time (90 min) with a hexokinase kit (Sigma) as per manufacturer's instructions and was quantitated using a standard glucose curve.

CUMULATIVE METHYL IODIDE PRODUCTION ASSAY

[0199] Long-term (>2 hour) methyl iodide production was measured by inducing cultures as above, assaying methyl iodide at 1 hour, and venting the culture to simulate product extraction. Cultures were then re-sealed and methyl iodide was measured again to determine how much methyl iodide had been vented. Cultures were again grown for 1 hour, measured, and vented. Data is displayed in the main text by summing the production each hour.

GROWTH AND METHYL IODIDE PRODUCTION ON CELLULOSIC STOCKS

[0200] *Actinotalea fermentans* was obtained from ATCC (43279). *A. fermentans* and *S. cerevisiae* cells were inoculated in either YP media + 2% glucose (for *S. cerevisiae*) or BH media + 2% glucose (for *A. fermentans*) and grown overnight. Cultures were diluted to OD_{600} = 0.05 in 50 mL of YP media with 20 g/L of cellulosic stock as the sole carbon source. Corn stover and poplar were pulverized using a

commercially available blender with a 1 HP, 1000W motor. Bagasse was aliquoted into the appropriate dry weight, then washed 3 times with hot water to remove soil and residual sugar. Cultures were incubated at 30 degrees with 250 rpm agitation for 36 hours. 9mL aliquots of cultures were placed in 14mL tubes with 1mL of 1M sodium chloride and sealed with a rubber stopper. Headspace samples were assayed for GC-MS production as above. *A. fermentans* and *S. cerevisiae* were quantitated as described below.

YEAST AND BACTERIA QUANTITATION

[0201] *S. cerevisiae* and *A. fermentans* were quantitated from cultures grown on cellulosic stocks by plating on selective media.. Cultures were diluted in sterile water and 100 uL was plated on either YPD agar + ampicillin (to quantitate *S. cerevisiae*) or brain-heart agar (to quantitate *A. fermentans*). Plates were incubated at 30 degrees for either 48 hours (for YPD) or 16 hours (for BH). Colonies were counted by hand and counts from at least 4 plates were averaged. In the switchgrass and corn stover grown cultures some unidentified background cultures were apparent but showed distinguishable morphology from *A. fermentans*.

Strains

[0202] *E. coli* (Invitrogen TOP10)

[0203] [F^- mcrA (mrr-hsdRMS-mcrBC) 80lacZM15 lacX74 recA1 ara139 (ara-leu)7697 galU galK rpsL (Str^R) endA1 nupG]

[0204] *S. cerevisiae* W303a

[0205] (MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15)

[0206] A. fermentans (ATCC 43279)

***

[0207] The examples given above are merely illustrative and are not meant to be an exhaustive list of all possible embodiments, applications or modifications of the invention. Thus, various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

[0208] The disclosures of all references and publications cited above are expressly incorporated by reference in their entireties to the same extent as if each were incorporated by reference individually.

CLAIMS

1. A bacteria-yeast co-culture comprising:

(i) *Actinotalea fermentans* bacteria which metabolize cellulose and produce one or more metabolic products, and

(ii) S. *cerevisiae* yeast,

wherein the yeast uses at least one metabolic product produced by the bacteria as a carbon source.

2. A co-culture system comprising a culture medium and

(i) a cellulosic bacterium component, wherein the bacteria metabolize cellulose and produce one or more metabolic products, and,

(ii) a yeast component, wherein the yeast uses at least one metabolic product of the bacteria as a carbon source.

3. The co-culture of claim 1 or 2 that comprises cellulose.

4. The co-culture system of any of claims 1-3 wherein the yeast is metabolically incapable of degrading cellulose.

5. The co-culture system of any of claims 1-3 wherein said at least one metabolic product is the sole or primary carbon and energy source for the yeast.

6. The co-culture system of any of claims 1-3 wherein the yeast is recombinantly modified to express a heterologous protein or over-express an endogenous protein.

7. The co-culture system of any of claims 1-3 wherein the yeast is a recombinantly modified to knock out expression of an endogenous protein.

8. The co-culture system of any of claims 1-3 wherein the bacteria and yeast grow together while maintaining a relatively constant ratio of species populations such that neither microorganism overtakes the other.

9. The co-culture system of any of claim 1-3 that is maintained under aerobic conditions.

10. The co-culture system of any of claims 1-3 that is maintained under anaerobic conditions.

11. The co-culture system of any of claims 1-3 wherein the yeast is from a genus selected from the group consisting of Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces

12. The co-culture system of claim 11 wherein the yeast is S. *cerevisiae*.

13. The co-culture of any of claims 1-3 in which the bacteria is a *Actinotalea* or *cellulomonas* species.

14. The co-culture of claim 13 in which the yeast is S. *cerevisiae* and the bacterium is *Actinotalea fermentans.*

15. The co-culture of any of claims 1- 3 in which the carbon source is molecule comprising 1-6 carbon atoms.

16. The co-culture of claim 15 wherein the carbon source is ethanol, acetate, lactate, succinate, citrate, formate or malate.

17. The co-culture system of any of claims 1-3 that comprises one species of yeast and one species of bacteria.

18. The co-culture system of claim 17 wherein the yeast and bacterium have a symbiotic relationship in culture.

19. The co-culture system of claim 6, wherein the yeast expresses a heterologous protein that is a mammalian protein.

20. The co-culture system of claim 6 in which the heterologous protein is a human protein used for treatment of patients.

21. The co-culture system of claim 6 wherein the heterologous protein is an enzyme.

22. The co-culture system of claim 21 in which the heterologous protein is a methyl halide transferase.

23. The co-culture system of any of claims 1-3 in which the yeast is genetically engineered to produce a commercially valuable small molecule compound.

24. The co-culture system of any of claims 1-3 in which the yeast is a naturally occurring or cultivated strain that is not recombinantly modified.

25. A yeast culture method comprising culturing cellulosic bacteria and yeast together in a liquid culture medium in the presence of cellulose or a cellulose-source, under conditions in which:

(i) the bacteria metabolize cellulose and produce one or more metabolic products, and,

(ii) the yeast component uses at least one metabolic product of the bacteria as a carbon source.

26. The method of claim 25 wherein the cellulose is microcrystaline cellulose.

27. The method of claim 25 in which the cellulose-source is biomass.

28. The method of claim 27 wherein the biomass is a pulverized feedstock selected from pulverized switchgrass, bagasse, elephant grass, corn stover, and poplar.

29. The method of claim 25 wherein the culture is maintained under aerobic conditions.

30. The method of claim 25 wherein the culture is maintained under anaerobic conditions.

31. The method of claim 25 wherein the yeast is metabolically incapable of degrading cellulose.

32. The method of claim 25 wherein the yeast and bacterium have a symbiotic relationship in culture.

33. The method of claim 25 wherein the yeast is from a genus selected from the group consisting of Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces

34. The method of claim 25 wherein the yeast is S. *cerevisiae*.

35. The method of claim 25 wherein the yeast is recombinantly modified to express a heterologous protein.

36. The method of claim 35 wherein the heterologous protein is a mammalian protein.

37. The method of claim 36 in which the heterologous protein is a human protein used for treatment of patients.

38. The method of claim 35 wherein the heterologous protein is an enzyme.

39. The method of claim 38 in which the heterologous protein is a methyl halide transferase.

40. The method of claim 25 wherein the yeast is a recombinantly modified to knock out expression of an endogenous protein.

41. The method of claim 25 wherein the yeast is a naturally occurring or cultivated strain that is not recombinantly modified.

42. The method of claim 25 in which the yeast is S. *cerevisiae* and the bacterium is *Actinotalea fermentans.*

43. The method of claim 25 in which the carbon source is molecule comprising 1-6 carbon atoms.

44. The method of claim 43 wherein the carbon source is ethanol, acetate, lactate, succinate, citrate, formate, or malate.

45. The method of any of claims 25-44 in which the bacterium is a *Actinotalea* or *cellulomonas* species.

46. The method of any of claims 25-41 and 43-44 in which the yeast is S. *cerevisiae* and the bacterium is *Actinotalea fermentans.*

47. The method of any of claims 25-44 further comprising recovering a product from the culture medium which product is produced by the yeast.

48. The method of claim 47 wherein the product is a recombinant protein expressed by the yeast.

49. The method of claim 47 wherein the product is a small molecule synthesized by the yeast cell.

50. The method of claim 47 wherein the synthesis requires expression of a heterologous protein in the yeast.

51. The method of claim 47 wherein the synthesis requires expression of an endogenous protein that is overexpressed in the yeast or deletion of one or more endogenous genes of the yeast.

52. The method of claim 50 wherein the product is a methyl halide.

53. The method of claim 47 wherein the product is a drug, food product, amino acid, cofactor, hormone, protein, vitamin, lipid, alkane, aromatic, olefin, alcohol, or biofuel intermediate.

54. A method for production of methyhalide comprising culturing a cellulosic bacteria which metabolizes cellulose and produces one or more metabolic products together with a yeast which does not metabolize cellulose and which is recombinantly modified to express a heterologous methyl halide transferase protein in a medium containing a cellulose source and a halide, under conditions in which methyl halide is produced.

55. The method of claim 54 wherein the halide is selected from the group consisting of chlorine, bromine and iodine.

56. A method comprising combining

i) a recombinant yeast comprising a heterologous gene encoding S-adenosylmethionine (SAM)-dependent methyl halide transferase (MHT),

ii) a halide selected from the group comprising chlorine, bromine and iodine; and

iii) a cellulolytic bacteria that produces a carbon source by metabolism of cellulose;

in a cultivation medium under conditions in which methyl halide is produced.

57. Method of claim 56 wherein the carbon source is molecule comprising 1-6 carbon atoms.

58. The method of claim 57 wherein the cellulolytic microorganism is a bacterium and the carbon source is ethanol, acetate, lactate, succinate, formate, citrate, or malate.

59. The method of claim 56 wherein the yeast is from a genus selected from the group consisting of Saccharomyces, Pichia, Hansenula, Kluyveromyces, Yarrowia, Trichoderma and Scizosacchromyces.

60. The method of claim 59 wherein the yeast is selected from the group consisting of *Saccharomyces cerevisiae, Pichia pastoris*, *Hansenula polymorpha*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Trichoderma reesei*, and *Scizosacchromyces pombe*.

61. The method of claim 60 wherein the yeast is S. *cerevisiae.*

62. The method of claim 56 wherein the bacterium is *Actinotalea fermentans.*

63. The method of claim 61 wheren MHT is from *Batis maritima.*

64. The method of any of claims 54-63 wherein the bacterium is *Actinotalea fermentans.*

65. The method of claim 54-63 wheren MHT is from *Batis maritima.*

66. The method of claim 54 or 56 in which the heterologous gene encodes a fusion protein comprising a MHT sequence and a targeting peptide sequence that targets the MHT sequence to the yeast vacuole.

67. The method of claim 66 wherein the targeting peptide sequence is the N-terminal peptide domain from carboxypeptidase Y.

68. The method of any of claims 54-63 further comprising recovering methyl halide from the culture medium.

69. The method of claim 68 further comprising the step of converting the methyl halide into a non-halogenated organic molecule or a mixture of non-halogenated organic molecules.

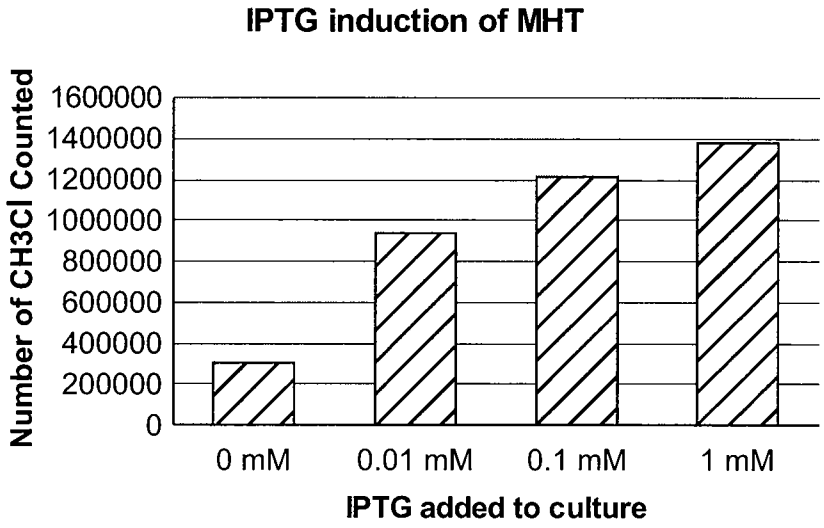

IPTG induction of MHT
Number of CH3Cl Counted
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0 mM
0.01 mM
0.1 mM
1 mM
IPTG added to culture

FIG. 1

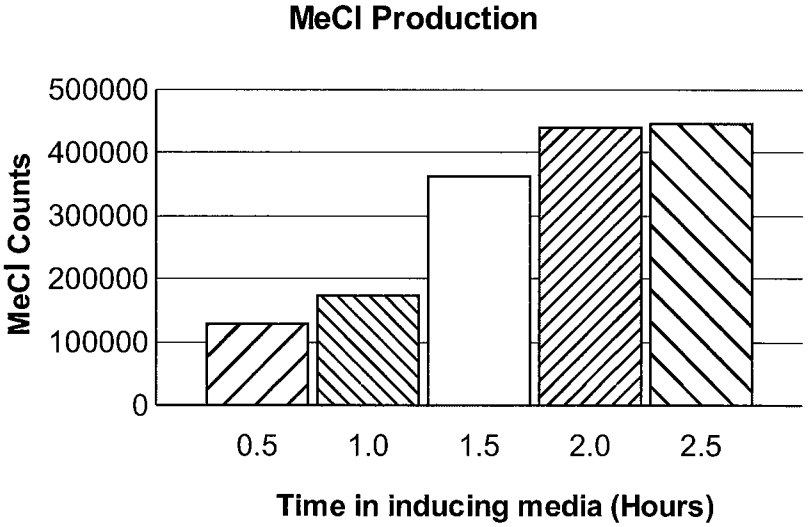

MeCl Production
MeCl Counts
500000
400000
300000
200000
100000
0
0.5
1.0
1.5
2.0
2.5
Time in inducing media (Hours)

FIG. 2

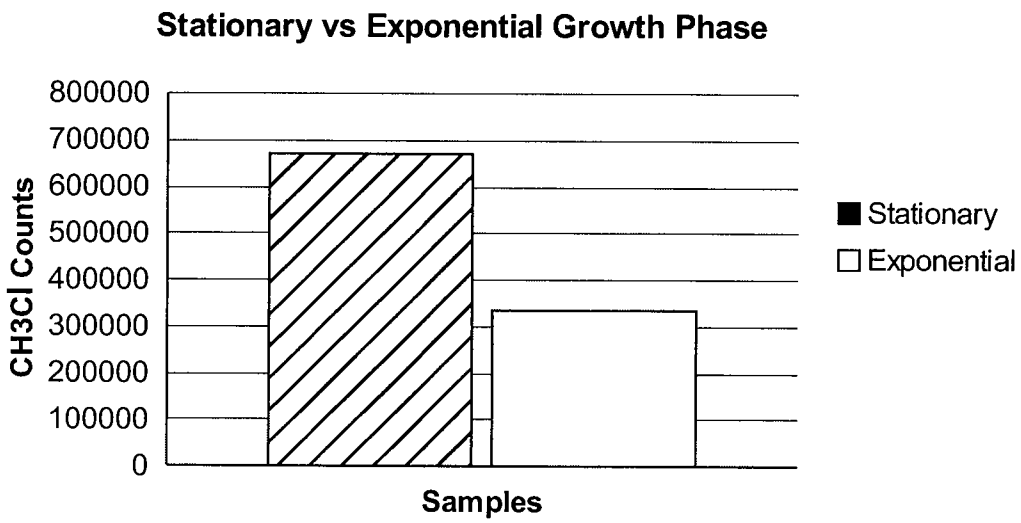

Stationary vs Exponential Growth Phase
800000
700000
600000
500000
400000
300000
200000
100000
0
CH3Cl Counts
Samples
Stationary
Exponential

FIG. 3

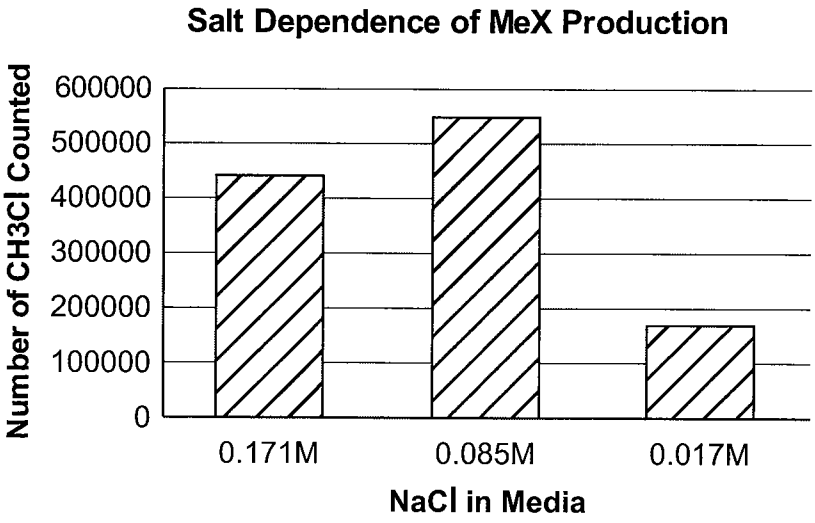

Salt Dependence of MeX Production
600000
500000
400000
300000
200000
100000
0
Number of CH3Cl Counted
0.171M
0.085M
0.017M
NaCl in Media

FIG. 4

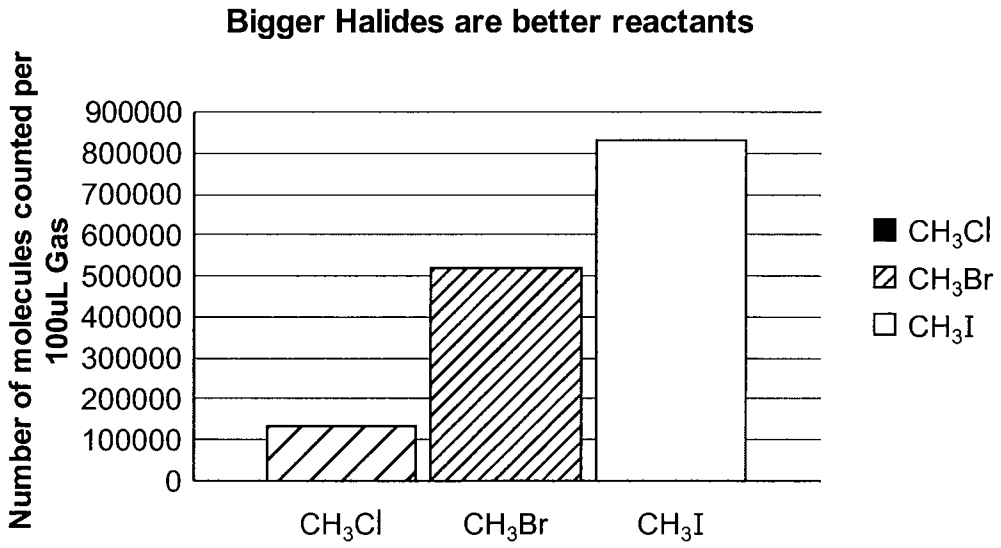

Bigger Halides are better reactants
Number of molecules counted per 100uL Gas
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
CH3Cl
CH3Br
CH3I
CH3Cl
CH3Br
CH3I

FIG. 5

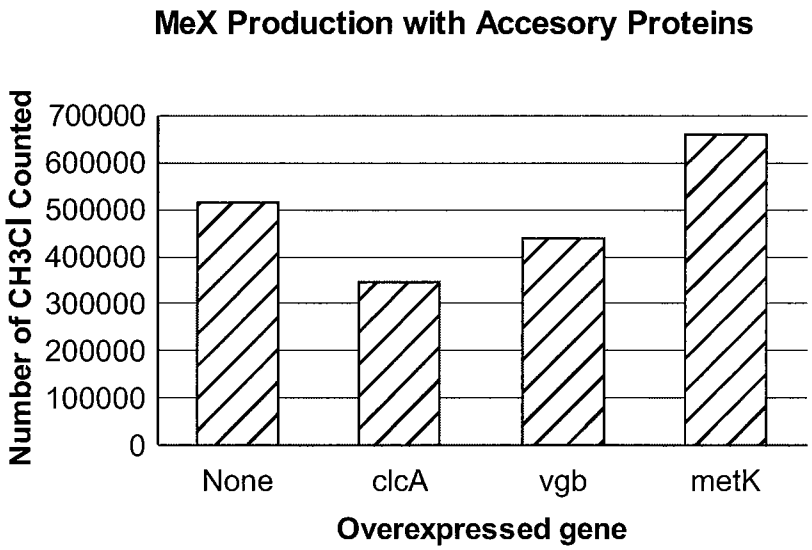

MeX Production with Accesory Proteins
Number of CH3Cl Counted
700000
600000
500000
400000
300000
200000
100000
0
None
clcA
vgb
metK
Overexpressed gene

FIG. 6

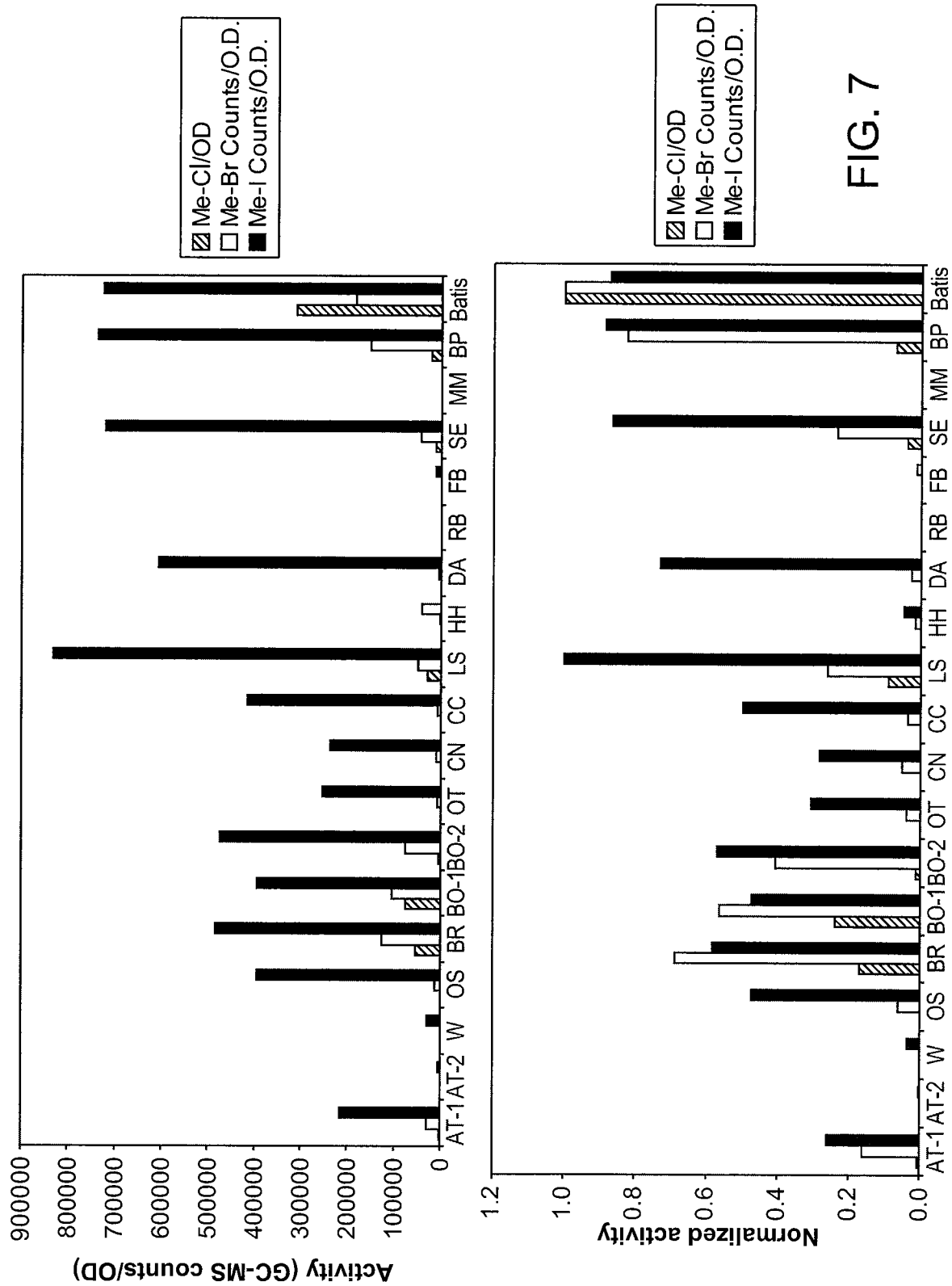

Me-Cl/OD
Me-Br Counts/O.D.
Me-I Counts/O.D.
Activity (GC-MS counts/OD)
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
AT-1 AT-2 W OS BR BO-1 BO-2 OT CN CC LS HH DA RB FB SE MM BP Batis
Normalized activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0

FIG. 7

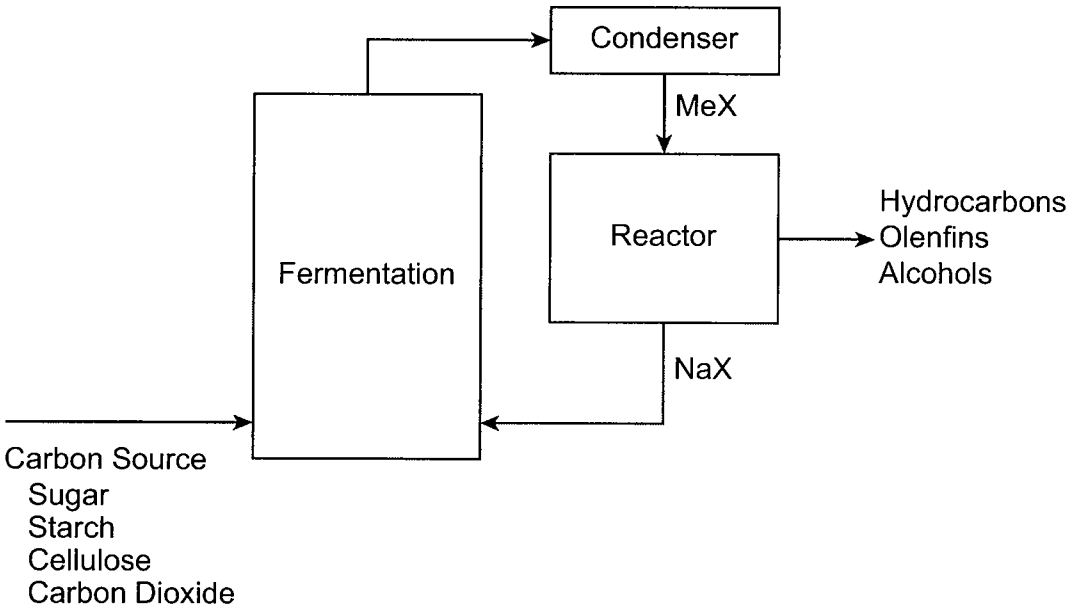

Condenser
MeX
Reactor
Hydrocarbons
Olenfins
Alcohols
Fermentation
NaX
Carbon Source
Sugar
Starch
Cellulose
Carbon Dioxide

FIG. 8

FIG. 9A

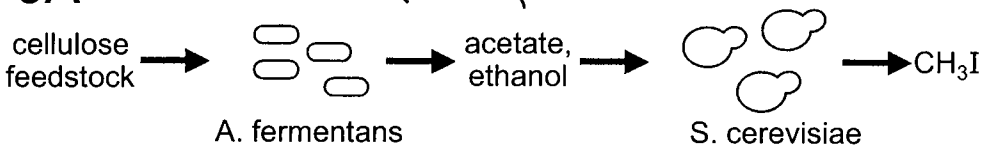


FIG. 9B

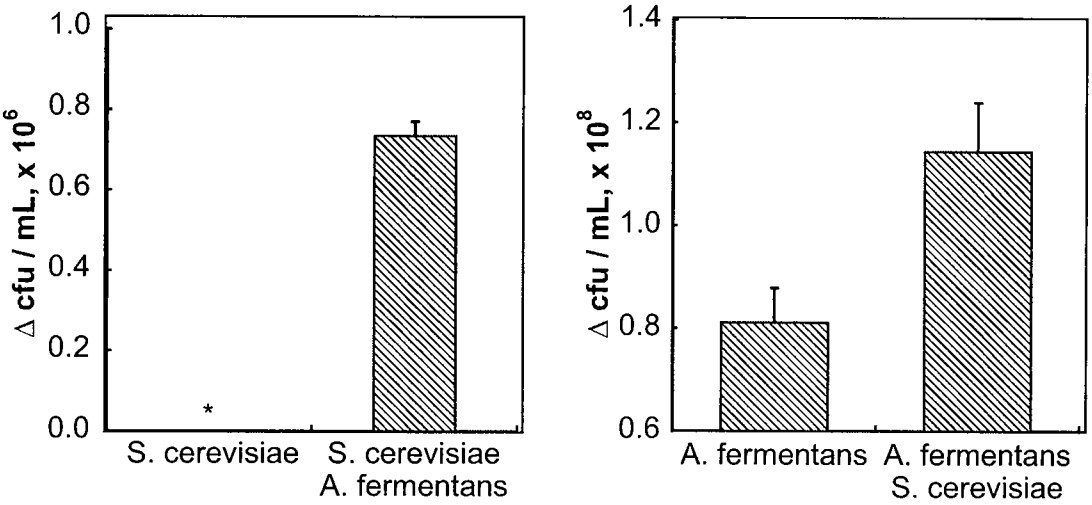
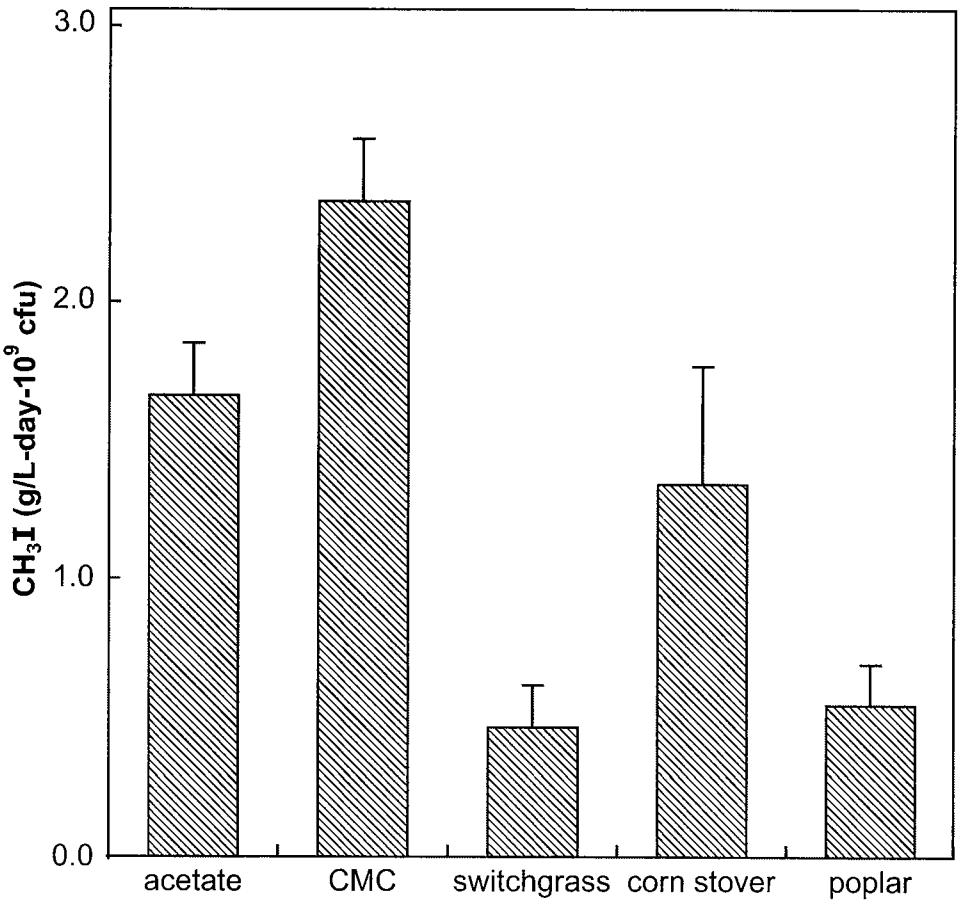


FIG. 9C



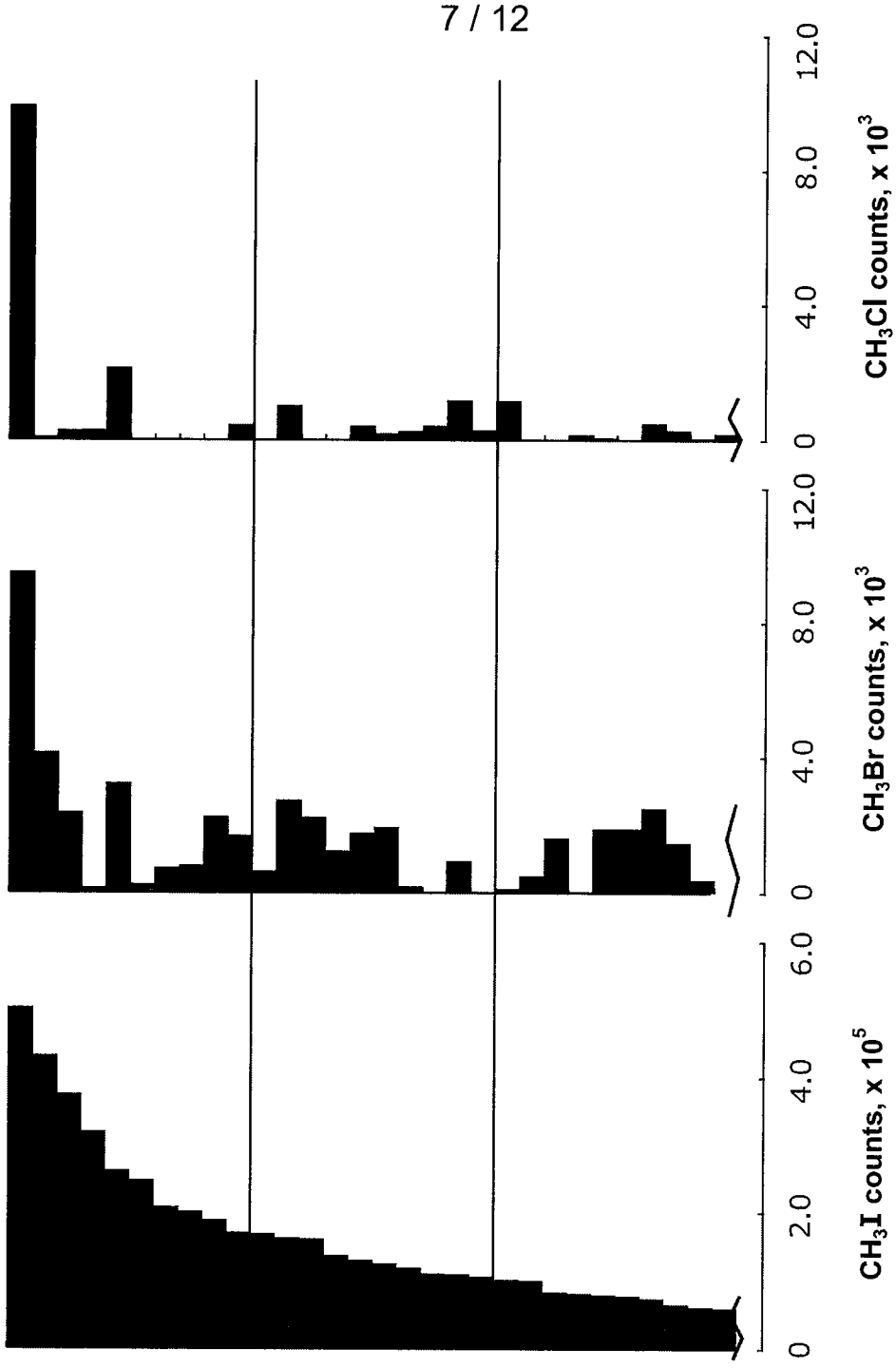

B. maritima
O. sativa 2
V. vinifera 2
B. xenovorans
B. rapa chinensis
B. pseudomallei
B. thailandensis
Marine bacterium HTCC2080
R. picketti
B. phytofirmans
B. vietnamensis
A. clavatus
P. nodorum
D. aromatica
K. algicida
M. petroleiphilum
B. dolosa
R. solanacearum
U. maydis
H. chejuensis
B. oleracea 2
B. ambifaria
R. ferrireducens
C. immitus
S. elongatus
R. eutropha
O. sativa japonica 2
B. multivorans
Cellulophaga sp.
N. fischeri
0
2.0
4.0
6.0
CH3I counts, x 10^5
0
4.0
8.0
12.0
CH3Br counts, x 10^3
0
4.0
8.0
12.0
CH3Cl counts, x 10^3

FIG. 10A

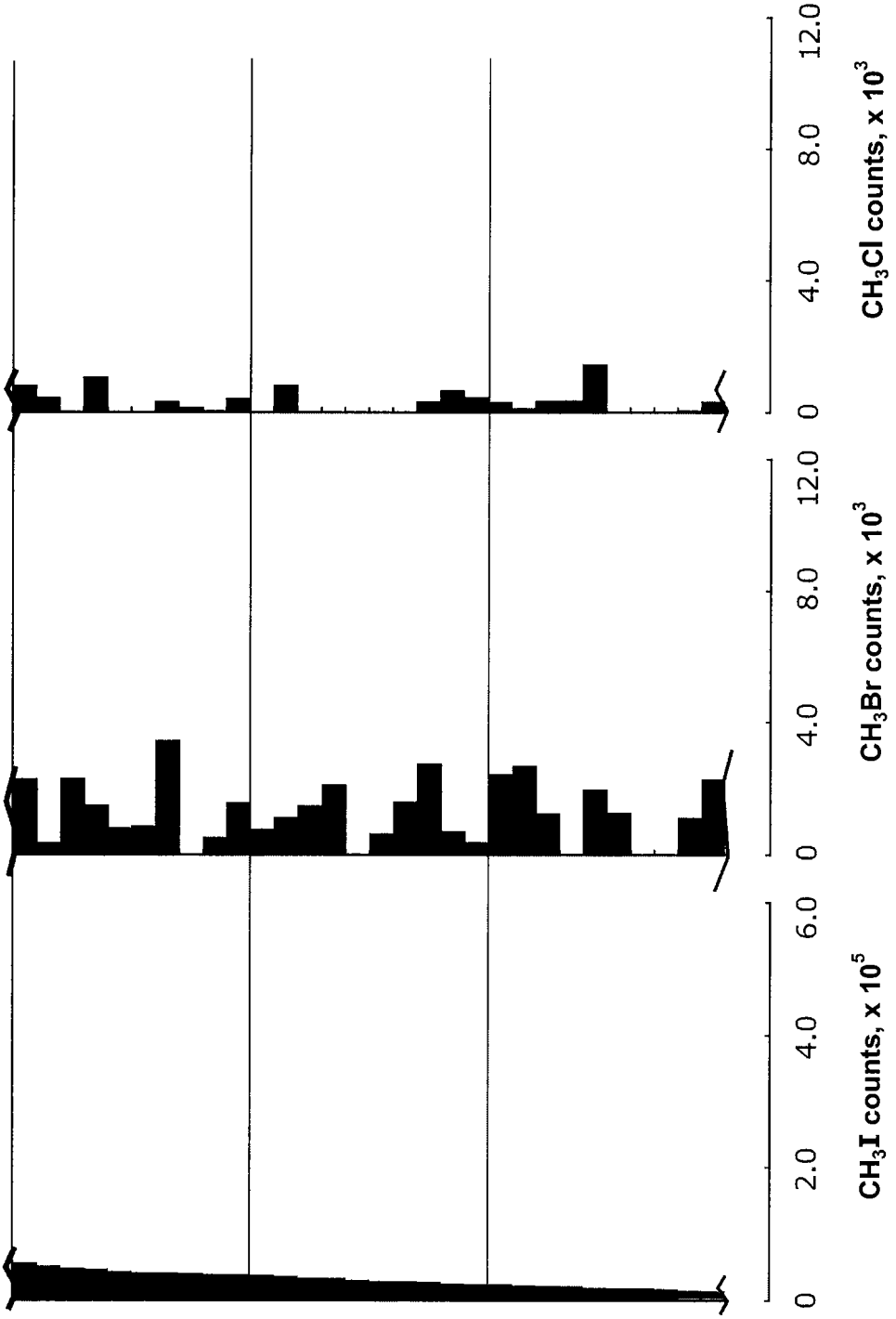

Tenacibaculum sp.
A. thaliana 3
G. zeae
L. pneumophila
B. cenocepacia
A. fumigatus
M. marina
O. alexandrii
B. cenocepacia
Leptospirillum sp.
M. ferooxydans
P. naphthalenivorans
Synechococcus sp. 1
F. bacterium
G. forsetii
B. oleracea 1
V. vinifera 3
Burkholderia sp.
F. bacterium
C. cinerea
Flavobacterium sp.
V. alginolyticus
C. neoformans
Algoriphagus sp.
V. parahaemolyticus
P. cryohalentis
C. atlanticus
A. niger 3
O. tauri
O. sativa 1
0
2.0
4.0
6.0
CH3I counts, x 10^5
0
4.0
8.0
12.0
CH3Br counts, x 10^3
0
4.0
8.0
12.0
CH3Cl counts, x 10^3

FIG. 10A (Cont.)

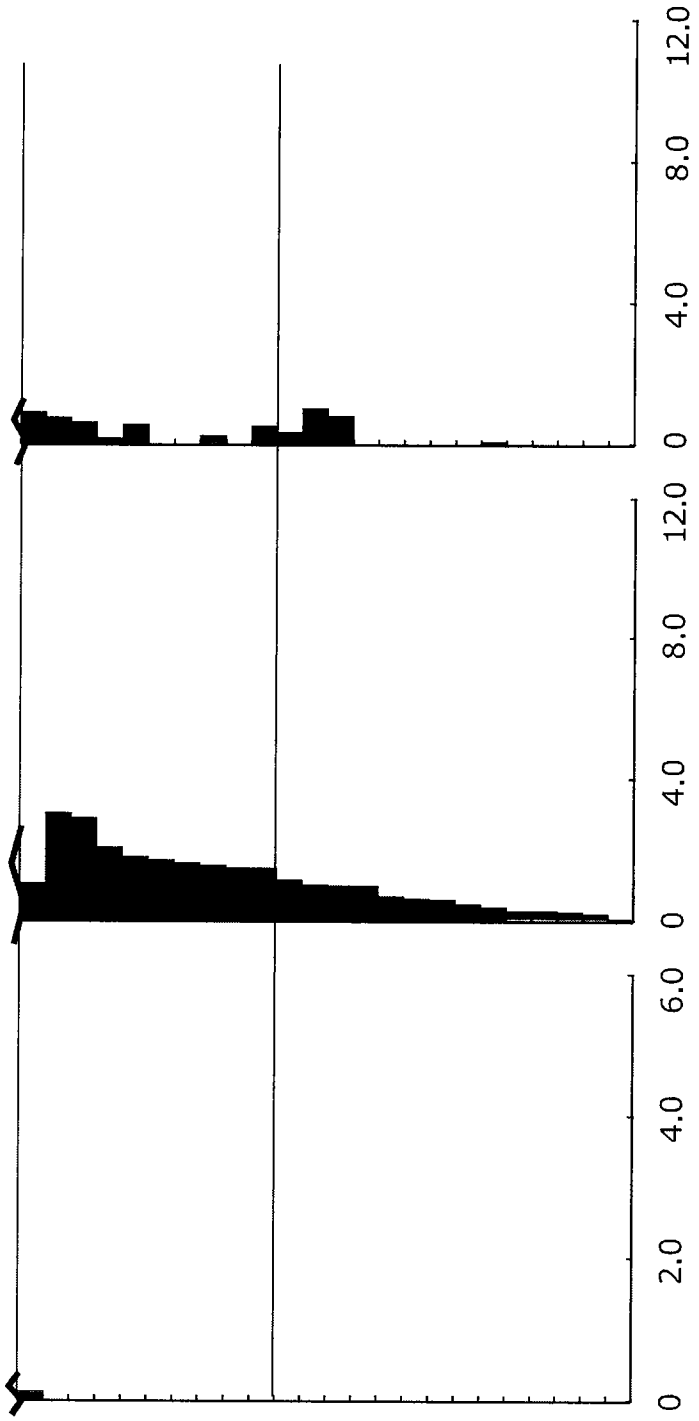

O. sativa japonica 1
P. irgensii
A. niger 1
A. nidulans 1
G. violaceus
A. dehalogenans
L. araneosa
P. arcticus
M. vanbaalenii
A. niger 2
M. acetovorans
Rhodococcus sp.
F. psychrophilum
Synechococcus sp. 2
A. metalliredigens
H. halophila
M. smegmatis
P. pacifica
A. thaliana 1
B. mallei
S. erythraea
Vibrio sp.
V. vinifera 1
A. thaliana 2
0
2.0
4.0
6.0
CH3I counts, x 10^5
0
4.0
8.0
12.0
CH3Br counts, x 10^3
0
4.0
8.0
12.0
CH3Cl counts, x 10^3

FIG. 10A (Cont.)

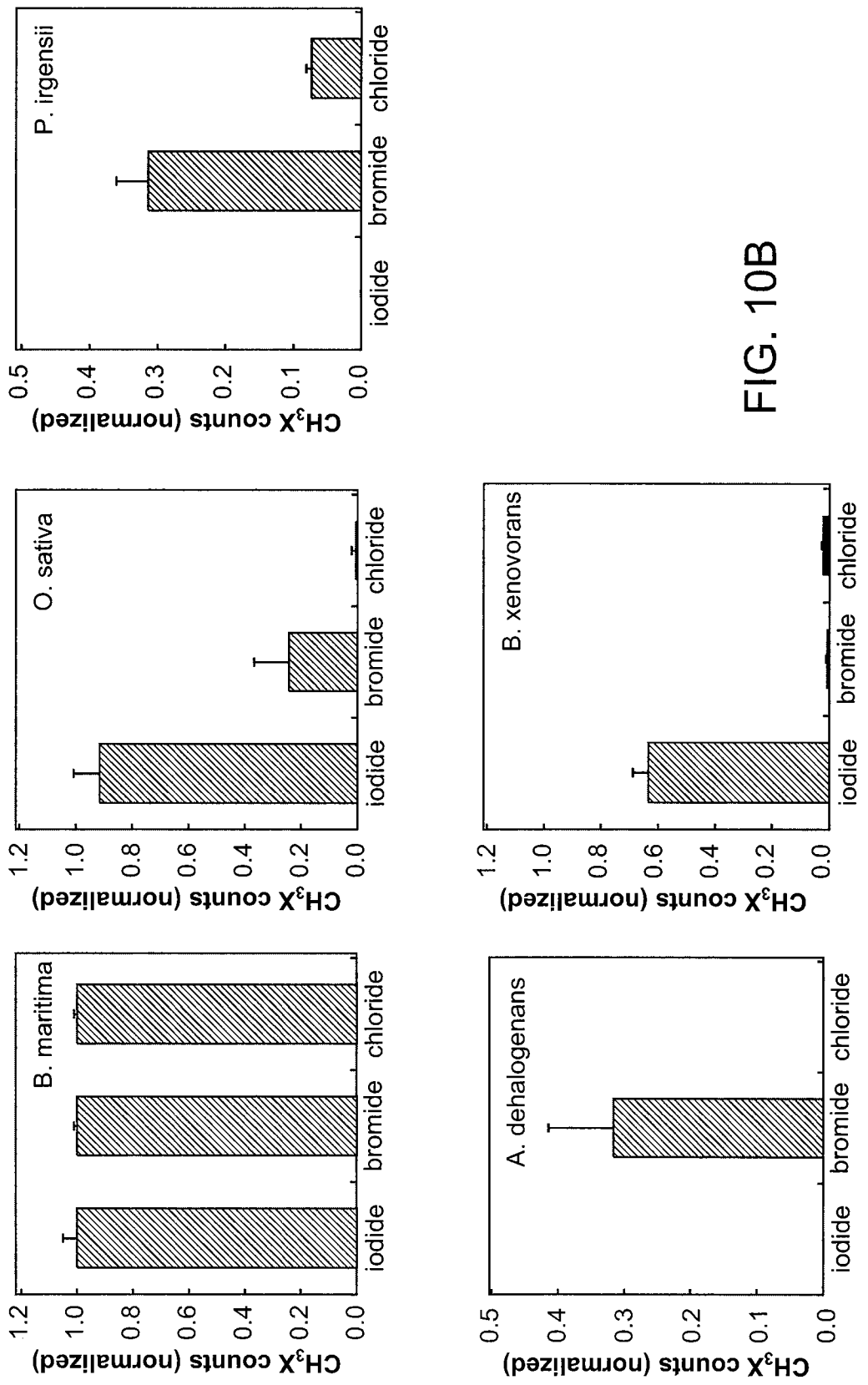

P. irgensii
O. sativa
B. xenovorans
B. maritima
A. dehalogenans
iodide
bromide
chloride
CH3X counts (normalized)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
1.0
1.2

FIG. 10B

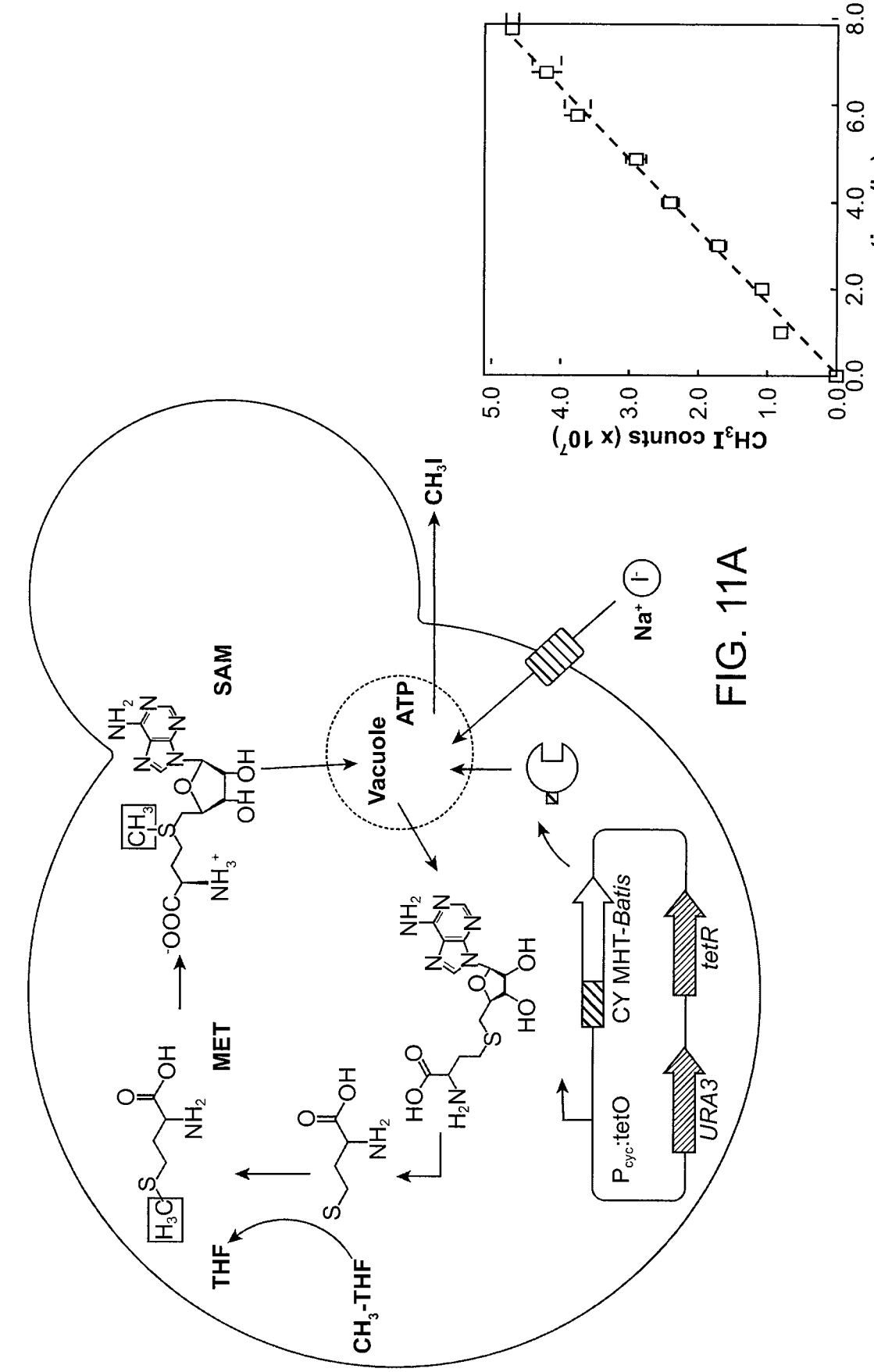


FIG. 11A

FIG. 11B

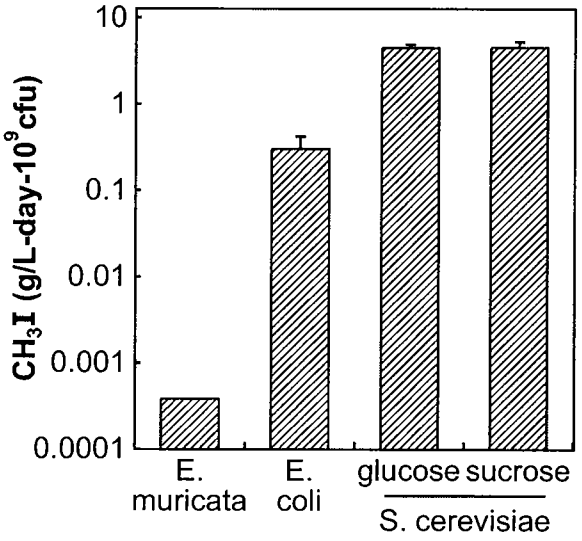


FIG. 11C

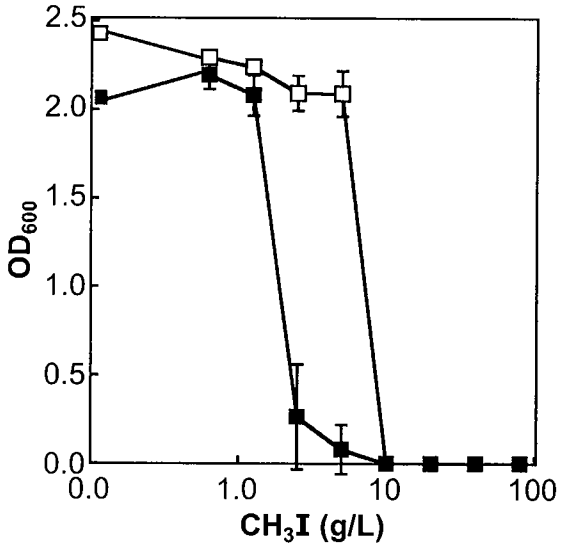


FIG. 11D

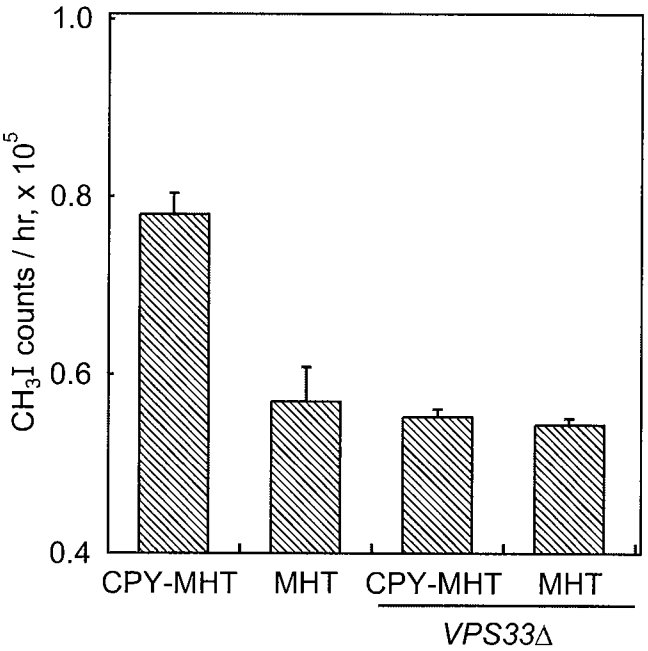


FIG. 12