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Synthesis of Methyl Halides from Biomass Using Engineered Microbes

Travis S. Bayer,[†] Daniel M. Widmaier,[†,‡] Karsten Temme,[†,§] Ethan A. Mirsky,[†,||] Daniel V. Santi,[†] and Christopher A. Voigt*[,†,‡,§,||]

Department of Pharmaceutical Chemistry, Chemistry and Chemical Biology Program, UCSF/UCB Joint Graduate Group in Bioengineering, and Biophysics Program, University of California, San Francisco, MC 2540, Room 408C, 1700 4th Street, San Francisco, California 94158-2330

Received December 11, 2008; E-mail: cavoigt@picasso.ucsf.edu

Abstract: Methyl halides are used as agricultural fumigants and are precursor molecules that can be catalytically converted to chemicals and fuels. Plants and microorganisms naturally produce methyl halides, but these organisms produce very low yields or are not amenable to industrial production. A single methyl halide transferase (MHT) enzyme transfers the methyl group from the ubiquitous metabolite S-adenoyl methionine (SAM) to a halide ion. Using a synthetic metagenomic approach, we chemically synthesized all 89 putative MHT genes from plants, fungi, bacteria, and unidentified organisms present in the NCBI sequence database. The set 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* results in productivity of 190 mg/L-h from glucose and sucrose. Using a symbiotic co-culture of the engineered yeast and the cellulolytic bacterium *Actinotalea fermentans*, we are able to achieve methyl halide production from unprocessed switchgrass (*Panicum virgatum*), corn stover, sugar cane bagasse, and poplar (*Populus* sp.). These results demonstrate the potential of producing methyl halides from non-food agricultural resources.

Introduction

The derivation of chemicals and fuels from petroleum poses a tremendous challenge for environmental and economic sustainability. The conversion of carbon in renewable biomass to valuable products will close the carbon cycle and relieve demand for petroleum.[1] A major focus of biofuels and bio-derived chemicals research is the discovery and engineering of enzymes and pathways for producing useful compounds.[2] Genetic and metabolic engineering has been used to construct microbial strains capable of converting biomass to ethanol,[3] butanol,[4,5] higher branched alcohols,[6] and propanediol.[7] These compounds can be used to supplement or replace the use of petroleum-derived chemicals.

The development of bioconversion processes requires the discovery of novel enzymes and metabolic pathways. The astonishing biodiversity in the microbial world has spurred searches for useful biocatalysts. Traditionally, researchers use culture-dependent methods, where strains are isolated depending on specific catalytic and metabolic functions they harbor.[8] However, culture-dependent methods are limited to a subset of microbes that can be cultured in laboratory conditions. To address this limitation, researchers have used metagenomic DNA libraries to screen for biocatalytic function.[9,10] Metagenomics is the sequence and functional analysis of DNA information from specific environments, such as soil or marine samples.[9] Metagenomic biocatalyst discovery strategies isolate DNA from particular environments and clone DNA fragments into vectors for expression in heterologous hosts.[9,10] The library can then be screened for metabolic functionality. Metagenomic DNA libraries have greatly enhanced the discovery of novel enzymes, pathways, and products from terrestrial and marine environments.[11]

An unexplored biocatalyst discovery approach involves searching the extensive sequence databases using bioinformatics and automated chemical DNA synthesis. We call this strategy "synthetic metagenomics" because the genes identified using this approach are often uncharacterized, annotated as putative or hypothetical, or from unknown organisms or metagenomic environmental samples. The genes are computationally optimized for expression, synthesized, cloned into a vector, and screened for function in *E. coli* or yeast.

[†] Department of Pharmaceutical Chemistry.
[‡] Chemistry and Chemical Biology Program.
[§] UCSF/UCB Joint Graduate Group in Bioengineering.
[||] Biophysics Program.

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In this work we use a synthetic metagenomic approach to identify and screen enzymes that enable *Escherichia coli* and yeast to convert biomass to methyl halides. As end products, methyl halides are used in a variety of applications, including use as solvents, silicone polymers, propellants, and soil fumigants.[12−18] On the bench scale, it has been demonstrated that methyl halides can also be used as intermediates for the chemical synthesis of more complex carbon compounds. Zeolite catalysts (e.g., ZSM-5 and SAPO-34) can be used to convert methyl halides to products including gasoline,[19] olefins,[20,22] aromatics,[19,23,24] alcohols,[25,26] and ethers.[25,26] Zeolites are widely used in the petrochemical industry and have been used on the industrial scale to convert methanol to gasoline and other commodity chemicals.,[19,23,25,27−32] A method to convert biomass to methyl halides would be make it possible to transform renewable sources of carbon into chemicals and liquid fuels. Ultimately, these products would be able to supplement or replace their petroleum-derived counterparts.

Methyl halides are naturally produced by a number of organisms, including marine algae, fungi, and halophytic plants.[33,34] The enzyme responsible for this production—methyl halide transferase (MHT)—has been studied in the context of the environmental production of methyl halides, which contribute to ozone depletion.[35,36] This led to the naming of the *Arabidopsis thaliana* gene identified by loss-of-function HOL-1 (Harmless to the Ozone Layer).[37] MHTs are small (~230 amino acids) monomeric enzymes that catalyze the formation of methyl halides using S-adenosyl methionine (SAM) to methylate chloride, bromide, or iodide.[38] SAM is a metabolite present in all cell types that is the "common currency" for methyl group transfers.[39] The universality of SAM could enable MHTs to be ported easily between organisms. Moving MHT into an industrial organism is critical because natural culturable sources, such as the marine red algae *Endocladia muricata*, are slow growing and show low methyl halide yields.[33,38]

Here, we designed a biological process for converting biomass to methyl halides by combining a synthetic metagenomic screen of putative enzymes with an engineered microbial co-culture. The synthetic microbial community involved symbiosis between the MHT-expressing yeast and a cellulolytic bacterium. This community is able to convert non-food agricultural lignocellulosic biomass (switchgrass, poplar, corn stover, and sugar cane bagasse) to methyl halides. This bioprocess could be used to produce methyl halides from renewable sources for use in industry or agriculture, or could be connected to chemical catalysis to produce a wide range of commodity chemicals or fuels.

Materials and Methods

Full methods are available in the Supporting Information.

Strains, Plasmids, and Gene Synthesis. Cloning was performed using standard procedures in *E. coli* TOP10 cells (Invitrogen). Primers are listed in the Supporting Information. The MHT coding regions were identified by a BLAST search of the NCBI database using known methyl chloride transferases and synthesized by DNA 2.0 (Menlo Park, CA) in the pTRC99a[40] inducible expression vector carrying a gene for chloramphenicol resistance. Constructs were transformed into DH10B strain for methyl halide production assays. For yeast expression, the *Batis maritima* MHT coding region was cloned into vector pCM190. Constructs were transformed into the *Saccharomyces cerevisiae* W303a background using standard lithium acetate technique and plated on selective media.[41]

Media and Growth Conditions. 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 °C for 3 h. Yeast-carrying MHT expression vectors were streaked on uracil dropout plates from freezer stocks (15% glycerol) and grown for 48 h. Individual colonies were inoculated into 2 mL of synthetic complete uracil dropout media and grown overnight at 30 °C. Cultures were next inoculated into 100 mL of fresh synthetic complete uracil dropout media and grown for 24 h. Cells were spun down and concentrated to high cell density ($OD_{600} = 50$) in fresh YP media with 2% glucose and 100 mM sodium iodide salt. Ten milliliter portions of this concentrated culture were aliquoted into 14 mL culture tubes and sealed with rubber stoppers. Cultures were grown at 30 °C with 250 rpm shaking.

Gas Chromatography−Mass Spectrometry. 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 (1 min) to 70 °C (10 deg/min). One hundred milliliters 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

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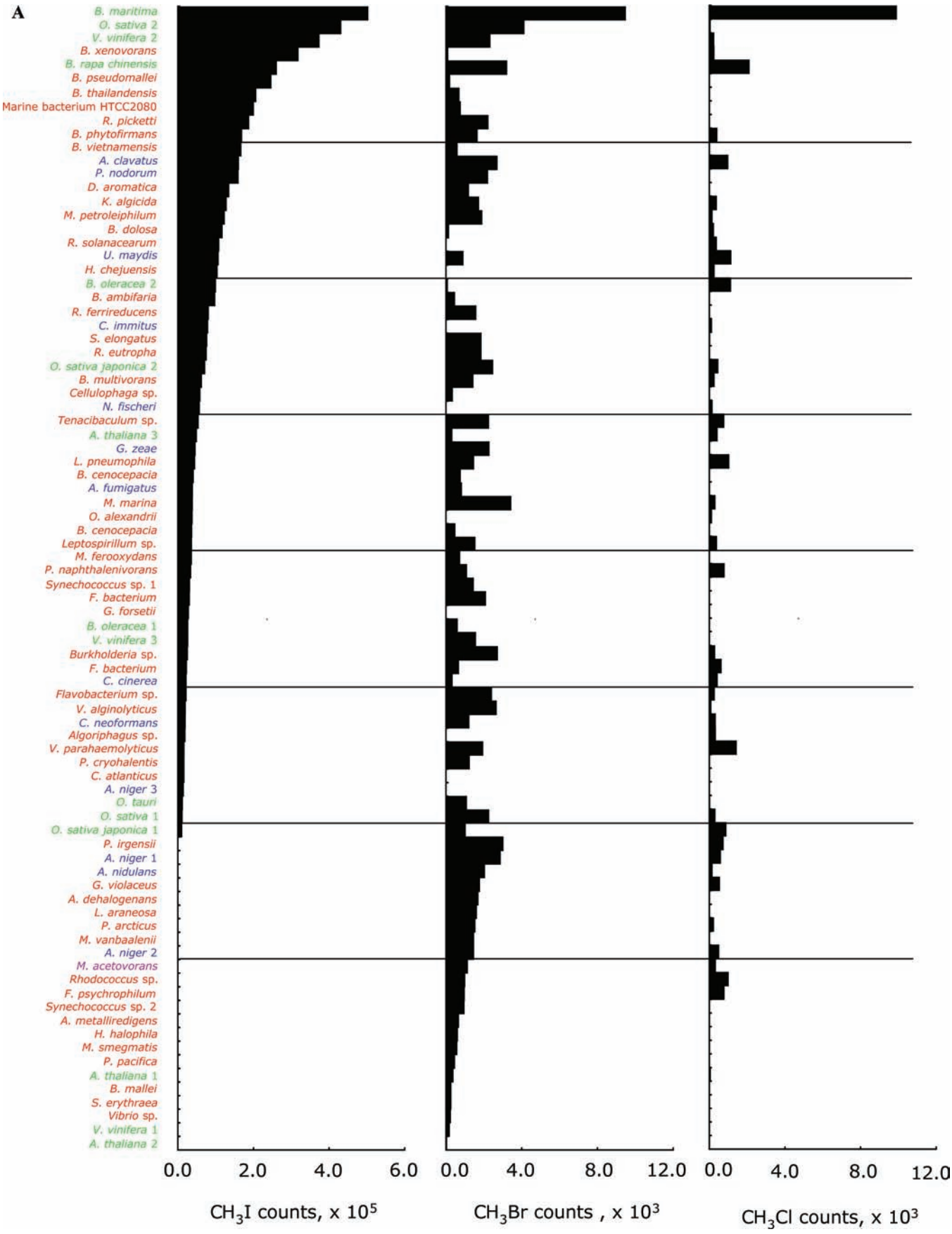
A
B. maritima
O. sativa 2
V. vinifera 2
B. xenovorans
B. rapa chinensis
B. pseudomallei
B. thailandensis
Marine bacterium HTCC2080
R. picketti
B. phytofirmans
B. vietnamensis
A. clavatus
P. nodorum
D. aromatica
K. algicida
M. petroleiphilum
B. dolosa
R. solanacearum
U. maydis
H. chejuensis
B. oleracea 2
B. ambifaria
R. ferrireducens
C. immitus
S. elongatus
R. eutropha
O. sativa japonica 2
B. multivorans
Cellulophaga sp.
N. fischeri
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
O. sativa japonica 1
P. irgensii
A. niger 1
A. nidulans
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
0.0
2.0
4.0
6.0
CH3I counts, x 10^5
0.0
4.0
8.0
12.0
CH3Br counts , x 10^3
0.0
4.0
8.0
12.0
CH3Cl counts, x 10^3

Figure 1

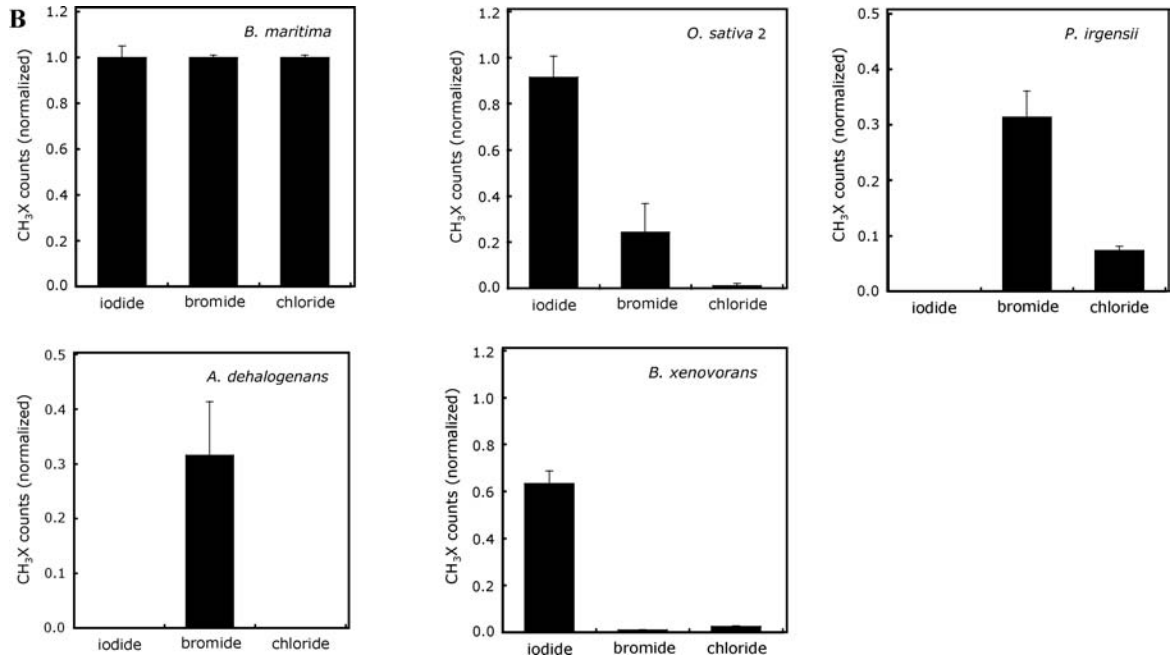

Figure 1. Screening the MHT library for methyl halide activity. (A) Putative methyl halide transferases (MHT) are identified from the NCBI sequence database, synthesized, and screened for activity in *E. coli*. The source organisms for the MHT genes are shown at left; bacteria are shown in red, plants in green, fungi in blue, and archae in purple. (B) The substrate specificies for iodide, bromide, and chloride differ for the MHTs. No enzyme showed activity on chloride. Measurements were performed in triplicate, and standard deviations are shown. Production of CH_3I, CH_3Br, and CH_3Cl is shown, normalized by the maximum achieved for each halide in the library.

by comparison with commercially obtained methyl iodide (Sigma), which had a retention time of 1.50 min and molecular weight of 142 (Supplemental Figure 1, Supporting Information). Methyl iodide production was compared to a standard curve of commercially available methyl iodide in YPD. Standards were prepared at 0.1, 0.5, 1.0, and 10 g/L in 10 mL YP media plus 2% glucose, aliquoted into 14 mL culture tubes, and sealed with rubber stoppers. Standards were incubated at 30 °C for 1 h, and methyl iodide in the headspace was measured as above. A standard curve was fit to the data to quantitate methyl iodide concentration with headspace counts.

Methyl Iodide Toxicity Assay. 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 °C with 250 rpm shaking for 24 h. OD_{600} was measured by spectometry with YP media used as a blank. Each data point was performed in triplicate. The RAD50Δ mutant[42] was obtained from the Saccharomyces Genome Deletion Project (Invitrogen).

Cellulosic Co-culture Methods. *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, 1000 W motor. Bagasse was aliquoted into the appropriate dry weight and then washed three times with hot water to remove soil and residual sugar. Cultures were incubated at 30 °C with 250 rpm agitation for 36 h. Nine milliliter aliquots of cultures were placed in 14 mL tubes with 1 mL of 1 M sodium chloride and sealed with a rubber stopper. Headspace samples were assayed for GC-MS production as above.

Yeast and Bacteria Quantitation. *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 μL samples were plated on either YPD agar + ampicillin (to quantitate *S. cerevisiae*) or brain-heart agar (to quantitate *A. fermentans*). Plates were incubated at 30 °C for either 48 h (for YPD) or 16 h (for BH).

Results and Discussion

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 identified a set of 89 genes from the NCBI sequence database by searching for genes homologous to known MHTs.[37,43,44] A BLAST search was run using a seed sequence, and all sequences down to 18% amino acid identity were saved. This was repeated with each sequence until the set was self-contained and iterations yielded no more sequences. The library contains a remarkable degree of sequence diversity, with an average of 28% amino acid identity between sequences. The library includes putative, hypothetical, and misannotated genes, as well as genes from uncharacterized organisms and metagenomic environmental samples.[45,46] The set included 61 genes from bacteria, 14 genes from plants, 13 genes from fungi, and 1 gene from archaea. These genes were computationally codon optimized for *E. coli* and yeast expression and constructed using automated whole gene DNA synthesis.[47,48] To our knowledge, this is the first

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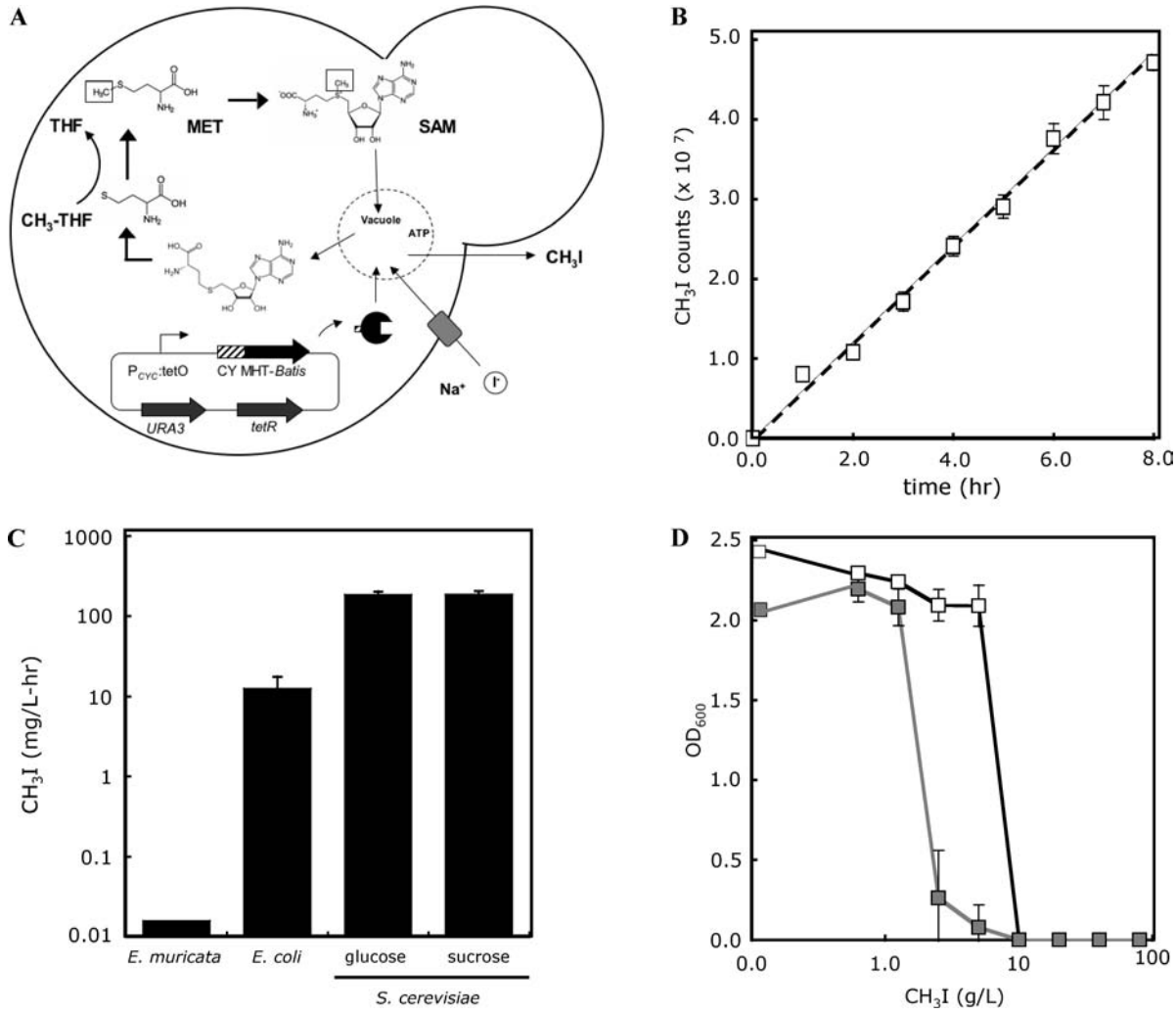

Figure 2. Methyl iodide pathway in recombinant *S. cerevisiae*. (A) CH_3I production pathway. The *B. maritima* MHT is expressed with the carboxypeptidase Y N-terminal vacuole targeting tag. Salts are transported across the membrane by ion channels. Iodide and SAM are transported to the vacuole, where the ATP-dependent MHT methylates iodide ions using SAM as a methyl donor, resulting in methyl iodide and S-adenosyl homocysteine (SAH) as products. SAM is replenished via the THF−methionine pathway. Carbon from fermentable sources (i.e., glucose) would enter the THF−methionine pathway via glycolysis and the tricarboxylic acid cycle (TCA), while non-fermentable carbon sources (ethanol, acetate) would enter the THF−methionine pathway via the TCA cycle. (B) CH_3I measured in culture headspace over time. Activity on glucose-grown cells is shown. (C) CH_3I productivity in milligrams per liter of culture per hour. Values for the culturable red algae *E. muricata* are taken from the literature.[38] Productivities from *B. maritima* MHT-expressing *E. coli* and *S. cerevisiae* are calculated by comparison to standard curves. (D) CH_3I is non-toxic below concentrations of 5 g/L. 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 h of growth. The W303a laboratory strain is shown in black, and the DNA methylation-sensitive *RAD50*Δ mutant is shown in gray. Errors bars are standard deviation and are calculated from three independent measurements.

example of constructing a synthetic metagenomic library by chemical synthesis and expression in a heterologous host.

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 (Supporting Information). We found a wide distribution of activities on each ion, with 56% of genes showing activity on chloride, 85% of genes showing activity on bromide, and 69% of genes showing activity on iodide (Figure 1A). Only 6% of genes showed zero activity on all ions. This is surprising because only one gene in the library was annotated "methyl halide transferase" in the sequence database and only 55% of the genes were annotated as methyltransferases. We did not observe any correlation between species and activity, with plants, bacteria, and fungi species showing a wide distribution of productivities on the ions.

The MHT from *B. maritima*,[44,49] a halophytic plant, displayed the highest activity of all genes on each ion. Several genes showed unique specificities for given ions (Figure 1B), a phenomenon that has also been observed on the organism level.[37] The highest activity for methyl iodide is about 10-fold higher than for methyl bromide and methyl chloride. This is consistent with the measured K_M of these enzymes: I^-, 8.5; Br^-, 18.5; and Cl^-, 155 mM.[43,44] Significant sequence diversity was retained for the subset of the library showing the highest activity for methyl iodide (an average of 36% amino acid among the top 10) and methyl bromide (31% identity). The top 10 enzymes for methyl iodide activity include plants (ice plant, rice, wine

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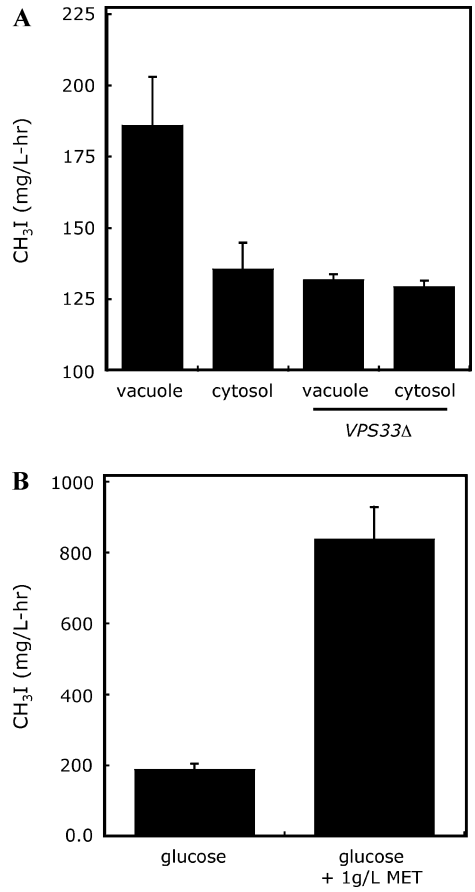

Figure 3. Improvements to methyl iodide production. (A) CH_3I productivity in milligrams per liter of culture per hour. Vacuole-targeted MHTs were constructed with the carboxypeptidase Y N-terminal vacuole targeting tag. Cytosolic MHTs lacked this tag. The enzymes were expressed in the W303a strain and in W303a strain harboring a *VPS33* deletion, which is known to disrupt vacuole formation. (B) CH_3I production in methionine-fed cells. Addition of methionine results in a 4.5-fold increase in methyl iodide production.

grapes, cabbage), soil bacteria (*Burkholderia* and *Ralstonia* species), and an uncharacterized organism (marine bacterium HTCC2080) isolated from a Sargasso Sea environmental sequencing effort.[50] Only the *B. maritima* enzyme showed strong activity for methyl chloride production.

We next transferred the *B. maritima* MHT to the yeast *S. cerevisiae* (Figure 2A). Yeast is a desirable organism for the industrial production of chemicals and fuels. We hypothesized that targeting the *B. maritima* MHT to the yeast vacuole could increase methyl iodide production: the majority of SAM is sequestered in the vacuole,[51] and halide ions are sequestered there as well.[52] We targeted the *B. maritima* MHT to the yeast vacuole using a 16 amino acid N-terminal tag from carboxypeptidase Y[53] (Figure 2A). Targeting the MHT to the vacuole resulted in a modest increase versus cytosolic expression (Figure 3A). We expressed the cytosol and vacuole-targeted MHT in a vacuole-deficient mutant strain (*VPS33*Δ) and found that the increase in methyl iodide productivity was abolished. Yeast displayed high production rate from glucose or sucrose and normal growth rates (doubling time of approximately 124 min versus 118 min for yeast carrying an empty vector). Methyl iodide productivity from glucose was measured at 190 mg/L-h, which is 15-fold higher than that obtained from our measurements of recombinant *B. maritima* MHT-expressing *E. coli* and 12000-fold over the best production rate from a culturable organism, *Endocladia muricata*.[38] Further, the rate can be increased to 860 mg/L-h by adding a small amount of methionine (1 g/L) to the media (Figure 3B). This has been shown previously to cause the overproduction of SAM.[54] This implies that the rate could be further increased by engineering yeast or *E. coli* to overproduce SAM or methionine. Although amino acid overproduction is a non-trivial undertaking, increasing SAM production has been achieved in *E. coli* and *S. cerevisiae*.[55−59]

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 from the balanced equation:

$$C_6H_{12}O_6 + 4I^- + 4H^+ + 8ATP \rightarrow 4CH_3I + 2CO_2 + 2H_2O \quad (1)$$

In addition, glucose is needed to generate ATP (32 ATP per glucose in aeorobic conditions) such that 1.25 glucose are needed to produce 4 methyl iodide, giving a mole fraction of 0.53. In comparison, the maximum efficiency of ethanol from glucose is 0.66. We measured the carbon conversion efficiency of glucose to methyl iodide to be 2.5%, indicating room for yield improvement by redirecting carbon flux to SAM (Supporting Information).

The response of the host organism to the toxic effects of product 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.[60] We found that yeast is naturally resistant to deleterious methylating effects of methyl iodide up to high levels. Growth is inhibited at concentrations above 5 g/L (Figure 3C). Because the fermentation is aerobic and methyl iodide is volatile,[61] it can be recovered from the fermentor off-gas while the concentration in the liquid remains below a toxic level. As a demonstration, we ignited methyl iodide from the headspace of a shake flask (Supplemental Movie 1, Supporting Information).

The production of fuels and chemicals from food-crop-derived sugars and starches (e.g., corn and sugar cane) may put stress on the global food supply.[62] Therefore, it is important that biofuels or commodity chemicals be derived from non-food sources of biocarbon, including cellulosic "energy crops", such as switchgrass (*Panicum virgatum*), elephant grass (*Miscanthus giganteus*), and poplar (*Populus* species), as well as agricultural

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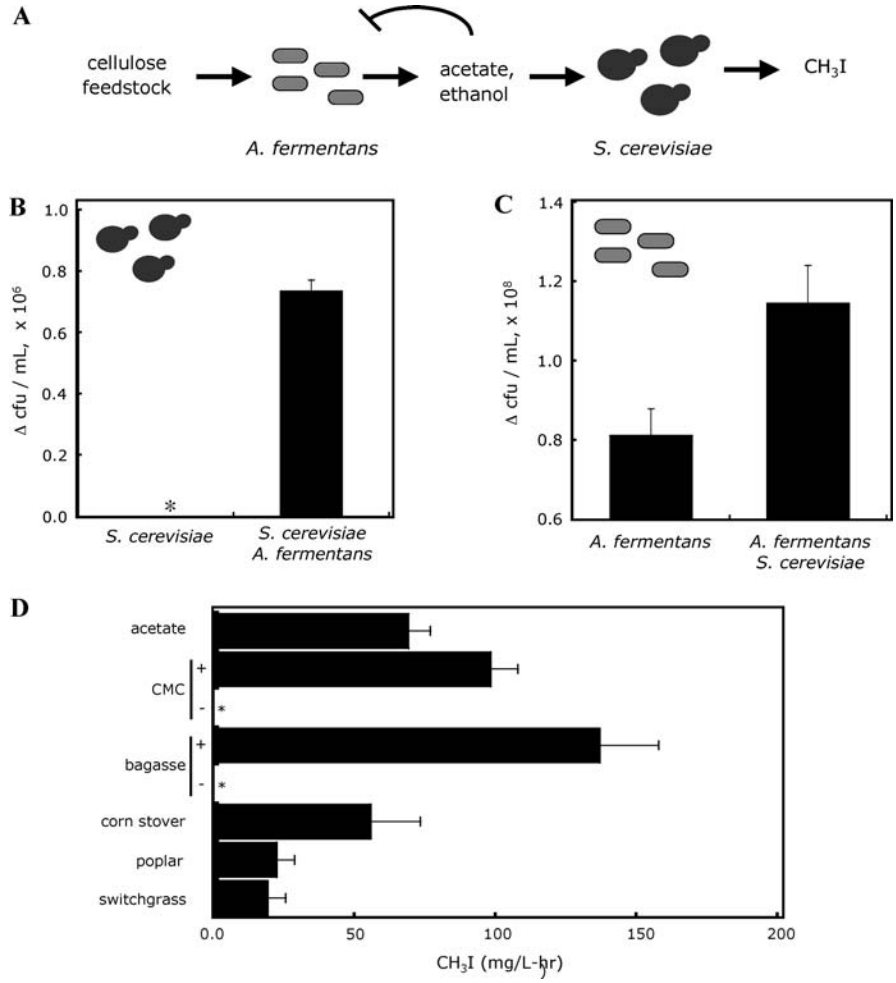

Figure 4. CH_3I production from cellulosic feedstocks using a microbial co-culture. (A) *A. fermentans* ferments cellulosic feedstocks to acetate and ethanol, which *S. cerevisiae* can respire as carbon and energy sources. (B) Yeast samples were inoculated on carboxymethylcellulose (CMC) as the sole carbon sources with and without *A. fermentans*. Growth was measured as colony-forming units (CFUs) and is represented as CFUs after growth minus CFUs before growth. (C) Growth of *A. fermentans* in co-culture. (D) CH_3I production from cellulosic feedstocks. Co-cultures were seeded at low density and grown for 36 h 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 activity is reported in milligrams per liter per hour. Yields are shown for the *A. fermentans*−*S. cerevisiae* co-culture on acetate, CMC, sugar cane bagasse, corn stover, poplar (*Populus* sp.), and switchgrass (*P. virgatum*). Controls are included for cultures grown on CMC and bagasse and are indicated by + (with *A. fermentans*) and − (without *A. fermentans*).

wastes such as corn stover and bagasse.[1,63−65] 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, the expense of using purified enzymes for biomass deconstruction, and the high energy cost for syngas conversion to liquid fuel. Yeast- and *E. coli*-expressing heterologous cellulases and pathways have shown some success in digesting cellulosic materials.[66,67]

To demonstrate the compatibility of methyl halide production with cellulolytic processes and organisms, we designed a novel co-culture of MHT-expressing yeast with a mesophyllic cellulolytic bacterium, *Actinotalea fermentans*, which was isolated from a landfill in France (Figure 4A).[68−72] *A. fermentans* ferments cellulose to acetate and ethanol aerobically. This organism was briefly explored as a potential route for the conversion of cellulose to ethanol, but the growth rate is product inhibited.[73,74] Under anaerobic conditions on cellobiose as the sole carbon source, *A. fermentans* is able to convert 37% of carbon consumed to acetate and ethanol.[70] Under aerobic conditions, 19% of the carbon consumed is converted to these

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fermentation products.[70] Both the yeast and bacterium have optimal growth rates at 30 °C.[70] *A. fermentans* has a doubling time of approximately 10 h on cellobiose under aerobic conditions.[70] *S. cerevisiae* grows on acetate with a doubling time of approximately 4 h.[75] 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 energy, and *A. fermentans* dependent on *S. cerevisiae* for metabolism of toxic waste products.

We inoculated *S. cerevisiae* with *A. fermentans* in media containing carboxymethylcellulose (CMC) 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 h increase to 10^6 cfu/mL, whereas yeast without the cellulolytic partner show little growth (Figure 4B). The presence of yeast also increases the growth rate of the bacterium by consuming toxic components (Figure 4C). Thus, we have created a synthetic symbiotic relationship between a yeast and a bacterium.

We next tested the co-culture conversion of cellulosic feedstocks to methyl iodide. We inoculated the co-culture at low density on media (20 g/L peptone, 10 g/L yeast extract, and 20 g/L switchgrass in sterile water) containing pulverized dry switchgrass as the sole carbon source.[64] At 36 h after inoculation, sodium iodide was added to the medium to induce methyl iodide production. Methyl iodide productivities on various cellulosic sources, including switchgrass, corn stover, and poplar, are shown in Figure 4D. Energy crops such as switchgrass offer several advantages over conventional crops by requiring fewer agricultural inputs and by growing on marginal land,[64] or by exhibiting extraordinary growth or genetic tractability (e.g., poplar).[76] Agricultural residues such as corn stover are another source of cellulosic carbon, with approximately 200 metric tons of stover produced in the United States each year.[63]

Conclusion

We have demonstrated that methyl halides can be produced at high titer from different forms of biomass using industrially relevant organisms. We used synthetic metagenomics to identify the highest activity biocatalysts from known amino acid sequences. Due to advances in DNA synthesis technology, an entire family of potentially functional genes can be synthesized at moderate cost. Chemical synthesis of genes from sequence databases enables functional screening of gene sequences from uncharacterized or unculturable organisms, as well as the ability to access genetic material without cloning from the host organism. This is an example of information-based cloning, where the results of bioinformatics searches can be physically "printed" by chemical DNA synthesis.[77]

Construction of a synthetic microbial co-culture, where a cellulolytic bacterium provided acetate to methyl iodide producing yeast, allowed diverse lignocellulosic feedstocks to be converted to methyl halides. This approach differs from other engineered examples of cellulose digestion where single species are engineered to perform cellulose cleavage, followed by utilization of the resulting five- and six-carbon sugars.[66,78,79] The co-culture described here divides the cellulolytic- and methyl halide-producing functions between two species. Metabolic division of labor is commonly observed in microbial consortia, including soil and termite gut microbiota that degrade cellulose, and has been used to engineer strains of *Bacillus subtilis* that cooperatively produce cellulosomes.[79−81]

Methyl halides are valuable industrial and agricultural chemicals in their own right and can also be used as precursors for the synthesis of more complex hydrocarbons. Zeolite catalysts can convert methyl halides into aromatics,[23,24] olefins,[20],,[22] alcohols,[26] alkanes,[19] and gasoline.[19,30] In one example, methyl chloride was reacted with a ZSM-5 catalyst to produce synthetic gasoline with an alkane and aromatic composition similar to that of gasoline from petroleum distillation.[19] There are two similar processes that could be used for biomass conversion. The first is the use of microbial methanogens to produce methane, which will then require chemical activation for downstream conversion into liquid fuels.[21,82] The second is the production of syngas by pyrolysis or microorganisms.[83] Both of these processes require large energy inputs to convert the gas to liquid fuels. We are proposing to bypass the energy-intensive steps of the Fischer−Tropsch process by having cells directly produce activated methane, using the chemical energy in ATP. Methyl halides may prove to be a fungible intermediate through which the carbon from different sources of biomass can be converted into a wide range of chemicals, consumer products, and liquid fuels.

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Supporting Information Available: Details of construction of MHT-expressing strains, growth and assay conditions, cumulative methyl iodide production, targeting the *B. maritima* MHT to the yeast vacuole, methyl halide production from cellulosic feedstocks, metabolic pathway to methyl iodide, and combustion of culture headspace; list of strains, plasmids, and primers used; MHT sequences; complete refs 10, 50, and 80; and Supplemental Movie 1, in. avi format, demonstrating combustion of the culture headspace. This material is available free of charge via the Internet at http://pubs.acs.org.

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