SUPPORTING INFORMATION

General Chemistry. For ^{1}H NMR quantification of solute concentrations, solutions were concentrated to dryness under reduced pressure, concentrated to dryness one additional time from D_2O, and then redissolved in D_2O containing a known concentration of the sodium salt of 3-(trimethylsilyl)propionic-*2,2,3,3*-d_4 acid (TSP, Lancaster Synthesis Inc.). Concentrations were determined by comparison of integrals corresponding to each compound with the integral corresponding to TSP (δ = 0.0 ppm) in the ^{1}H NMR. All ^{1}H NMR spectra were recorded on a Varian VXR-500 NMR Spectrometer (500 MHz). Samples analyzed by gas chromatography were derivatized by bis(trimethylsilyl)trifluoroacetamide and quantified relative to an internal standard of dodecane. Gas chromatography was performed on an Agilent 6890N equipped with an HP-5 capillary column (30 m x 0.25 mm x 0.25 micron). Temperature programming began with an initial temperature of 120 °C for 3 min. The temperature was increased to 210 °C at a rate of 15 °C/min, and held at the final temperature for 1 min. The split injector was maintained at a temperature of 300 °C and the FID detector was kept at 350 °C.

High-Pressure Hydrogenation of D,L-Malic Acid. A solution of D,L-malic acid (13.4 g, 0.1 mol) in distilled, deionized water (100 mL) was placed in a glass liner along with 5 wt % Ru on C (2.68 g, 1.33 mmol). The liner was inserted into a 500 mL Parr 4575 stainless steel high-temperature, high-pressure reactor and the vessel sealed. A Parr 4842 controller maintained temperatures and stirring rates. Hydrogen was bubbled through the reaction mixture for 10-15 min to remove air while stirring at 100 rpm. The vessel was then pressurized with 4000 psi H_2. After heating the reaction to 135 °C, the H_2 pressure increased to 5000 psi. The reaction was subsequently stirred at 200 rpm for 10 h at 135 °C under 5000 psi H_2. After removal of the catalyst by filtration, the reaction mixture was concentrated under vacuum to afford a colorless oil. Individual products in this oil were separated by flash chromatography ($MeOH/CH_2Cl_2$, 1:9, v/v) and identified by ^{1}H NMR as ethylene glycol; 1,2-propanediol; 1,3-butanediol; 1,4-butanediol; 3-hydroxy-γ-butyrolactone and 1,2,4-butanetriol. Product yields were determined by gas chromatography after derivatization. The colorless oil resulting from hydrogenation of D,L-malic acid (~50 mg) was dissolved in pyridine (1 mL, 12.4 mmol) followed by the addition of dodecane (0.1 mL, 0.44 mmol) and bis(trimethylsilyl)trifluoroacetamide (2 mL, 7.53 mmol). The reaction was stirred at rt for 3 h and then analyzed by gas chromatography. Based on response factors determined for authentic samples relative to dodecane as the internal standard, product yields resulting from the high-pressure hydrogenation of D,L-malic acid were as follows: ethylene glycol (3%); 1,2-propanediol (11%); 1,3-butanediol (3%); 1,4-butanediol (8%); 3-hydroxy-γ-butyrolactone (1%); 1,2,4-butanetriol (74%).

General Microbiology. All solutions were prepared in distilled, deionized water. LB medium (1 L) contained Bacto tryptone (10 g), Bacto yeast extract (5 g), and NaCl (10 g). LB-glucose medium contained glucose (10 g), $MgSO_4$ (0.12 g), and thiamine hydrochloride (0.001 g) in 1 L of LB medium. M9 salts (1 L) contained Na_2HPO_4 (6 g), KH_2PO_4 (3 g), NH_4Cl (1 g), and NaCl (0.5 g). M9 D-xylonic acid medium contained potassium D-xylonate (10 g), $MgSO_4$ (0.12 g), and thiamine hydrochloride (0.001 g) in 1 L of M9 salts. M9 L-arabinonic acid medium contained potassium L-arabinonate (10 g), $MgSO_4$ (0.12 g), and thiamine hydrochloride (0.001 g) in 1 L of M9 salts. Except where specifically mentioned, antibiotics were added where appropriate to the following final concentrations: ampicillin (Ap), 50 μg/mL; kanamycin (Kan),

50 μg/mL. Isopropyl-β-D-thiogalactopyranoside (IPTG) was prepared as a 500 mM stock solution. Solutions of LB medium, M9 salts, $MgSO_4$, and glucose were autoclaved individually and then mixed. Solutions of potassium D-xylonate, potassium L-arabinonate, thiamine hydrochloride, antibiotics, and IPTG were sterilized through 0.22-μm membranes. Nutrient agar (Oxiod) plates were prepared according to procedure recommended by the manufacture. Other solid media were prepared by addition of Difco agar to a final concentration of 1.5% (w/v) to the liquid media.

Standard protocols were used for construction, purification, and analysis of plasmid DNA.[1] PCR amplifications were carried out as previously described.[1] Primers were synthesized by the Macromolecular Structure Facility at Michigan State University. DNA sequencing was performed at the Genomic Technology Support Facility at Michigan State University.

Isolation of *aadh* and *aatp* Genes. Genomic DNA of *Pseudomonas fragi* (ATCC4973) was isolated according to the procedure described by Wilson.[2] This DNA was partially digested with *Sau*3A to afford fragments in the range of 30-42 kb. The resulting DNA fragments were ligated into *Bam*HI-digested cosmid vector SuperCos I (Stratagene). Ligated DNA was packaged *in vitro*, using the Gigapack® III XL packaging extract (Stratagene). *E. coli* BL21(DE3) was transfected with the packaging mix, and colonies were selected on solid M9 medium containing L-arabinonate as the sole source of carbon for growth. Restriction enzyme mapping of three cosmids isolated from three colonies that grew on L-arabinonate indicated a common 5.0-kb DNA fragment. Further subcloning guided by assaying for L-arabinonate dehydratase activity was employed to minimize the size of the DNA fragment containing the *aadh* gene.

An open reading frame possessing high homology to a sugar transport protein was identified in the 5.0-kb DNA fragment employing BLAST (Basic Local Alignment Search Tool) program on the NCBI (National Center for Biotechnology Information) search engine. To further determine the function of this putative transport protein, plasmids pWN6.086A and pWN6.126A were constructed. Both plasmids contained genes encoding L-arabinonate dehydratase and benzoylformate decarboxylase, while plasmid pWN6.126A also contained the gene encoding the putative transport protein. Single colonies of *E. coli* BL21(DE3)/pWN6.086A and *E. coli* BL21(DE3)/pWN6.126A were inoculated into 5 mL LB medium containing Ap and cultured at 37 °C with agitation at 250 rpm. When the OD_{600} of cell cultures reached 0.4-0.6, IPTG was added (t = 0) to a final concentration of 0.5 mM along with 0.5 mL of L-arabinonate (1 M). During the subsequent 36 h of cultivation, samples (1 mL) of each cell culture were removed at 12 h time intervals. Solute concentrations in culture media were determined by ^{1}H NMR. The concentration of L-arabinonate decreased in the culture medium of *E. coli* BL21(DE3)/pWN6.126A, while the concentration of L-arabinonate remained unchanged during the initial 24 h of culturing *E. coli* BL21(DE3)/pWN6.086A. Formation of 1,2,4-butanetriol was detected in the culture medium of *E. coli* BL21(DE3)/pWN6.126A at 24 h. It reached a concentration of 4.5 mM at 36 h. A decrease of the concentration of L-arabinonate was observed at 36 h in the culture medium of BL21(DE3)/pWN6.086A, but no formation of 1,2,4-butanetriol was detected. The results indicated that expression of the putative transport protein enabled *E. coli* BL21(DE3)/pWN6.126A to transport L-arabinonate and produce 1,2,4-butanetriol. The

open reading frame encoding this potential L-arabinonic acid transport protein was designated *aatp*.

Enzyme Assays. Cells were collected by centrifugation at 4 000*g* and 4 °C. Harvested cells were resuspended in buffer containing Tris-HCl (50 mM, pH 8.0) and $MgCl_2$ (10 mM) for assay of D-xylonate and L-arabinonate dehydratase while harvested cells were resuspended in sodium phosphate (50 mM, pH 6.5) for assay of D,L-3-deoxy-*glycero*-pentulosonate decarboxylase activity. Resuspended cells were then disrupted two times using a French press (16,000 psi). Cellular debris was removed by centrifugation at 48 000*g* for 20 min. Protein concentrations were determined using the Bradford dye-binding method.[3] Protein assay solution was purchased from Bio-Rad. Protein concentrations were determined by comparison to a standard curve prepared using bovine serum albumin.

D-Xylonate and L-arabinonate dehydratase were assayed according to procedures described by Dahms.[4] The 2-keto acids 3-deoxy-D-*glycero*-pentulosonate and 3-deoxy-L-*glycero*-pentulosonate formed during enzyme-catalyzed dehydration were measured as semicarbazone derivatives. Two solutions were prepared and incubated separately at 30 °C for 3 min. The first solution (150 μL) contained Tris-HCl (50 mM), $MgCl_2$ (10 mM) and an appropriate amount of cell lysate at pH 8.0. The second solution (25 μL) contained potassium D-xylonate or L-arabinonate (0.1 M). After the two solutions were mixed (time = 0), aliquots (30 μL) were removed at timed intervals and mixed with semicarbazide reagent (200 μL), which contained 1% (w/v) of semicarbazide and 0.9% (w/v) of sodium acetate in water. Following incubation at 30 °C for 15 min, each sample was diluted to 1 mL with H_2O. After removing the precipitated protein by centrifugation, the absorbance of semicarbazone was measured at 250 nm. One unit of D-xylonate or L-arabinonate dehydratase activity was defined as the formation of 1 μmol of 3-deoxy-D-*glycero*- or 3-deoxy-L-*glycero*-pentulosonate per min at 30 °C. A molar extinction coefficient of 10,200 $M^{-1}cm^{-1}$ (250 nm) was used for 2-keto acid semicarbazone derivatives.

D,L-3-Deoxy-*glycero*-pentulosonate decarboxylase was assayed by coupling the decarboxylation reaction with D,L-3,4-dihydroxybutanal-dependent oxidation of NADH catalyzed by equine liver alcohol dehydrogenase. Synthesis of D,L-3-deoxy-*glycero*-pentulosonic acid followed a procedure described by Stoolmiller.[5] The enzyme assay solution (1 mL) contained sodium phosphate (50 mM, pH 6.5), potassium D,L-3-deoxy-*glycero*-pentulosonate (100 mM), $MgCl_2$ (10 mM), thiamine pyrophosphate (0.15 mM), NADH (0.2 mM), 500 U of equine liver alcohol dehydrogenase, and an aliquot of cell lysate. One unit of D,L-3-deoxy-*glycero*-pentulosonic acid decarboxylase activity was defined as the decarboxylation of 1 μmol of D,L-3-deoxy-*glycero*-pentulosonic acid per min at 24 °C as measured by the oxidation of NADH at 340 nm. A molar extinction coefficient of 6,220 $M^{-1}cm^{-1}$ (340 nm) was used for NADH.

General Fermentations. Fermentations employed a 2.0 L working capacity B. Braun M2 culture vessel. Utilities were supplied by a B. Braun Biostat MD controlled by a DCU-3. Data acquisition utilized a Dell Optiplex GX200 personal computer (PC) equipped with B. Braun MFCS/Win software (v2.0). Temperature, pH and glucose addition were controlled with PID control loops. Dissolved oxygen (D.O.) was monitored using a Mettler-Toledo 12 mm

sterilizable O_2 sensor fitted with an Ingold A-type O_2 permeable membrane. Samples (5-10 mL) of fermentation broth were removed at 3 or 6 h intervals. Cell densities were determined by dilution of fermentation broth with water (1:100) followed by measurement of absorption at 600 nm (OD_{600}). The remaining fermentation broth was centrifuged to obtain cell-free broth. Solute concentrations in the cell-free broth were determined by ^{1}H NMR or GC analysis.

Prior to purification of microbe-synthesized D-xylonate, purification of microbe-synthesized L-arabinonate, and analysis of the enantiomeric purity of microbe-synthesized D- and L-1,2,4-butanetriol, fermentation broth was centrifuged at 14 000*g* for 20 min and the cells were discarded. Color and protein were removed from the resulting supernatant by addition of Darco KB-B activated carbon (20 g/L) followed by agitation at 250 rpm for 2 h. After filtration to remove activated carbon, the filtrate was treated with activated carbon a second time in the same fashion.

Microbial Oxidation of Pentoses. For microbial oxidation of D-xylose or L-arabinose, fermentation medium (1 L) contained K_2HPO_4 (2 g), KH_2PO_4 (1 g), $(NH_4)_2SO_4$ (5 g) and yeast extract (5 g). Solutions of D-xylose (100 g) or L-arabinose (100 g) and $MgSO_4$ (0.24 g) were autoclaved separately and added immediately prior to initiation of the fermentation. Inoculants were started by introduction of a *Pseudomonas fragi* single colony picked from a nutrient agar plate into 5 mL of fermentation medium. Cultures were grown at 30 °C with agitation at 250 rpm until they were turbid (~24 h) and subsequently transferred to 100 mL of fermentation medium. Cultures were grown at 30 °C and 250 rpm for an additional 12 h. The inoculant (OD_{600} = 1.0-3.0) was then transferred into the fermentation vessel and the batch fermentation was initiated (t = 0 h). The fermentation control settings were: 30 °C, stirring speed at 650 rpm, and airflow at 0.5 L/L/min. The culture medium was maintained at pH 6.4 by addition of 2 N H_2SO_4 and a base solution, which was 30% $CaCO_3$ for oxidation of D-xylose or concentrated NH_4OH for oxidation of L-arabinose. A standard curve was determined for each metabolite using solutions of authentic, chemically synthesized samples.[6] Compounds were quantified by ^{1}H NMR using the following resonances: D-xylonic acid (δ 4.08, d, 1 H); L-arabinonic acid (δ 4.24, d, 1 H); and L-arabino-1,4-lactone (δ 4.64, d, 1 H).

A modified procedure by Buchert[7] was employed for the purification of D-xylonate. Following treatment with activated carbon and concentration (1-1.1 L to 250 mL) of D-xylonate-containing fermentation broth, EtOH (3:1, v/v) was added. After 12 h at 4 °C, the precipitated calcium xylonate was filtered and dried under vacuum (95% recovery based on D-xylonate in the crude fermentation broth). Potassium D-xylonate was obtained by passing an aqueous solution of calcium xylonate through a Dowex 50 (K^+ form) column.

After treatment with activated carbon and concentration (1-1.1 L to 100 mL) of fermentation broth resulting from microbial oxidation of L-arabinose, the solution was adjusted to pH 12.0 by addition of solid KOH for hydrolysis of the L-*arabino*-1,4-lactone. The hydrolysis reaction was carried out at rt overnight. Following neutralization of the hydrolysis solution with concentrated HCl addition, a 5:1 (v/v) amount of MeOH was added relative to the L-arabinonate solution. After 12 h at 4°C, precipitated potassium L-arabinonate was filtered and dried under vacuum (92% recovery based on L-arabinonate and L-*arabino*-1,4-lactone in the crude fermentation broth).

Microbial Synthesis of 1,2,4-Butanetriol. For microbial synthesis of D- or L-1,2,4-butanetriol, fermentation medium (1 L) contained Bacto tryptone (20 g), Bacto yeast extract (10 g) and NaCl (5 g). Solutions of K_2HPO_4 (3.75 g), glucose, and $MgSO_4$ (0.24 g) were autoclaved separately and added prior to initiation of the fermentation. Thiamine hydrochloride (0.34 g) and kanamycin (0.1 g) were added into the culture medium at the same time. Inoculants were started by introduction of a single colony picked from an agar plate into 5 mL of LB-glucose medium containing kanamycin. Cultures were grown at 37 °C with agitation at 250 rpm until they were turbid. A 0.5 mL of this culture was subsequently transferred to 100 mL of LB-glucose medium containing kanamycin, which was grown at 37 °C and 250 rpm for an additional 10 h. The inoculant (OD_{600} = 1.0-3.0) was then transferred into the fermentation vessel and the batch fermentation was initiated (t = 0 h). The fermentation control settings were: 33 °C, dissolved oxygen (D.O.) at 20% of air saturation, and pH 7.0. Addition of concentrated NH_4OH or 2 N H_2SO_4 was employed to maintain pH. The initial glucose concentration in the fermentation media ranged from 15-22 g/L.

Maintenance of D.O. at 20% of air saturation proceed through three stages during the fermentations. Stage 1 began with an airflow setting of 0.06 L/L/min. The D.O. concentration was maintained during Stage 1 by increasing the impeller speed from its initial set point of 50 rpm to its preset maximum of 1100 rpm. Stage 2 began with the impeller rate at 1100 rpm. D.O was then maintained at 20% of air saturation during Stage 2 by use of the mass flow controller to increase the airflow rate from 0.06 L/L/min to a preset maximum of 1.0 L/L/min. At constant impeller speed and constant airflow rate, the D.O. concentration was maintained at 20% of air saturation during Stage 3 by O_2 sensor-controlled glucose feeding. At the beginning of Stage 3, the D.O. concentration fell below 20% of air saturation due to residual glucose in the media. This lasted for approximately 10 - 30 min before glucose (65% w/v) feeding commenced. The glucose feed PID control parameters were set to 0.0 s (off) for the derivative control (τ_D) and 999.9 s (minimum control action) for the integral control (τ_I). X_P was set to 950% to achieve a K_c of 0.1. IPTG stock solution (1 mL), and D-xylonate or L-arabinonate solution was added to the culture media upon initiation of Stage 3. The concentration of 1,2,4-butanetriol was determined by GC analysis.

Enatiomeric Purity Analysis of Microbial Synthesized 1,2,4-Butanetriol. Following concentration of partially purified 1,2,4-butanetriol fermentation broth (200 mL) to 20 mL, the solution was eluted through a Dowex1 1X8-400 (OH^- form) column with water. The eluant was neutralized by addition of Dowex 50 (H^+ form) resin. After removing the resin by filtration, the filtrate was concentrated under vacuum. To 1,2,4-butanetriol (0.0027 g) in pyridine (0.2 mL), CH_2Cl_2 (0.3 mL), 4-(dimethylamino)pyridine (0.005 g), and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.026 g) were sequentially added. The mixture was stirred at rt overnight and passed through a disposable pipette containing silica gel, which was eluted with 3 mL of CH_2Cl_2. After removing CH_2Cl_2 under vacuum, the residue was redissolved in CH_2Cl_2 and washed with 1% $NaHCO_3$ (5 mL) and H_2O (2 x 5 mL). The CH_2Cl_2 layer was concentrated under vacuum to give the Mosher ester. The Mosher esters of D- and L-1,2,4-butanetriol were analyzed employing an Agilent 1100 HPLC equipped with a Chiralpak AD column (Daicel Chemical, 4.6 mm x 250 mm), which had been equilibrated with hexane:2-propanol = 98:2 (v/v). The column was eluted with the same solvent mixture at a rate of 1.25

mL/min, while the eluant was monitored at 260 nm. The retention time of D- and L-1,2,4-butanetriol Mosher ester were 14.4 min and 8.1 min, respectively. Mixtures containing varying amounts of authentic D- and L-1,2,4-butanetriol were derivatized using Mosher's reagent and analyzed by HPLC. A calibration curve was generated by plotting the ratios of integrated peak areas of eluted Mosher esters prepared from mixtures of authentic D- and L-1,2,4-butanetriol against the weight ratio of D- and L-1,2,4-butanetriol in these samples. Based on this calibration curve, the percent enantiomeric excess of microbe-synthesized D- and L-1,2,4-butanetriol were determined to be 99% and >99%, respectively.

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