2.7.7.4 coded by cysNcysD). The APS is then converted to PAPS by APS kinase (EC 2.7.1.25 encoded by cysC). This step requires one ATP. PAPS is converted to sulfite by a PAPS reductase (EC 1.8.4.8 coded by cysH) and sulfite is reduced to sulfide by NADPH-sulfite reductase (EC 1.8.1.2 coded by cysIcysJcysG). The alternate pathway, shown on the right side of FIG. **6**, converts APS directly to sulfite using an adenylyl sulfate reductase (EC 1.8.9.92 or 1.8.4.9). One of ordinary skill in the art will appreciate that any adenylyl sulfate reductase that can convert APS to sulfite will work. For example, the adenylyl sulfate reductase from *Bacillus subtilis* (Accession number CAA04409), or from *Pseudomonas aeruginosa* (Accession number NP_250447).

Adenylyl sulfate reductase encoding nucleic acid sequences can be introduced into any microorganism used to produce methionine. For example the strains described herein, as well as the strains described in WO2005/108561 and WO2006138689 by Metabolic Explorer, and those described by Kumar and Gomes, *Biotechnology Advances* 23:41-61, 2005, can benefit from the disclosed route bypassing PAPS and thus requiring one less ATP molecule for sulfate assimilation.

EXAMPLES

Example 1

Multiple Methionine Production Pathways, One of which Utilizes Direct Sulfhydrylation, Using Exogenously Expressed Nucleic Acid Sequences

A. Construction of a Microorganism Having Both metABC (Transsulfuration) and metAZ (Direct Sulfhydrylation)

As described before, endogenous production of methionine in *E. coli* occurs mainly through the transulfuration reaction. This Example describes the engineering of *E. coli* to increase direct sulfhydrylation while also maintaining the endogenous metABC pathway.

Direct sulfhydrylation was increased by cloning O-succinylsulfhydrylase (EC 4.2.99.-) which converts O-succinylhomoserine to homocysteine by reacting with hydrogen sulfide. This enzyme is codified by metZ and can be found in some *Pseudomonas* species (Vermeij and Kertesz, *J Bacteriol.* 181:5833-5837, 1999 and Inoue et al., *J. Bacteriol.* 179:3956-3962, 1997).

More specifically, metZ from *Pseudomonas aeruginosa* was cloned into methionine auxotrophs of strain TF4076BJF, which was derived from threonine-producing strain TF4076, (additionally modified by the deletion of thrB and metJ, and the insertion of metF under the control of the pTrc promoter, further described in Example 3, below). These auxotrophs have deletion of either the metB or the metB and metC genes. metZ from *Pseudomonas aeruginosa* enhanced the growth of the metB and the metBC deletion mutants in minimal medium. Even though in flask cultures methionine production was not fully recovered, metZ expression induces the methionine production up to ~100 mg/L in metBC deletion mutant, as shown in Table 1. This indicates that metZ is responsible for the production of homocysteine in the cell.

Low methionine production of the deletion mutants transformed with metZ may be due to the limitation of sulfide in the intracellular fraction (methods of increasing sulfide concentration are provided below). This is supported by the finding that the growth of the metBC deletion strain transformed with metZ was enhanced in M9 media in the presence of 2 mM sodium sulfide. In in-vitro assays, the O-succinylsulfhydrylase had low sulfide affinity. Through directed evolution, it is possible to develop improved O-succinylsulfhydrylases with higher sulfide affinity and also higher activity. A highly active O-succinylsulfhydrylase could replace metB and metC in the methionine pathway, or could complement the pathway to increase the carbon flux to methionine.

TABLE 1

Growth complementation and methionine production on TF4076BFJ-ΔBC

		glucose used	met intermediate (mg/L)		GA and HS (g/L)	
TF4076BJF-ΔBC	OD	(g/L)	OSH	met	HS	GA
empty vector	2.5	10.0	3867	0.0	0.0	0.4
pCL-metB	20.9	38.1	0.0	0.0	0.6	0.2
pCL-metB-metC	9.7	40.0	0.0	670	4.36	2.4
pPro-metZ	13.0	40.0	0.0	101	3.1	4.3

pCL-metB: metB with its own promoter in pCL1920
pCL-metB-metC: metB and metC with their own promoters in pCL1920
pPro-Z: metZ from *Pseudomonas aeruginosa* in pProLar vector (ClonTech)

B. Construction of a Microorganism Having Both metABC (Transulfuration) and metXY (Direct Sulfhydrylation)

This example shows simultaneous methionine production from two pathways in *E. coli*. One pathway is the endogenous metABC pathway and the second pathway allows for direct sulfhydrylation via the expression of metY and metX from various organisms.

As shown in FIG. **1** *E. coli* produces methionine endogenously using the transsulfuration pathway genes metA, metB and metC and goes through OSHS. Genetic engineering was used to add an additional pathway to *E. coil* by cloning and expressing the genes metX and metY into *E. coli*, which resulted in a host organism that makes methionine through both transsulfuration and direct sulfhydrylation simultaneously.

The metY and metX genes used to construct the heterologous pathway were cloned from *Leptospira meyeri, Deinococcus radiodurans, Chloroflexus aurantiacus, Brevibacterium linens, Nostoc punctiforme* and *Pseudomonas aeruginosa* DNA as described below, and several different strains were constructed to analyze the impact of the addition of these genes on methionine production. The homocysteine synthase from *Corynebacterium glutamicum* and *Saccharomyces cerevisiae* were also cloned and tested. Both pathways were demonstrated to work simultaneously and methionine production was improved with this addition.

To evaluate whether the *L. meyeri* metX and metY enzymes could complement the growth of an *E. coli* methionine auxotroph, the *L. meyeri* metYX gene cluster was amplified from plasmid metXY-pCR2.0-TOPO and cloned into the pPRO-Nde-del vector. The transcription of the metYX genes in this plasmid was initiated by a lac/ara promoter located on the vector.

Four *E. coli* strains including W3110 ΔmetA (stopping production of OSHS), TF4076BJF (increased homoserine production), TF4076BJF ΔmetA (stopping production of OSHS), and TF4076BJF ΔmetAmetB (stopping production of OSHS and cystathionine from OAHS or OSHS) were evaluated. Strain TF4076BJF is a threonine auxotroph, deregulated for methionine production with an increase