utilize sucrose as a carbohydrate source, it is desirable to engineer the microorganism so that it can utilize sucrose.

Recombinant bacteria that have been engineered to utilize sucrose by incorporation of sucrose utilization genes have been reported. For example, Livshits et al. (U.S. Patent No. 6,960,455) describe the production of amino acids using *Escherichia coli* strains containing genes encoding a metabolic pathway for sucrose utilization. Additionally, Olson et al. (*Appl. Microbiol. Biotechnol.* 74:1031-1040, 2007) describe *Escherichia coli* strains carrying genes responsible for sucrose degradation, which produce L-tyrosine or L-phenylalanine using sucrose as a carbon source. Eliot et al. (U.S. Patent Application No. 2011/0136190 A1) describe recombinant bacteria that produce glycerol and glycerol-derived products from sucrose.

However, problems remain in engineering production microorganisms so that they can utilize sucrose effectively. Specifically, high levels of expression of sucrose transport genes result in poor growth on sucrose because excess sucrose transport is inhibitory. On the other hand, low levels of sucrose transport also result in sub-optimal growth on sucrose. Therefore, it is difficult to obtain the proper sucrose transporter gene expression level. Additionally, expression of sucrose transport genes under conditions at which sucrose transport is in excess, such as at high sucrose concentrations, may inhibit growth even at gene expression levels at which growth is not inhibited at lower sucrose concentrations. Therefore, a need also exists for a sucrose transporter that can enable growth on sucrose over a broad range of sucrose concentrations.

SUMMARY OF THE INVENTION

One ~~embodiment~~ aspect of the disclosure provides a variant sucrose transporter polypeptide having an amino acid sequence that has at least 95% identity to an amino acid sequence as set forth in SEQ ID NO:26 based on

a Clustal W method of alignment and having an amino acid change from arginine to alanine or arginine to leucine at position 300, and comprising:

(a) at least one additional amino acid change selected from the group consisting of:

(i) glutamine to histidine at position 353

(ii) leucine to proline at position 61;

(iii) phenylalanine to leucine at position 159;

(iv) glycine to cysteine at position 162;

(v) proline to histidine at position 169;

(vi) leucine to tryptophan at position 61;

(vii) leucine to histidine at position 61;

(viii) leucine to phenylalanine at position 61; and

(ix) leucine to tyrosine at position 61; or

(b) a length of 402 to 407 amino acids from the N-terminus; or

(c) a length of 402 to 407 amino acids from the N-terminus, and having at least one of the amino acid changes of (a).

Another ~~embodiment~~ aspect provides a variant sucrose transporter polypeptide having an amino acid sequence that has at least 95% identity based on a Clustal W method of alignment to an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98, and comprising an amino acid at an equivalent position when compared with a reference amino acid sequence of SEQ ID NO:26 selected from the group consisting of:

(a) alanine at a position equivalent to position 300; and

(b) leucine at a position equivalent to position 300.

Another ~~embodiment~~ aspect provides a recombinant bacterium comprising in its genome or on at least one recombinant construct:

(a) a nucleotide sequence encoding a variant sucrose transporter polypeptide having an amino acid sequence that has at least 95% identity based on a Clustal W method of alignment to an amino acid sequence selected from the group consisting of SEQ ID NOs:26, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98,and

an amino acid at an equivalent position when compared with a reference amino acid sequence of SEQ ID NO:26 selected from the group consisting of:

(i) alanine at a position equivalent to position 300; and

(ii) leucine at a position equivalent to position 300; and

(b) a nucleotide sequence encoding a polypeptide having sucrose hydrolase activity;

wherein (a) and (b) are each operably linked to the same or a different promoter, further wherein said recombinant bacterium is capable of metabolizing sucrose.

In one embodiment, the recombinant bacterium produces 1,3-propanediol, glycerol, and/or 3-hydroxypropionic acid.

Another ~~embodiment~~ aspect provides a process for making glycerol, 1,3-propanediol and/or 3-hydroxypropionic acid from sucrose comprising:

a) culturing the recombinant bacterium that produces 1,3-propanediol, glycerol, and/or 3-hydroxypropionic acid, disclosed herein, in the presence of sucrose; and

b) optionally, recovering the glycerol, 1,3-propanediol and/or 3-hydroxypropionic acid produced.

BRIEF SEQUENCE DESCRIPTIONS

The following sequences conform with 37 C.F.R. 1.821 1.825 ("Requirements for Patent Applications Containing Nucleotide Sequences and/or Amino Acid Sequence Disclosures - the Sequence Rules") and consistent with World Intellectual Property Organization (WIPO) Standard ST.25 (2009) and the sequence listing requirements of the EPO and PCT (Rules 5.2 and 49.5(a bis), and Section 208 and Annex C of the Administrative Instructions). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.