PRODUCTION AND SECRETION OF GLUCOSE IN PHOTOSYNTHETIC PROKARYOTES (CYANOBACTERIA)

TECHNICAL FIELD OF THE INVENTION

[0001] The present invention relates in general to the field of exogenous gene expression, and more particularly, to the expression of exogenous cellulose synthase genes in cyanobacteria which result in the production and extracellular production of glucose.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to and is a Divisional Application of U.S. patent application Ser. No. 11/866,872, filed Oct. 3, 2007 and U.S. Provisional Patent Application Ser. No. 60/849,363, filed Oct. 4, 2006, the entire contents of each of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0003] Without limiting the scope of the invention, its background is described in connection with cellulose production.

[0004] Cellulose biosynthesis has a significant impact on the environment and human economy. The photosynthetic conversion of CO_2 to biomass is primarily accomplished through the creation of the cellulosic cell walls of plants and algae (Lynd et al., 2002). With approximately 10^{11} tons of cellulose created and destroyed annually (Hess et al., 1928), this process ameliorates the adverse effects of increased production of greenhouse gasses by acting as a sink for CO_2 (Brown, 2004). Although cellulose is synthesized by bacteria, protists, and many algae; the vast majority of commercial cellulose is harvested from plants.

[0005] Timber and cotton are the primary sources of raw cellulose for a number of diverse applications including textiles, paper, construction materials, and cardboard, as well as cellulose derived products such as rayon, cellophane, coatings, laminates, and optical films. Wood pulp from timber is the most important source of cellulose for paper and cardboard. However, extensive processing is necessary to separate cellulose from other cell wall constituents (Klemm et al. 2005; Brown, 2004). Both the chemicals utilized to extract cellulose from associated lignin and hemicelluloses from wood pulp and the waste products generated by this process pose serious environmental risks and disposal problems (Bajpai, 2004). Additionally, the cultivation of other cellulose sources, such as cotton, entails the extensive use of large tracts of arable land, fertilizers and pesticides (both of which require petroleum for their manufacture), and dwindling fresh water supplies for irrigation.

SUMMARY OF THE INVENTION

[0006] More particularly, the present invention includes compositions, methods, systems and kits for the production of microbial cellulose using cyanobacteria that include a portion of an exogenous cellulose operon sufficient to express bacterial cellulose. Examples of cyanobacteria for use with the present invention include those that are photosynthetic, nitrogen-fixing, capable of growing in brine, facultative heterotrophs, chemoautotrophic, and combinations thereof.

[0007] In one embodiment, the present invention includes compositions and methods for isolated cyanobacteria that include a portion of an exogenous bacterial cellulose operon sufficient to express bacterial cellulose, whereby the cyanobacterium is capable of the extracellular production of glucose. In one aspect, the cyanobacterium is further defined as producing extracellular glucose in the form of monosaccharides, disaccharides, oligosaccharides or polysaccharides from photosynthesis. In another aspect, the cyanobacterium is further defined as making monosaccharides, disaccharides, oligosaccharides or polysaccharides that comprise glucose and cellulose. Examples of cyanobacteria for use with the present invention include *Synechococcus* sp. PCC 7002, *Synechococcus leopoliensis* strain UTCC100, *Agmenellum quadruplicatum* UTEX B2268, and *Synechococcus* sp. ATCC 27264. Furthermore, the glucose, the cyanobacterial extracellular sheath or both are further processed as a renewable feedstock for biofuel production. In one aspect, the cyanobacterium can fix CO_2 while producing cellulose and reducing atmospheric CO_2 that are quantified as carbon credits which are then sold in the open market, e.g., a carbon credit market. In one aspect, the cyanobacteria increase the extracellular production of monosaccharides, disaccharides, oligosaccharides or polysaccharides upon exposure to acidic conditions.

[0008] Another embodiment of the present invention includes an isolated cyanobacterium, which includes a *Synechococcus* sp., with a portion of an exogenous bacterial cellulose operon sufficient to express bacterial cellulose, whereby the cyanobacterium is capable of secreting monosaccharides, disaccharides, oligosaccharides or polysaccharides that include glucose. In one aspect, the cyanobacterium is further defined as producing extracellular glucose in the form of monosaccharides, disaccharides, oligosaccharides or polysaccharides from photosynthesis. In another aspect, the cyanobacterium is further defined as making monosaccharides, disaccharides, oligosaccharides or polysaccharides that comprise glucose and cellulose. Example of cyanobacteria include *Synechococcus* sp. PCC 7002, *Synechococcus leopoliensis* strain UTCC100, *Agmenellum quadruplicatum* UTEX B2268, and *Synechococcus* sp. ATCC 27264. The cellulose, the cyanobacterial extracellular sheath or both are further processed as a renewable feedstock for biofuel production.

[0009] Another method of the present invention includes producing a photobiomass that may include monosaccharides, disaccharides, oligosaccharides or polysaccharides that include glucose, by modifying a cyanobacterium with a portion of an exogenous bacterial cellulose operon sufficient to express and produce extracellular glucose; growing the cyanobacterium under conditions that promote extracellular glucose production; and exposing the cyanobacteria to an acidic condition, wherein the acid increases glucose secretion. The method may further include the step of processing the glucose into ethanol. For example, the glucose is used as a renewable feedstock for biofuel production, to fix CO_2 and thus atmospheric CO_2 or even as a renewable feedstock for animals.

[0010] Another embodiment of the present invention includes a method of fixing carbon by growing a cyanobacterium comprising a portion of an exogenous bacterial cellulose operon sufficient to make cellulose and produce extracellular glucose in a CO_2-containing growth medium; generating glucose with said cyanobacterium, wherein CO_2 is fixed into glucose at a level higher than an unmodified cyanobacterium; and calculating the amount of CO_2 fixed into the