CLAIMS

We claim:

1. A bacterial cell comprising increased levels of phosphoenol pyruvate carboxykinase (*pck*) gene transcripts and one or more genetic modifications in one or more genes selected from the group consisting of *ptsG*, *ptsH*, *ptsI*, *crr* and *crp*, said transcripts encoding active phosphoenol pyruvate carboxykinase (PCK) and wherein said cell exhibits increased PCK activity.

2. The bacterial cell of claim 1, wherein said bacterial cell is a non-ruminant bacterial cell.

3. The bacterial ~~strain~~cell of claim 1, wherein said bacterium is *Escherichia coli, Gluconobacter oxydans, Gluconobacter asaii, Achromobacter delmarvae, Achromobacter viscosus, Achromobacter lacticum, Agrobacterium tumefaciens, Agrobacterium radiobacter, Alcaligenes faecalis, Arthrobacter citreus, Arthrobacter tumescens, Arthrobacter paraffineus, Arthrobacter hydrocarboglutamicus, Arthrobacter oxydans, Aureobacterium saperdae, Azotobacter indicus, Brevibacterium ammoniagenes, divaricatum, Brevibacterium lactofermentum, Brevibacterium flavum, Brevibacterium globosum, Brevibacterium fuscum, Brevibacterium ketoglutamicum, Brevibacterium helcolum, Brevibacterium pusillum, Brevibacterium testaceum, Brevibacterium roseum, Brevibacterium immariophilium, Brevibacterium linens, Brevibacterium protopharmiae, Corynebacterium acetophilum, Corynebacterium glutamicum, Corynebacterium callunae, Corynebacterium acetoacidophilum, Corynebacterium acetoglutamicum, Enterobacter aerogenes, Erwinia amylovora, Erwinia carotovora, Erwinia herbicola, Erwinia chrysanthemi, Flavobacterium peregrinum, Flavobacterium fucatum, Flavobacterium aurantinum, Flavobacterium rhenanum, Flavobacterium sewanense, Flavobacterium breve, Flavobacterium meningosepticum, Micrococcus sp. CCM825, Morganella morganii, Nocardia opaca, Nocardia rugosa, Planococcus eucinatus, Proteus rettgeri, Propionibacterium shermanii, Pseudomonas synxantha, Pseudomonas azotoformans, Pseudomonas fluorescens, Pseudomonas ovalis, Pseudomonas stutzeri, Pseudomonas acidovolans, Pseudomonas mucidolens, Pseudomonas testosteroni, Pseudomonas aeruginosa, Rhodococcus erythropolis, Rhodococcus rhodochrous, Rhodococcus sp. ATCC 15592, Rhodococcus sp. ATCC 19070, Sporosarcina ureae, Staphylococcus aureus, Vibrio metschnikovii, Vibrio tyrogenes, Actinomadura madurae, Actinomyces violaceochromogenes, Kitasatosporia parulosa,*

Streptomyces coelicolor, Streptomyces flavelus, Streptomyces griseolus, Streptomyces lividans, Streptomyces olivaceus, Streptomyces tanashiensis, Streptomyces virginiae, Streptomyces antibioticus, Streptomyces cacaoi, Streptomyces lavendulae, Streptomyces viridochromogenes, Aeromonas salmonicida, Bacillus pumilus, Bacillus circulans, Bacillus thiaminolyticus, Bacillus licheniformis, Bacillus subtilis, Bacillus amyloliquifaciens, Bacillus coagulans, Escherichia freundii, Microbacterium ammoniaphilum, Serratia marcescens, Salmonella typhimurium, Salmonella schottmulleri, or *Xanthomonas citri.*

4. The bacterial cell of claim 1, wherein increased levels of said *pck* transcripts result from replacement of native regulatory sequences with altered regulatory sequences of the *pck* gene, wherein the altered regulatory sequence causes an ~~that~~ increase in the *pck* transcription.

5. The bacterial cell of claim 4, wherein said regulatory sequences are in the promoter region of said *pck* gene.

6. The bacterial cell of claim 1, wherein said increased levels of said *pck* transcripts result from one or more mutations in the promoter region of the *pck* gene.

7. The bacterial cell of claim 6, wherein said one or more mutations are point mutations comprising replacement of nucleotide ~~AG~~ with nucleotide ~~G~~A at position ~~68~~64 up stream of the *pck* gene start codon.

8. The bacterial cell of claim 1, wherein increased levels of said *pck* transcripts result from replacement of the native promoter sequence with an exogenous promoter sequence.

9. The bacterial cell of claim 8, wherein said exogenous promoter is a constitutive promoter.

10. The bacterial cell of claim 8, wherein said exogenous promoter is an inducible promoter.

11. The bacterial cell of claim ~~8~~10, wherein the said inducible promoter is a *lac* promoter.

12. The bacterial cell of claim 1, further comprising one or more genetic modification that disrupts the functioning of PEP-dependent phosphotransferase system.

13. The bacterial cell of claim 12, wherein said genetic modification is in one or more genes coding for the structural components of PEP-dependent phosphotransferase system.

~~14. The bacterial cell of claim 12, wherein said genetic modification is in one or more genes coding for proteins that regulate the expression of PEP-dependent phosphotransferase system.~~

~~15. The bacterial cell of claim 12, wherein the said genetic modification is in one or more genes selected from the group consisting of *ptsG*, *ptsH*, *ptsI*, *err* and *crp*.~~

~~16.~~14. The bacterial cell of claim 1, further comprising: (a) one or more genetic modification that disrupts the functioning of PEP-dependent phosphotransferase system; and (b) one or more genetic modifications that upregulate the expression of one or more genes encoding sugar transporters.

~~17.~~15. The bacterial cell of claim ~~16~~14, wherein said sugar transporter is a member of ATP binding cassette transporters.

~~18.~~16. The bacterial cell of claim ~~16~~14, wherein said sugar transporter is a member of major facilitator super family.

~~19.The bacterial cell of claim 1, further comprising genetic modification leading to the inactivation of gene expression in one or more genes involved in the fermentative pathway.~~

~~20.~~17. The bacterial cell of claim 1, further comprising a genetic modification leading to the inactivation of gene expression in one or more genes selected from ~~a~~the group consisting of *adhE*, *ldhA*, *focA*, *pflA*, *ack*, *pta*, *pdh*, *mgsA*, *tdcD*, *tdcE* and *poxB*.

~~21.~~18. The bacterial cell of claim 1, further comprising: (a) one or more genetic modification that disrupts the functioning of PEP-dependent phosphotransferase system; and (b) mutation in one or more genes involved in the fermentative pathway.

~~22.~~19. The bacterial cell of claim 1, further comprising: (a) genetic modification in one or more genes selected from a group consisting of *ptsG*, *ptsH*, *ptsI*, *crr* and *crp*; and (b) genetic modifications leading to the inactivation of gene expression in one or more genes selected from a group consisting of *adhE*, *ldhA*, *focA*, *pflA*, *ack*, *pta*, *pdh*, *mgsA*, *tdcD*, *tdcE* and *poxB*.

~~23.~~20. The bacterial cell of claim 1, further comprising genetic modification in one or more genes associated with the operation of the TCA cycle.

~~24.~~21. The bacterial cell of claim 1, further comprising a genetic modification in one or more genes selected from ~~a~~the group consisting of *mdh*, *fumA*, *fumB*, *fumC*, *frdABCD*, *a*~~e~~*ceAB*, *acnAB*, *icd*, *iclR*, *aspC*, and *scfA*.

~~25.~~22. The bacterial cell of claim 1, said bacterial cell further comprising : ~~(a) genetic modification in one or more genes selected from a group consisting of *ptsG*, *ptsH*, *ptsI*, *crr* and *crp*;~~ (~~b~~a) genetic modifications leading to the inactivation of gene expression in one or more genes selected from ~~a~~the group consisting of *adhE*, *ldhA*, *focA*, *pflA*, *ack*, *pta*, *pdh*, *mgsA*, *tdcD*, *tdcE* and *poxB*; and (~~e~~b) a genetic modification in one or more genes selected from ~~a~~the group consisting of *mdh*, *fumA*, *fumB*, *fumC*, *frdABCD*, *a*~~e~~*ceAB*, *acnAB*, *icd*, *iclR*, *aspC*, and *scfA*.

~~26.~~23. The bacterial ~~strain~~cell of claim 1, further comprising a genetic modification in one or more genes selected from ~~a~~the group consisting of phosphoenol pyruvate carboxylase, NADH dependent malic enzyme and NADPH dependent malic enzyme.

~~27.~~24. The bacterial ~~strain~~cell of claim 1, further comprising an exogenous pyruvate carboxylase.

~~28.~~25. The bacterial ~~strain~~cell of claim ~~27~~24, wherein said pyruvate carboxylase is from *Lactobacillus lactis* or *Sorghum vulgare* or *Rhizopium etli*.

~~29.~~26. A genetically modified bacterial cell, wherein said genetically modified bacterial cell is NRRL B-50267, NRRL B-50268, NRRL B-50269, NRRL B-50271, NRRL B-50272, NRRL B-50273, NRRL B-50274, NRRL B-50275, NRRL B-50276, NRRL B-50277, NRRL B-50278, NRRL B-50279, NRRL B-50280, NRRL B-50281~~XZ320, XZ332, XZ3431, XZ468, XZ469, XZ470, XZ613, XZ615, XZ616, XZ618, XZ620, XZ647, XZ7121, or XZ723~~.

~~30.~~27. An *Escherichia coli* bacterial ~~strain~~cell comprising: (a) an inactivated *ldhA*; (b) inactivated *focA*; (c) an inactivated *pflB*; (d) inactivated *ackA*; (e) an inactivated *mgsA*; (f) an inactivated *adhE*; (g) an inactivated *tdcD*; (h) an inactivated *tdcE*; (i) an inactivated *aspC*; (j) an inactivated *sfcA*; (k) an inactivated *ptsH*; (l) an inactivated *citD* and (m) an up regulated *pck*.

~~31.~~28. The bacterial cell as in claim ~~29~~26 or claim ~~30~~27 wherein said bacterial ~~strain~~cell produces at least 0.1 moles of succinic acid per mole of glucose in a minimal salt medium comprising between 2% and 20% (w/v) glucose.

~~32.~~29. A method of producing succinic acid comprising:

(a) Culturing a bacterial ~~strain~~cell of claim 1;

(b) Providing a carbon source;

(c) Allowing said bacteria to metabolize said carbon source; and

(d) Isolating succinic acid

~~33.~~30. The method of claim ~~32~~29, wherein said bacterial ~~strain~~cell is cultured in anaerobic condition, aerobic condition, microaerobic condition or combinations thereof.

~~34.~~31. The method of claim ~~32~~29, wherein said bacterial ~~strain~~cell is cultured in minimal salt growth medium.

~~35.~~32. The method of claim ~~32~~31, wherein said minimal salt growth medium comprises between 2% and 20% (w/v) carbon source.

~~36.~~33. The method of claim ~~32~~29, wherein the said carbon source is glucose, fructose, xylose, arbinose, galactose, mannose, rhamnose, sucrose, cellobiose, hemicelluloses, glycerol or combination thereof.

~~37.~~34. The method of claim ~~32~~29, wherein said culturing is a batch process or fed batch process.

~~38.~~35. The method of claim ~~32~~29, wherein said culturing is a continuous process.

~~39.~~36. A bacterial cell as in claim 1 further comprising mutation in *gldA* gene.

~~40.~~37. A bacterial cell as in claim 1 further comprising a mutation in *dhaKLM* operon.

~~41.~~38. A bacterial cell as in claim 1 further comprising: (a) a mutation in *gldA*; and (b) a mutation in *dhaKLM* operon.

~~42.~~39. A bacterial cell as in claim 1 further comprising: (a) a mutation in *gldA*; and (b) a mutation in *dhaKLM* operon~~; and (c) a mutation in one or more genes coding for a protein in phosphotransferase system~~.

~~43.~~40. A bacterial cell as in claim 1 further comprising: (a) a mutation in *gldA*; (b) a mutation in *dhaKLM* operon; and (c) ~~a mutation in one or more genes coding for a protein in phosphotransferase system; and (d)~~ a mutation in one or more genes coding for the proteins involved in fermentative pathway.

~~44.~~41. The bacterial cell according to any one of claims 1-~~30~~27 or ~~39-43~~36-40, wherein said *pck* transcripts are endogenous *pck* transcripts.