UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/560,937	11/17/2006	Keita FUKUI	P31186	6604

7055 7590 02/21/2013
GREENBLUM & BERNSTEIN, P.L.C.
1950 ROLAND CLARKE PLACE
RESTON, VA 20191

EXAMINER
RAGHU, GANAPATHIRAM

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
02/21/2013	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

gbpatent@gbpatent.com
greenblum.bernsteinplc@gmail.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte KEITA FUKUI, JUN NAKAMURA, KAYO AKIYAMA, and HIROYUKI KOJIMA

Appeal 2011-005094
Application 11/560,937
Technology Center 1600

Before DONALD E. ADAMS, ERICA A. FRANKLIN, and JACQUELINE WRIGHT BONILLA, *Administrative Patent Judges*.

ADAMS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims 8, 10, 13, 14, and 16 (App. Br. 5; Ans. 2).[1] We have jurisdiction under 35 U.S.C. § 6(b).

[1] Pending claims 1-7 stand withdrawn from consideration (App. Br. 5).

STATEMENT OF THE CASE

The claims are directed to a method for producing succinic acid. Claim 8 is representative and is reproduced in the Claims Appendix of Appellants' Brief.

Claims 8, 10, 13, 14, and 16 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Dusch,[2] Tomar,[3] '888,[4] and '385.[5]

We affirm.

ISSUE

Does the preponderance of evidence on this record support a conclusion of obviousness?

---

[2] Dusch et al., JP 2001-161386, published June 19, 2001 (as translated in ZA 20006039, lodged October 26, 2000; *see generally* Office Action entered October 14, 2008).

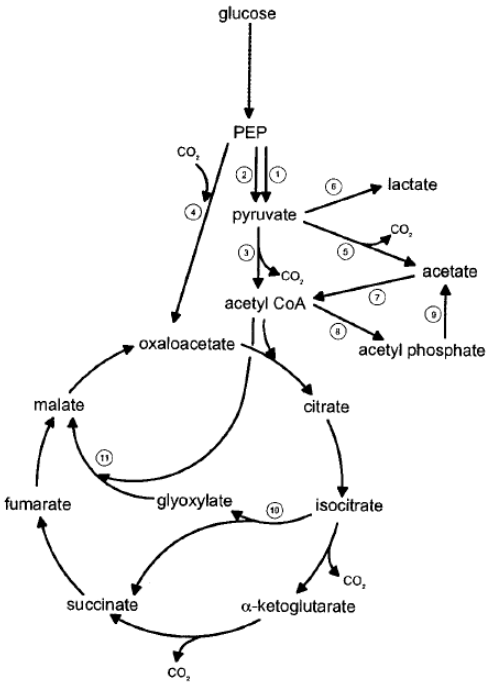
[3] A. Tomar et al., *The effect of acetate pathway mutations on the production of pyruvate in Escherichia coli*, 62 APPL. MICROBIOL. BIOTECHNOL. 76-82 (2003).

[4] Kobayashi et al., JP 11-196888, published July 27, 1999 (as translated at http://dossier1.ipdl.inpit.go.jp/cgi-bin/tran_web_cgi...edical_NG2V13 &Ntt9=gakujutsuV13&Ntt10=&Ntt11=&Ntt12=, accessed March 23, 2009; *see generally* Office Action entered October 14, 2008).

[5] Hatakeyama et al., JP 11-206385, published August 3, 1999 (as translated at http://dossier1.ipdl.inpit.go.jp/cgi-bin/tran_web_cgi_...ience_NG1_v5& Ntt0=science_NG2_v5&Ntt10=science_NG3_v5, accessed July 17, 2008; *see generally* Office Action entered October 14, 2008).

FACTUAL FINDINGS (FF)

FF 1. Tomar's Figure 1 is reproduced below:



Tomar's Figure 1 illustrates "[b]iochemical pathways involved in pyruvate accumulation in *Escherichia coli*. *1* Phosphotransferase system, *2* pyruvate kinase, *3* pyruvate dehydrogenase, *4* phosphoenol pyruvate carboxylase, *5* pyruvate oxidase, *6* lactate dehydrogenase, *7* acetyl CoA synthase, *8* phosphotransacetylase, *9* acetate kinase, *10* isocitrate lyase, *11* malate synthase" (Tomar 77: col. 1, Fig. 1, legend).

FF 2. '888 suggests a

> method for production of succinic acid in [] coryneform bacteria having succinic acid producing ability…, said method comprising allowing said coryneform bacterium … to act on an organic raw material in a reaction liquid containing carbonate ion, bicarbonate ion or carbon dioxide to produce and accumulate succinic acid in the reaction liquid under <u>anaerobic conditions</u> … <u>polymerizing the obtained succinic acid</u>

(Ans. 4-5 (citing '888 claims 1-6 and entire document).)

FF 3. Tomar's Figure 1 illustrates, *inter alia*, that "pyruvate oxidase and lactate dehydrogenase … divert … pyruvate (a key intermediate precursor for succinate/succinic acid production) to form acetate or lactate (unwanted by-products) respectively" (Ans. 6; *see* Tomar 77: col. 2, ll. 17-18; *see also* Tomar 77, Fig. 1).

FF 4. '385 suggests a "method for production of succinic acid in a coryneform bacteria having succinic acid producing ability" that involves

> disruption of [the] lactate dehydrogenase gene responsible for catalyzing the formation of lactate, an unwanted by-product of fermentation…, said method comprising allowing said genetically modified coryneform bacterium … to act on an organic raw material in a reaction liquid containing carbonate ion, bicarbonate ion or carbon dioxide to produce and accumulate succinic acid in the reaction liquid under <u>anaerobic conditions</u>.

(Ans. 5 (citing '385 claims 1-6 and entire document).)

FF 5. "Pyruvate oxidase is encoded by the *poxB* gene and … would be an obvious next candidate for mutation as a means to generate additional pyruvate" (Tomar 81: col. 1, l. 7 - col. 2, l. 2; Ans. 6).

FF 6. Dusch suggests the amino acid sequence of pyruvate oxidase, which corresponds to Appellants' SEQ ID NO: 49 (Ans. 4).

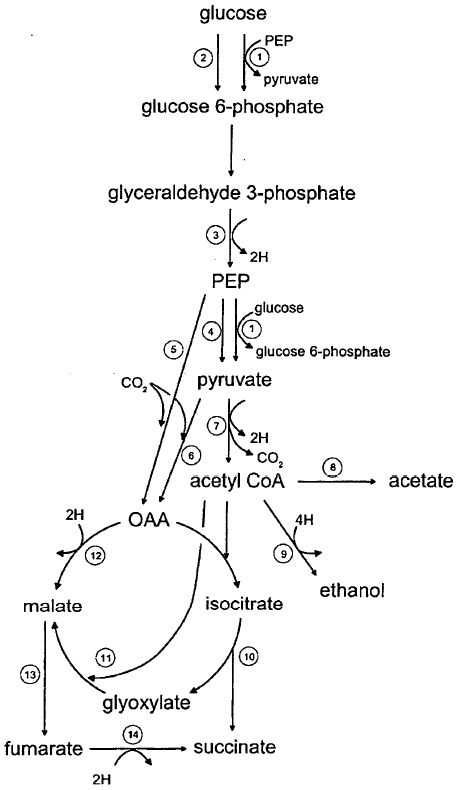
FF 7. Dusch suggests "coryneform bacteria … wherein the activity of [the] *poxB* gene encoding the pyruvate oxidase enzyme has been attenuated/decreased as a result of integration mutagenesis" (*id.*; *see* Dusch translation 5: 18-27 (Dusch's "invention … relates to a process for the fermentative production of amino acids … using coryneform bacteria … [wherein] the nucleotide sequences which code for the poxB gene are attenuated…. [T]he term 'attenuation' means reducing or suppressing the

intracellular activity of one or more enzymes … in a microorganism"); *see also id.* at 19: 10 ("Integration mutagenesis of the poxB gene")).

FF 8. Examiner finds that the "pyruvate carboxylase gene (*pyc*) [is] one of the key genes involved in the production of organic acids such as succinic acid" (Ans. 5).

FF 9. Dusch and '888 both suggest "the overexpression/increased activity of [the] *pyc* gene" in coryneform bacteria (*id.* at 4-5)

FF 10. Vemuri's[6] Figure 4 is reproduced below:



Vemuri's Figure 4 illustrates "[b]iochemical pathways for the synthesis of succinate from glucose in *E. coli*" (Vemuri 1722: FIG. 4, legend).

---

[6] G. N. Vemuri, et al., *Effects of Growth Mode and Pyruvate Carboxylase on Succinic Acid Production by Metabolically Engineered Strains of Escherichia coli*, 68(4) APPLIED AND ENVIRONMENTAL MICROBIOLOGY 1715-1727 (2002).

ANALYSIS

Appellants provide separate arguments for the following groups of claims: (I) claims 8, 13, 14, and 16; and (II) claim 10.[7] Claims 8 and 10 are representative.

Based on the combination of Dusch, Tomar, '888, and '385, Examiner concludes that, at the time Appellants' invention was made, it would have been prima facie obvious to produce succinic acid by a method that allows a coryneform bacterium, comprising: (1) modifications that disrupt the genes encoding, and thereby decrease the activity of, pyruvate oxidase and lactate dehydrogenase and (2) having a succinic acid-producing ability, to act on an organic raw material in a reaction liquid containing carbonate ion, bicarbonate ion or carbon dioxide under anaerobic conditions to produce and accumulate succinic acid in the reaction liquid (*see* Ans. 6-8; FF 1-9).

Claim 8:

Appellants contend "that the cited documents, either alone or in combination, fail to suggest that a decrease in pyruvate oxidase activity, much less a decrease in pyruvate oxidase activity as a result of modification of a gene encoding SEQ ID NO: 49 or a variant thereof, plays a role in succinic acid biosynthesis" (App. Br. 17). We are not persuaded.

Methods of producing succinic acid, wherein coryneform bacteria are allowed to act on an organic raw material in a reaction liquid containing

[7] Appellants separately list claims 13, 14, and 16 and contend that "the obviousness rejection based upon [these] claim[s] … is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 8" (App. Br. 31-32). Therefore, claims 13, 14, and 16 stand or fall with claim 8.

carbonate ion, bicarbonate ion or carbon dioxide to produce and accumulate succinic acid in the reaction liquid under anaerobic conditions, were known at the time of Appellants' claimed invention (FF 2 and 4).

Tomar's Figure 1 illustrates that pyruvate is an intermediate, i.e., a precursor, for succinic acid production (FF 1 and 3). Therefore, at the time of Appellants' claimed invention, a person of ordinary skill in this art would have recognized that pathways diverting pyruvate away from the production of succinic acid, or otherwise reducing the intracellular concentration of pyruvate, would have been detrimental to the production of succinic acid (*see, e.g.*, FF 4). Tomar's Figure 1 illustrates that *pyruvate oxidase* and *lactate dehydrogenase* divert pyruvate away from the production of succinic acid (FF 1).

Disruption of *lactate dehydrogenase* inhibits the conversion of pyruvate to lactate (*see* FF 1 and 3). '385 suggests a method of producing succinic acid, wherein a coryneform bacteria comprising a disruption of the *lactate dehydrogenase* gene is allowed to act on an organic raw material in a reaction liquid containing carbonate ion, bicarbonate ion or carbon dioxide to produce and accumulate succinic acid in the reaction liquid under anaerobic conditions (FF 4).

Tomar suggests that pyruvate oxidase would have been "an obvious next candidate for mutation as a means to generate additional pyruvate" (FF 5). Stated differently, Tomar suggests the disruption of *pyruvate oxidase* to inhibit the conversion of pyruvate to acetate (*id.*; *see* FF 3). Dusch suggests the modification of the pyruvate oxidase gene, which encodes a protein having the amino acid sequence of Appellants' SEQ ID NO: 49, in coryneform bacteria (FF 6-7).

Therefore, notwithstanding Appellants' contentions to the contrary, the combination of prior art relied upon by Examiner suggests that decreases in pyruvate oxidase and lactate dehydrogenase activities play a role in succinic acid biosynthesis by inhibiting pyruvate from entering pathways that result in unwanted products (*Cf.* App. Br. 17).

For the foregoing reasons we are not persuaded by Appellants' contention that "it was not known that … the enhancement or attenuation of any gene involved in L-amino acid production [(e.g., pyruvate oxidase)] was *also* effective in succinic acid production" (App. Br. 17; *see also id.* at 23; *Cf.* FF 5-7).

For the same reasons we are not persuaded by Appellants' contention that Examiner "fail[ed] to establish or provide a reason as to *why* one of ordinary skill in the art would have combined the art cited to arrive at the invention *as claimed*" (*id.* at 18; *see also id.* at 21 "the cited art fails to specifically motivate the skilled artisan to practice … [the claimed] method").

Appellants failed to establish that succinate/succinic acid production in coryneform bacteria follows a different pathway than that set forth in Tomar's figure 1 (*see* FF 1; *Cf.* Ans. 11 (the "TCA cycle is conserved across various organisms including prokaryotes and eukaryotes")). Therefore, we are not persuaded by Appellants' contentions that "Tomar is not germane to the claimed subject matter" or that "[u]nder anaerobic conditions the TCA cycle does not proceed well" (App. Br. 18 and Reply Br. 2).

We recognize, but are not persuaded by, Appellants' reference to "the Official Action mailed September 3, 2008" in support of their contention that the obviousness rejection before this panel "can only be characterized as

a 180 degree shift in [Examiner's] position" (*id.* at 18-19; *see also* Reply Br. 3-4). We recognize that in response to Examiner's September 3, 2008 Office Action, Appellants amended the claims and contended that "any (and every) coryneform bacteria *can* be used, as the succinic acid-producing pathway including pyruvate oxidase and lactate dehydrogenase is conserved in coryneform bacteria" (December 10, 2008, Amendment Under 37 C.F.R. § 1.11: 8-9; *Cf.* Ans. 19-21). As discussed above, Appellants failed to establish that succinate/succinic acid production in coryneform bacteria follows a different pathway than that set forth in Tomar's figure 1. Therefore, we are not persuaded by Appellants' contention that "the documents in combination fail to establish any nexus between pyruvate oxidase function in *E. coli* and the role of *poxB* disruption in coryneform bacteria in succinic acid production" (App. Br. 20; Reply Br. 4; *Cf.* Ans. 12-13 ("The key points … of entry or diversion of the desired precursors, the metabolic pathway, the specific enzymes, their structure correlated to function are also known in the art for many of the industrially important microorganisms such as *E.*[ ]*coli* and *Corynebacteria*")).

Notwithstanding, Appellants' unsupported contentions regarding the differences between *E. coli* and coryneform bacteria, discussed above, we recognize Appellants' reliance on Vemuri, which addresses the "Effects of Growth Mode and Pyruvate Carboxylase on Succinic Acid Production by Metabolically Engineered Strains of *Escherichia coli*" (Vemuri, Title; App. Br. 24). To be clear, Vermuri illustrates part, but not all, of the pathway set forth in Tomar (*see* FF 10; *Cf.* FF 1; *see also* App. Br. 27). Nevertheless, Appellants contend that "Vermuri fails to disclose pyruvate oxidase['s] involvement in succinate production *at all*, much less as a 'key enzyme' in

succinic acid production" (App. Br. 24; Reply Br. 4-5). We are not persuaded. The question is not whether pyruvate oxidase is involved in succinic acid production, but is instead whether pyruvate concentration in a cell is reduced or otherwise diverted away from succinic acid production, by entering a pathway, involving pyruvate oxidase, that leads to an unwanted product, e.g., acetate. As discussed above, the combination of prior art relied upon by Examiner suggests that succinic acid production will benefit from inhibiting pyruvate from entering into pathways leading to the production of unwanted products, e.g., lactate and acetate (FF 3; *Cf.* App. Br. 27 ("the Office has failed to acknowledge Appellant's arguments and evidence … that one of ordinary skill in the art would not have been motivated by the teachings of Tomar to attenuate *pyruvate oxidase* activity in a method of succinic acid production")).

For the foregoing reasons and those set forth by Examiner, we are not persuaded by Appellants' contentions relating to Bott[8] (*see* Ans. 27-29; *Cf.* App. Br. 25-26 and 27-28; Reply Br. 5-6).

Claim 10:

We are not persuaded by Appellants' contention that the combination of prior art relied upon by Examiner fails to suggest "'polymerizing the obtained succinic acid'" (App. Br. 30; *Cf.* FF 2).

[8] Michael Bott and Axel Niebisch, *The respiratory chain of* <u>*Corynebacterium glutamicum*</u>, 104 J. BIOTECH. 129-153 (2003).

CONCLUSION OF LAW

The preponderance of evidence on this record supports a conclusion of obviousness. The rejection of claims 8 and 10 under 35 U.S.C. § 103(a) as unpatentable over the combination of Dusch, Tomar, '888, and '385 is affirmed. Claims 13, 14, and 16 are not separately argued and fall with claim 8.

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

cdc