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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte HAROLD E. SMITH and FRANK T. ROBB[1]

Appeal 2015-004934
Application 12/999,185
Technology Center 1600

Before ULRIKE W. JENKS, TIMOTHY G. MAJORS, and RACHEL H. TOWNSEND, *Administrative Patent Judges*.

TOWNSEND, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method of enhancing protein folding in a bacteria host, which have been rejected as obvious. We have jurisdiction under 35 U.S.C. § 6(b).

We affirm.

STATEMENT OF THE CASE

"Protein production in bacteria, typically *Escherichia coli*, offers a number of advantages over other systems: ease of transformation and culture growth, a wide range of inducible expression vectors that produce large

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[1] Appellants identify the Real Parties in Interest as the University of Maryland, Baltimore and University of Maryland, College Park. (Appeal Br. 3.)

amounts of protein, and a variety of epitope tags that permit one-step affinity purification." (Spec. ¶ 3.) However, "protein insolubility remains a major stumbling block for recombinant protein production." (Spec. ¶ 4.) In particular, "[t]he failure to fold properly leads to protein aggregation." (Spec. ¶ 6.) According to Appellants' Specification "[i]nsolubility of recombinant proteins is likely a consequence of limited folding capacity in the bacterial host." (Spec. ¶ 7.)

It is known that protein chaperones promote "[p]rotein folding *in vivo* . . . in all organisms." (Spec. ¶ 6.) Appellants' invention is directed at the use of such chaperones to enhance protein folding of a non-native protein in a bacterial host. (Spec. ¶ 9.)

Claims 1, 2, 7, and 8 are on appeal. Claim 1 is representative and reads as follows:

> 1. A method of enhancing protein folding in a bacteria host, the method comprising:
>
> providing at least one expression vector comprising:
>
> > nucleic acid sequences encoding a single chaperone from a hyperthermophilic or psychrophilic archaeon, wherein the single chaperone is selected from the group consisting of *Pyrococcus furiosus* Heat Shock Protein 60 (HSP60) having the amino acid sequence of SEQ ID NO: 14, and *Pyrococcus furiosus* NAC having the amino acid sequence of SEQ ID NO: 16 and *Methanococcoides burtonii* HSP60 having the amino acid sequence of SEQ ID NO: 4; and nucleic acid sequences encoding a non-native protein for expression in the host bacteria, wherein the non-native protein is non-native to both the bacterial host and archaeon;
>
> transforming the bacteria host with the at least one expression vector; and culturing the bacteria host at a temperature of 37 °C

> and conditions sufficient for expression of the non-native protein and the single chaperone.

(Appeal Br. 16.)

The following ground of rejection by the Examiner is before us on review:

Claims 1, 2, 7, and 8 under 35 U.S.C. § 103(a) as unpatentable over Robb[2] and Imanaka.[3]

DISCUSSION

The Examiner finds that Imanaka teaches co-transforming a bacterial cell with a gene encoding a single chaperone protein that aids in protein folding and solubility and a gene encoding a desired protein. (Final Action 5; Ans. 5.) The Examiner further finds that Imanaka teaches culturing such bacterial transformants at 37 °C. (Final Action 5; Ans. 4, 7.) The Examiner also notes that Imanaka teaches that Hsp60 is a known heat shock chaperone protein. (Final Action 5; Ans. 4.)

The Examiner finds that Robb teaches Hsp60 is a chaperone protein that catalyzes protein folding and can be used in that manner, as well as teaching an Hsp60 chaperone protein that has "an amino acid sequence that is 100% identical to SEQ ID NO:14" recited in the claims. (Final Action 5; Ans. 5.) The Examiner further notes that Robb teaches that sHsp is a chaperone protein that is "known to be used to protect proteins . . . from high heat stress" but is "unable to refold proteins in a catalytic fashion." (Ans. 7.)

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[2] WO 2007/028069 A2, published March 8, 2007.
[3] EP 0774512 A2, published May 21, 1997.

The Examiner notes that Robb teaches combining sHsp to provide thermostability with Hsp60 to provide a composition that promotes refolding at "*high temperatures of 100 °C.*" (*Id.* (emphasis in original).)

The Examiner finds that it would have been obvious to one having ordinary skill in the art to use the Hsp60 chaperone protein taught in Robb in the method of Imanaka with a reasonable expectation of achieving success, i.e., recombinantly co-expressing Hsp60 with a non-native protein in a bacterial host to aid in protein folding and solubility. (Final Action 5–6; Advisory Action 3–5; Ans. 6–8.)

We agree with the Examiner's factual findings and conclusion that the claimed method, which involves transforming a bacterial host cell with "at least one expression vector" comprising a single chaperone "selected from the group consisting of *Pyrococcus furiosus* Heat Shock Protein 60 (HSP60) having the amino acid sequence of SEQ ID NO: 14 . . ." would have been obvious from the teachings of Imanaka and Robb.

Appellants seem to argue that Robb cannot be interpreted as teaching that Hsp60 alone provides for protein folding because "it has always been understood in the past that Hsp60 (GroEL) must be combined with co-factor chaperone Hsp10 (GroES) to provide for protein folding," which is a fact implicit from Robb's citation to "Hartl (1996) and Hartl/Hayer-Hartl (2002)" and comports with statements "in the present application" at paragraphs 8 and 64 (Reply Br. 3) as well as Imanaka's disclosure of using GroESL, which is a combination of GroEL and GroES (Reply Br. 5–6). We do not find this argument persuasive. It cannot be disputed that Robb discloses "Hsp60s[, as compared to sHsps, which can prevent denatured proteins from

aggregating but are unable to refold non-native proteins in a catalytic fashion,] on the other hand catalyze ATP-dependent protein folding," regardless of what articles are cited after that statement. (Robb 1.)

Moreover, Imanaka also notes that GroEL, which is in the HSP60 chaperonin family, is "involved in the conformation and the conformational change of a protein." (Imanaka 2.) Furthermore, we note that Robb demonstrates that Hsp60 works by itself to promote protein folding of Taq polymerase at 100 °C. (Robb 13 ("The level of protection by the Hsp60 alone, Figure 2, closed and open squares, was comparable to that of the sHsp alone (Figure 2, crosses). . . ."); Robb 14 ("The Hsp60 alone caused a slight improvement of approximately 2 fold compared to controls (Figure 3 closed and open squares, respectively).").)

In addition, we disagree with Appellants' argument that Robb "teaches the **necessity of using two chaperones in combination** and in light of this reference one would never consider the use of a single chaperone, for example Hsp60, without a co-chaperone." (Appeal Br. 10 (emphasis in original).) As the Examiner noted "Robb teaches a method that is conducted at *high temperatures of 100 °C*." (Ans. 7 (emphasis in original); Robb 11.) As explained in Robb, Taq polymerase is a thermophilic bacterium that can denature at exposure to 95 °C, but that sHsp provides thermal stability by preventing precipitation of denatured Taq polymerase. (Robb 11–12.) The experimentation in Robb proceeded to examine "the cooperative effects of several *P. furiosus* molecular chaperones," noting that Hsp60s are known to be "active proteins," whereas sHsps are "passive." (Robb 13.) And as Appellants recognize, it was

determined that at 100 °C, "using co-chaperones such as prefoldin or sHsp **in combination with Hsp60** was essential for optimal Hsp60 turnover . . . facilitat[ing] its performance by five-fold," whereas, by itself, Hsp60 "was comparable to that of the sHsp alone." (Appeal Br. 9–10 (emphasis in original); Robb 14).) Thus, Robb does not teach that Hsp60, by itself, fails to promote protein folding of Taq polymerase at 100 °C. Rather, Robb is reasonably interpreted as teaching that performance is *optimized* with the addition of sHsp or pre-foldin. (Robb 13–14 (referring to results depicted in Figs. 2 and 3 and noting that "[t]he present results indicated that prefoldins and sHsps have analogous roles as they both improve the efficiency of Hsp60 catalysis.") Indeed, Appellants appear to concede as much. (Reply Br. 4 ("Clearly, using a single chaperone did not provide effective results *when compared to the use of a combination of sHsp and Hsp60*") (emphasis added); *see also* Appeal Br. 10 ("The level of protection by the Hsp60 alone, Robb Figure 2, closed and open squares, was comparable to that of the sHsp alone (Robb Figure 2, crosses). . . . It is evident that when Hsp60 and sHsp were used alone by Robb, the results were not much better than the control.").) We note that Appellants' claims do not require "optimal" protein folding or specify any particular degree of folding required. Thus, with respect to Appellants' claimed invention, we disagree with Appellants that Robb teaches the necessity of two chaperones.

Second, while Appellants argue that using Hsp60 alone was not much better than the control (Reply Br. 5), we note that Robb indicates that enzyme activity was 2 fold better when Hsp60 was added compared to the control where no Hsp60 was added and no pre-foldin. (Robb 14 (referring

to Fig. 3).) Appellants argue that a "between 2 to 2.5 fold" increase demonstrated in the Specification is "impressive" (Appeal Br. 7 (referring to Figure 2 and Spec. ¶ 59 ("GFP fluorescence was increased between 2 to 2.5 fold compare[d] to cells expressing GFP alone.").) Thus, we disagree with Appellants that Robb teaches away from using Hsp60 by itself (Appeal Br. 12). We do not agree with Appellants that, in light of Robb, "one would never consider the use of a single chaperone, for example Hsp60, without a co-chaperone" (Appeal Br. 10). And, we disagree with Appellants that "the Robb reference never recognized that the use of the Hsp60 chaperone 'alone' was a result-effective variable" (Appeal Br. 10).

Moreover, even if it were true that Hsp60 used alone "were not much better than the control," we disagree that one of ordinary skill in the art would not consider using the Hsp60 alone. Even if better alternatives exist in the prior art, that "does not mean that an inferior combination is inapt for obviousness purposes." *In re Mouttet*, 686 F.3d 1322, 1334 (Fed. Cir. 2012).

Where "a patent claims a structure already known in the prior art that is altered by the mere substitution of one element for another known in the field, the combination must do more than yield a predictable result." *See KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 416 (2007). Robb teaches that Hsp60 by itself promotes folding of protein, and that, even at 100 °C, Hsp60 is two-fold better than in the absence of Hsp60. (Robb 14.) Thus, we do not find Appellants' argument that a "2 to 2.5 fold" increase in expression of and folding of proteins compared to cells that did not include

Hsp60 was surprising or unexpected (Appeal Br. 13–14). Rather, the evidence of record indicates that the result is predictable.

We also disagree with Appellants that Robb teaches away from the effectiveness of using Hsp60 by itself in Imanaka's method. (Appeal Br. 12.) As just discussed, Robb demonstrates that Hsp60 functions by itself even at 100°C. Appellants do not argue, nor do we find anything in Robb, that teaches Hsp60 would not be expected to function at lower temperatures. As the Examiner noted (Final Action 5), Imanaka teaches an *in vivo* method where co-transformation takes place at 37 °C. (*See*, *e.g.*, Imanaka at 9 (Ex. 6 (noting that transformed cells were cultured at 37 °C in NZCYM medium)), 10 (Ex. 9 (noting that cells co-transformed with a protein of interest and a chaperone were "cultured in NZCYM medium in the same manner as in Example 6")), (Ex. 10) (same)). Thus, we agree with the Examiner that "since Robb teaches sHsp protects proteins from the effects of high heat stress (100 °C) and sHsp does not catalyze protein folding, one would have recognized that Robb's Hsp60 alone is sufficient to catalyze protein folding in lmanaka's method, which is conducted at low temperature (37 °C)." (Ans. 7.)

Appellants further argue that there is nothing in the prior art that teaches the use of a single chaperone to increase protein folding of a non-native protein by a bacterial host. (Appeal Br. 13.) According to Appellants, Imanaka "**<u>did not</u>** use just a SINGLE [Hsp60] chaperone." (Reply Br. 5 (emphasis in original).) While it may be the case that in Example 7, Imanaka described the use of GroEL with GroES, as Appellants

concede (Reply Br. 6), Imanaka's examples 9 and 10 employed Hsp from KOD-1, as did example 11. (Imanaka 10–11.)[4]

Moreover, in light of example 11 of Imanaka (referred to by the Examiner (Ans. 9)), which teaches expression of soluble sFV in a bacterial host using a single Hsp, we disagree with Appellants that the prior art does not teach using a single chaperone protein in a bacterial host to produce a non-native protein.

In their Reply Brief, Appellants argue that the fact that Imanaka teaches production of proteins as soluble proteins, it does not teach the proteins produced by the bacterial host were properly folded. (Reply Br. 6.) This argument is deemed waived. 37 C.F.R. § 41.41(b)(2) ("Any argument raised in the reply brief which was not raised in the appeal brief, or is not responsive to an argument raised in the examiner's answer, including any designated new ground of rejection, will not be considered by the Board for purposes of the [present] appeal, unless good cause is shown.").

In light of the foregoing reasons, we conclude the Examiner established by a preponderance of the evidence that claim 1 would have been obvious over Robb and Imanaka.

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[4] We also agree with the Examiner, however, that the claims by the use of the transitional phrase comprising and the phrase "at least one expression vector" do not exclude provision of an additional expression vector that can encode an additional chaperone. (Ans. 8.) Thus, to the extent that Imanaka is deemed to require both GroEL and GroES, we agree with the Examiner that Imanaka envisions providing them individually as well as by a recombinant expression of both as GroESL. Thus, such a method would also be deemed obvious.

Claims 2, 7, and 8 have not been argued separately and, therefore, fall with claim 1. 37 C.F.R. § 41.37(c)(1)(iv).

SUMMARY

We affirm the rejection of Claims 1, 2, 7, and 8 under 35 U.S.C. § 103(a) as unpatentable over Robb and lmanaka.

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