

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 54

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte DENNIS G. KLEID, DANIEL G. YANSURA,
HERBERT L. HEYNEKER and MIOZZARI F. GIUSEPPE

Appeal No. 1999-1157
Application No. 08/482,321

ON BRIEF

Before WILLIAM F. SMITH, ADAMS and MILLS, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL¹

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1, 9, 10, and 35-37, which are all the claims pending in the application.

Claim 1 is illustrative of the subject matter on appeal and is reproduced below:

1. The method of producing a polypeptide product by the expression in bacteria of a structural gene coding therefor which comprises:
 - (a) providing a bacterial inoculant transformed with a replicable plasmidic expression vehicle having a sequence of double-stranded DNA

¹ This application has a filing date of June 6, 1995. However, we note that this application has an effective filing date of March 24, 1980.

comprising, in phase from a first 5' to a second 3' end of the coding strand thereof, the elements:

- (i) a bacterial trp promoter-operator system from a bacterial trp operon;
 - (ii) nucleotides coding for a ribosome binding site for translation of element (iv);
 - (iii) nucleotides coding for a translation start signal for translation of element (iv); and
 - (iv) a structural gene encoding the amino acid sequence of a heterologous polypeptide;
said sequence comprising neither any trp attenuation capability nor nucleotides coding for the trp E ribosome binding site, provided however, that if the heterologous polypeptide includes any polypeptide also includes polypeptide sequence not encoded by the trp operon;
- (b) placing the transformed inoculant in a fermentation vessel and growing the same to a predetermined level in a suitable nutrient media containing additive tryptophan sufficient in quantity to repress said promoter-operator system; and
- (c) depriving said bacteria of said additive while maintaining the viability of said bacteria so as to derepress said system and occasion the expression of the product encoded by said structural gene.

The references relied upon by the examiner are:

Cohen et al. (Cohen) 4,237,224 Dec. 2, 1980

Bertrand et al. (Bertrand), "Regulation of Transcription Termination in the Leader Region of the Tryptophan Operon of Escherichia coli Involves Tryptophan or its Metabolic Product," J. Mol. Biol., Vol. 103, pp. 339-349 (1976)

Miozzari et al. (Miozzari (R)), "Translation of the Leader Region of the Escherichia coli Tryptophan Operon," J. Bacteriology, Vol. 133, No. 3, pp. 1457-1466 (1978)

Miozzari et al. (Miozzari (S)), "Tryptophan Biosynthesis in Saccharomyces cerevisiae: Control of the Flux Through the Pathway," J. Bacteriology, Vol. 134, No. 1, pp. 48-59 (1978)

Goeddel et al. (Goeddel), "Direct expression in Escherichia coli of a DNA sequence coding for human growth hormone," Nature, Vol. 281, pp. 544-548 (1979)

The reference relied upon by the Board is:

Backman et al. (Backman), "Construction of plasmids carrying the *cl* gene of bacteriophage ϕ ," Proc. Natl. Acad. Sci. USA, Vol. 73, No. 11, pp. 4174-4178 (1976)

GROUND OF REJECTION

Claims 1, 9, 10 and 35-37 stand rejected under 35 U.S.C. § 103 as being unpatentable over Cohen in view of Bertrand and Goeddel.

Claims 1, 9, 10 and 35-37 stand rejected under 35 U.S.C. § 103 as being unpatentable over Cohen in view of Miozzari (R), Miozzari (S) and Goeddel.

We reverse.

DISCUSSION

In reaching our decision in this appeal, we have given careful consideration to the appellants' specification and claims, and to the respective positions articulated by the appellants and the examiner. We make reference to the examiner's Answer² for the examiner's reasoning in support of the rejections. We further reference appellants' Brief³ for the appellants' arguments in favor of patentability.

² Paper No. 53, mailed January 3, 1997.

³ Paper No. 52, received November 13, 1996.

At the outset we note that this application was filed on June 6, 1995. However, through a number of continuation applications, this application has an effective filing date of March 24, 1980. Accordingly, we look back over 20 years to evaluate the obviousness rejections of record from the perspective of a person of ordinary skill in the art at the time this invention was made.

Initially, we note that appellants agree with the examiner with regard to the teachings in Goeddel and the disclosure of Cohen. Appellants state (Brief, page 7) that “[t]he Examiner cites Goeddel et al solely for the proposition that it teaches the expression of chemically synthesized genes for human insulin in E. coli. As limited to this teaching, Appellants concur with the Examiner.” Appellants also “agree [Answer, page 8] with the Examiner that Cohen et al. teach ‘the formation of plasmids containing promoters and eukaryotic genes used to transform cells and produce proteins’. Appellants also agree with the Examiner that Cohen et al. teach ‘the use of trp operons with various deletions’.”

Cohen in view of Bertrand and Goeddel:

The examiner argues (Answer, page 9) that “Bertrand et al. disclose attenuator-deleted trp operons, and the step of inducing transcription from the trp operons by trp starvation.” However, we note the limitation of appellants’ claim 1 section (iv) which states in part “a structural gene ... comprising 6 amino acids of the trp leader peptide, [and] ... at least about the distal third of the trp E polypeptide....” The examiner does not explain, and we do not find, where Bertrand

meets this limitation of appellants' claimed invention. Cohen and Goeddel fail to make up for this deficiency in Bertrand.

The initial burden of presenting a prima facie case of obviousness rests on the examiner. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). In satisfying this initial burden, every limitation positively recited in a claim must be given effect in order to determine what subject matter that claim defines. In re Wilder, 429 F.2d 447, 450, 166 USPQ 545, 548 (CCPA 1970). Here the examiner failed to meet her burden of establishing a prima facie case of obviousness. Accordingly, we reverse the rejection of claims 1, 9, 10 and 35-37 under 35 U.S.C. § 103 as obvious over Cohen in view of Bertrand and Goeddel. Cohen in view of Miozzari (R), Miozzari (S) and Goeddel:

Miozzari (R), however, teach (Miozzari (R), figure 1) two constructs trp?LE1417, and trp?LE1413. Both of these constructs meet the limitation of appellants' claim 1 section (iv) with respect to "a structural gene ... comprising 6 amino acids of the trp leader peptide, [and] ... at least about the distal third of the trp E polypeptide...." In fact, we note that appellants' specification (page 10, lines 28-34) discloses:

Two particularly useful plasmids from which the attenuator region has been deleted are the plasmids pGM1 and pGM3, G.F. Miozzari et al, J. Bacteriology 133, 1457 (1978). These respectively carry the deletions trp?LE1413 and trp?LE1417 and express (under the control of the trp promoter-operator) a polypeptide comprising approximately the first six amino acids of the trp leader and distal regions of the E polypeptide. In the most preferred case, pGM1, only about the last third of the E polypeptide is expressed whereas pGM2 expresses almost the distal one half of the E polypeptide codons.

As appellants' argue (Brief, page 9):

[T]he 'process' of Cohen et al referenced by the Examiner is heterologous gene expression under the control of the lac promoter. The essential issue with regard to the outstanding rejections, therefore, is whether it would have been obvious to one of ordinary skill in the art on 24 March 1980 to substitute the attenuator- and trpE ribosome binding site-deleted trp promoter for the lac promoter actually employed in the 'process' of Cohen et al.

Much of appellants', as well as the examiner's, arguments are directed to an increase in expression resulting from the use of the trp promoter mutants relative to the lac promoter. However, we note that nothing in the claims require an increase in expression. The examiner argues (Answer, page 8) that it would have been obvious to substitute the lac promoter with the trp promoter as taught by Miozzari. We note that, where the prior art recognizes two components to be equivalent, an express suggestion to substitute one for another need not be present in order to render such substitution obvious. In re Fout, 675 F.2d 297, 301, 213 USPQ 532, 536 (CCPA 1982). Therefore, it appears that the examiner has made out a plausible prima facie case of obviousness.

However, a conclusion of prima facie obviousness, does not end a patentability determination under 35 U.S.C. § 103. As stated in In re Hedges, 783 F.2d 1038, 1039, 228 USPQ 685, 686 (Fed. Cir. 1986):

If a prima facie case is made in the first instance, and if the applicant comes forward with reasonable rebuttal, whether buttressed by experiment, prior art references, or argument, the entire merits of the matter are to be reweighed. In re Piasecki, 745 F.2d 1468, 1472, 223 USPQ 785, 788 (Fed. Cir. 1984).

Here, appellants provide evidence of unexpected results. Specifically, appellants argue (Brief, page 6) that “the surprisingly high yields of polypeptides obtained using the claimed invention turned a laboratory phenomenon into an industrially [sic] process [of] producing recombinant polypeptides.”

In response to appellants’ arguments concerning the lack of motivation to combine Miozzari with Cohen, the “Examiner strongly disagrees [Answer, page 6] that the record does not contain a motivation for the combination. ... Miozzari et al. show that deletions of a sequence between the operator and the trp E coding region can increase the expression of structural genes.”

With respect to the examiner’s arguments concerning increased expression due to the trp promoter, appellants argue (Brief, bridging paragraph, pages 10-11) that:

Appellants cannot stress strongly enough that Bertrand et al’s and Miozzari et al’s demonstration of increased expression from an attenuator-deleted trp promoter as compared to an intact trp promoter does not, in any way, suggest that an attenuator-deleted trp promoter would provide increased expression as compared to the **lac promoter** employed by Cohen et al. It is essential for the Examiner to

keep in mind that the issue is whether there would have been motivation to substitute the attenuator-deleted trp promoter for Cohen et al's lac promoter, not for an intact trp promoter. Thus, while the ordinarily skilled artisan might possibly have been motivated to substitute the attenuator-deleted trp promoter for an intact trp promoter in order to achieve increased gene expression ... he or she simply would not have known whether substitution of the lac promoter of Cohen et al with the attenuator-deleted trp promoter would have provided increased gene expression. In other words, as of the filing date of the present application, there simply was no way to compare the efficiencies of the attenuator-deleted trp promoter and the lac promoter employed by Cohen et al.

We agree with appellants. The examiner has provided no evidence suggesting that the trp promoter mutants of appellants' claimed invention would provide an increase in the expression of a heterologous gene relative to the lac promoter of Cohen. As appellants explain, each of the examiner's arguments concerning an increased level of expression resulting from a trp promoter mutant are relative to the wild-type trp promoter.

Appellants provide the Kleid Declaration⁴ to support their argument concerning unexpected results. Kleid explains (Declaration, page 5) that "[t]he yields obtained by the method of the present invention were unpredictable and unexpected at the time this invention was made ... [t]he relative heterologous protein yields from small-scale fermentations are apparent from Figures 3, 7 and 11 of the present application."

Figure 7, of the specification, compares the level of expression of a heterologous gene, human growth hormone (HGH), resulting from the use of the lac

promoter or the trp promoter. According to the specification (pages 22-23) figure 7 is a photograph of a stained gel wherein:

Lanes 1 and 7 contain protein markers of various known sizes; lane 2 is a control ... Lane 3 segregates protein from E. [c]oli 294/pHGH 107 [a lac promoter construct] grown in LB media [which contains tryptophan, resulting in reduced expression from the trp promoter due to the trp repressor/operator interactions that occur in the presence of tryptophan]; Lane 4 segregates protein from E. [c]oli 294/pHGH 107 grown in M9 media [which lacks tryptophan]; Lane 5 segregates protein from E. [c]oli 294/pHGH 207 [a trp promoter/operator construct] grown in LB media; and Lane 6 segregates protein from E. [c]oli 294/pHGH 207 grown in M9. The dense band in Lane 6 is human growth hormone, as shown by comparison to the similar bands in Lanes 2-4. As predicted by the invention, the organism E. [c]oli 294/pHGH 207 when grown in tryptophan-rich LB media produces less human growth hormone by reason of tryptophan repressor/operator interactions, and when grown in M9 media produces considerably more HGH than E. [c]oli 294/pHGH 107 owing to the induction of the stronger tryptophan promoter-operator system vs the lac promoter-operator system in pHGH 107.

Kleid explains (Declaration, page 7) that figure 7 “demonstrates the unexpectedly better results obtained with the present trp system (Lane 6, yield estimate about 10%) as compared with the original lac system (Lane 3, yield estimate about 2%).”

In response to appellants’ presentation of unexpected results, the examiner argues (Answer, page 9-10) that:

[a]ppellants’ arguments regarding unexpectedly large yields are not convincing because no support is found in the specification, as originally filed. ... Because the repressor protein is directly regulated by the level of tryptophan available one can up regulate transcription by decreasing tryptophan, an analogous mechanism is not available in the lac promoter-operator system. The examiner’s position is supported in the decision of In re O’Farrell (7 USPQ 2d 1673, CAFC

⁴ Executed April 17, 1989, attached to appellants’ Brief as Exhibit A.

1988) where the court found that the prior art was required only to “reveal reasonable expectation of success in producing the claimed invention”. One would have expected a stronger attenuator deleted tryptophan promoter-operator system because intracellular tryptophan regulates repressor affinity for operator as well as trp E and trp D by feedback inhibition-thus two points of regulation exist that do not exist in an analogous way in the lac-operon system.

We are not persuaded by the examiner’s arguments. With regard to the examiner’s argument concerning two points of regulation, appellants’ specification (page 4) explains that “[i]n wild-type E. coli, the tryptophan operon is under at least three distinct forms of control.” Two of which are regulated by tryptophan. With regard to the point of control not regulated by tryptophan, the specification (page 4) explains that this “control is effected by a process known as attenuation under the control of the ‘attenuator region’ of the gene, a region within the trp leader sequence.” Appellants’ claimed invention specifically excludes this “attenuator region,” see claim 1 “said sequence comprising neither any trp attenuation capability nor nucleotides coding for the trp E ribosome binding site.” With regard to the two points of control regulated by tryptophan, the specification discloses (page 4) that one is “by a process of feedback inhibition, tryptophan binds to a complex of the trp E and trp D enzymes, prohibiting their participation in the pathway [of tryptophan] synthesis.” Therefore, since appellants’ construct is not involved in tryptophan synthesis this point of regulation does not appear to be a relevant point of control for the instant invention.

With regard to the second point of control regulated by tryptophan, appellants’ specification (page 4) explains “tryptophan acts as a corepressor and

binds to its aporepressor to form an active repressor complex which, in turn, binds to the operator, closing down the pathway in its entirety.” It is this point of control that is relevant to appellants’ construct as demonstrated by the results obtained in figure 7 of the specification.

We do not agree with the examiner’s position (Answer, page 9) that an “analogous mechanism [to tryptophan regulation of the trp promoter-operator] is not available in the lac promoter-operator system.” As explained by appellants⁵ during prosecution of the 07/773,740, of which this case is a File Wrapper Continuation:

The attenuator-deleted operon is bereft of the attenuator control system (it’s deleted), so control only operates through the trp repressor. This situation is no different than the lac repressor controlling the expression of the lac promoted protein where, IPTG is used to derepress lac and promote expression, just as trp deprivation promotes expression from the trp promoter.”

⁵ Paper No. 34, received May 26, 1982, at page 3.

Thus as explained by appellants the lac promoter-operator is regulated by the lac repressor and this repression of gene expression from the lac promoter/operator is relieved by IPTG.

This, however, raises the question of what effect IPTG would have had on appellants' comparison of the lac promoter-operator with the trp promoter-operator as explained in the specification (pages 22 and 23), figure 7 and the Kleid Declaration. This comparison tests the expression of the lac construct in the presence or absence of tryptophan (which regulates the trp promoter-operator), not IPTG (which regulates the lac promoter-operator). Appellants' specification, however, resolves this issue. As explained in appellants' specification (page 23) E. coli 294 cells were used to compare the expression of the lac and trp constructs. The specification (page 16) makes reference to Backman with regard to E. coli K-12 strain 294.

Backman use a lac promoter-operator construct to drive expression of the ? repressor. Backman teach in Table 2 (page 4176) that "Strain 294 makes wild-type lac repressor levels." However, Backman also teach (page 4176, column 2) in reference to Table 2 that "[a] strain bearing a wild-type lac operon does not make enough lac repressor to repress significantly the synthesis of ? repressor from the lac promoters...."

Therefore, it does not appear that IPTG would have significantly affected appellants evidence of unexpected results. Absent a reasoned statement by the examiner as to why appellants' results are not unexpected, we find that the examiner

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failed to meet her burden of establishing a prima facie case of obviousness under 35 U.S.C. § 103, in view of all the facts in evidence, including appellants' evidence of unexpected results. In re Piasecki, 745 F.2d 1468, 1472, 223 USPQ 785, 788 (Fed. Cir. 1984).

Accordingly, we reverse the examiner's rejection of claims 1, 9, 10 and 34-37 under 35 U.S.C. § 103 over Cohen in view of Miozzari (R), Miozzari (S) and Goeddel.

Other Issues:

A number of 1449 forms are present in this administrative file with no indication of being reviewed by the examiner. Upon return of this application, the examiner should review the 1449 forms and take appropriate action.

REVERSED

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WILLIAM F. SMITH)	
Administrative Patent Judge)	
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)	BOARD OF PATENT
DONALD E. ADAMS)	
Administrative Patent Judge)	APPEALS AND
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