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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte TATIANA A. EGOROVA-ZACHERNYUK

Appeal 2019-006193
Application 10/564,012
Technology Center 1600

Before HUBERT C. LORIN, FRANCISCO C. PRATS, and JAMIE T. WISZ, *Administrative Patent Judges.*

WISZ, *Administrative Patent Judge.*

DECISION ON APPEAL

STATEMENT OF THE CASE

Pursuant to 35 U.S.C. § 134(a), Appellant[1] appeals from the Examiner's decision to reject claims 13–15, 39 and 40. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

CLAIMED SUBJECT MATTER

The Specification describes "the labelling of biological compounds with stable isotopes." Spec. 1. Uniform labelling of biological compounds "with stable isotopes allows the determination of the three-dimensional structure by NMR spectroscopy of the biological compound." *Id.* Claim 13, the only independent claim, is illustrative of the claimed subject matter and is reproduced below:

> 1. A method for producing a biomolecule in mammalian or insect cells, whereby at least 95% of the atoms in the biomolecule, for at least one of H, C or N, are isotopically labelled, the method consisting of the steps of:
>
> a) growing an organism selected from the group consisting of *Pichia pastoris, Hansenula polymorpha, Cyanidium caldarium, Galdieria sulpuraria, Scenedesmus obliquus,* and *Methylobacillus flagellatus* on a chemically-defined or mineral medium which supports growth of the organism, whereby in the medium contains at least one of:
>
> i) a sole carbon source which is ^{13}C-glucose or NaH[^{13}C]O_3, wherein at least 95% of the atoms in the carbon source is isotopically labelled, and

[1] We use the word "Appellant" to refer to "applicant" as defined in 37 C.F.R. § 1.42. Appellant identifies the real party in interest as Tatiana Egorova-Zachernyuk. Appeal Br. 2.

> ii) a sole nitrogen source which is $^{15}NH_4Cl$, wherein at least 95% of the N atoms in the nitrogen source is isotopically labelled, to produce labelled biomass;
>
> (b) autolysing the biomass of the organism grown as in (a) to produce an autolysate;
>
> (c) composing a nutrient medium for mammalian or insect cells by combining the autolysate as obtained in (b) with further components necessary for growth of the mammalian or insect cells;
>
> (d) growing a culture of the mammalian or insect cells producing the biomolecule under conditions conducive to the production of the biomolecule, in the nutrient medium ; and
>
> (e) recovery of the biomolecule,
>
> whereby the biomolecule is a mammalian polypeptide produced as a result of genetic engineering of the mammalian or insect cells.

Appeal Br. 13 (Claims App.).

REJECTIONS

The Examiner rejected claims 13 and 40 under 35 U.S.C. § 103(a) as obvious over Hansen,[2] in view of Skladnev.[3]

The Examiner rejected claims 14 and 15 under 35 U.S.C. § 103(a) as obvious over Hansen and Skladnev, and further in view of Werten.[4]

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[2] Andrew P. Hansen et al., *A Practical Method for Uniform Isotopic Labeling of Recombinant Proteins in Mammalian Cells*, 31 Biochemistry 12713–12718 (1992) ("Hansen").
[3] D.A. Skladnev et al., *Preparation of $^{13}C+^{15}N$-Modified Peptide Antibiotic Zervamycin II*, 5 Biotechnology in Russia, 41–50 (2002) ("Skladnev").
[4] Paul J.L. Werten et al., *Large-scale purification of functional recombinant human aquaporin-2*, FEBS Letters 504, 200–205 (2001) ("Werten").

The Examiner rejected claim 39 under 35 U.S.C. § 103(a) as obvious over Hansen and Skladnev, and further in view of Iding[5] and Castro[6] and as evidenced by Chung.[7]

ISSUES AND ANALYSIS

Rejection of claims 13 and 40 under 35 U.S.C. § 103(a) as obvious over Hansen in view of Skladnev

The Examiner finds that "Hansen teaches a method of growing biomass (*E. coli*) in a medium consisting of only [^{15}N] ammonium chloride as the nitrogen source . . . so 100% of the available nitrogen would be labeled." Final Act. 6 (citing Hansen 12714). The Examiner also finds that "Hansen teaches ^{15}N/^{13}C labeled amino acids obtained from lyophilized algae" and "enzymatic and acid hydrolysis of bacterial and algal biomass to produce a[n] isotopically-labeled protein lysate." *Id.* (citing Hansen 12714). The Examiner further finds that "Hansen teaches a nutrient medium comprising the isotopically-labeled hydrolyzed protein" and "teaches culturing Sp2/0 mammalian hybridoma cells transfected with a urokinase-expressing construct . . . in a media containing acid-hydrolyzed bacterial and algal extracts that have been labeled with ^{13}C and ^{15}N, then recovering the labeled urokinase." *Id.* (citing Hansen 12713–12715). According to the

[5] K. Iding et al., *An Automatic System for the Assessment of Complex Medium Additives under Cultivation Conditions*, 73 Biotech. and Bioeng. (2), 442–448 (2001) ("Iding").
[6] Paula Maria Lima e Castro, *Optimisation of CHO Cell Growth and Recombinant Interferon-γ Production*, Ph.D. Thesis, University College London (1993) ("Castro").
[7] John D. Chung et al., Extension of Sp2/0 Hybridoma Cell Viability Through Interleukin-6 Supplementation, 55 Biotech. and Bioeng. (2), 439–446 (1997) ("Chung").

Examiner, Hansen also "teaches isolation of isotopically-labeled urokinase" and that the "urokinase produced using Hansen's method allows for study with NMR techniques." *Id.* (citing Hansen 12715, 12717).

The Examiner acknowledges that Hansen does not teach *Methylobacillus flagellates* as claimed, but finds that this deficiency is cured by Skladnev. Final Act. 7. According to the Examiner, Skladnev, which "teaches methods of making ^{13}C and ^{15}N-labeled Zervamycin II," also

> teaches growing *Methylobacillus flagellates* on ^{13}C-methanol and ^{15}N-ammonium chloride as exclusive carbon and nitrogen sources respectively on a chemically-defined (e.g. minimal medium) . . . preparing an autolysate of the ^{13}C and ^{15}N-labeled *M. flagellates* . . . adding the ^{13}C and ^{15}N-labeled *M. flagellates* autolysate to a *E. salmosynnemata* nutrient medium . . . growing *E. salmosynnemata* with the nutrient medium comprising ^{13}C and ^{15}N-labeled *M. flagellates* autolysate to yield ^{13}C and ^{15}N labeled-Zervamycin II.

Id. (citing Skladnev 43–45).

The Examiner further finds that "it would have been obvious before the invention was made to substitute the ^{13}C and ^{15}N *M. flagellatus* autolysate of Skladnev for the ^{13}C and ^{15}N hydrolyzed bacterial and algal biomass of Hansen." Final Act. 7. The Examiner also finds that a person of ordinary skill in the art would have had a reasonable expectation of success in doing so "because Hansen and Skladnev are both directed towards methods of making radiolabeled proteins by growing cells with a ^{13}C and ^{15}N radiolabeled nutrient source such that ^{13}C and ^{15}N are incorporated into the protein of interest." *Id.* The Examiner concludes:

> The skilled artisan would have been motivated to [do so] because Hansen teaches there are known limitations of radiolabeled hydrolyzed biomass (e.g. acid hydrolysis of glutamine, asparagine, cysteine and tryptophan) and so the

> substitution of the autolyzed biomass of Skladnev would improve Hansen's methods as the lack of any acid hydrolysis step would preserve those radiolabeled acid-labile amino acids for incorporation into the downstream mammalian cell culture and radiolabeled protein production methods of Hansen.

Id. at 7–8.

Appellant argues that Skladnev does not teach autolysing as defined in the Specification and as commonly used in the field despite Skladnev's use of the term. Appeal Br. 6–7. According to Appellant, "[a]utolysis is a term that is commonly used in the field of biology, it is also known as self-digestion and refers to the destruction of a cell through the action of its own enzymes." *Id.* at 6. Appellant further asserts that "[a]utolysis of cells usually comprises an incubation of the cells at an elevated temperature (30-50°C) for a prolonged period of time (3-18 hours) in the presence of a plasmolysing agent, such as *e.g.* NaCl, ethanol, ethyl acetate, chloroform or dextrose" and that "[d]uring the incubation cellular components are hydrolysed by the cell's endogenous hydrolytic enzymes, the cell wall breaks and disintegrates and releases the proteinaceous content into the aqueous environment." *Id.* (citing Spec. 20:19–31).

Appellant further contends that the Specification "demonstrates that the process of autolysis is not carried out at an acidic pH" and cites to the Specification which states, "'[s]ubsequently the yeast cells are allowed to autolyse at a pH of 6.5-10.0, preferably a pH of 7.5-8.0 and at 10% w/v NaCl'" and to "Example 1 wherein the pH during autolysis is pH 8.0." *Id.* at 7 (quoting Spec. 21–22) (emphasis omitted). According to Appellant, "Skladnev clearly teaches to use acid hydrolysis to prepare isotopically labelled amino acids for mammalian cell growth" because, in Skladnev, "*the*

cells were subjected to a mild autolysis with a ***solution of 0,3 M HCl***." *Id.* (quoting Skladnev 43). Appellant contends that "[a]utolysis of cells does not comprise adding a strong acid such [as] HCl to the cells as this would interfere with the process of autolysing." *Id.* Therefore, according to Appellant, Skladnev "teaches that the cells are lysed through acidic hydrolysis" which is "similar to the process used by Hansen who correctly refers to this process as the *acid hydrolysis* of bacterial and algal cells." *Id.* at 7–8.

Appellant also asserts that the combined teaching of Skladnev and Hansen do not yield the claimed method. Appeal Br. 8. According to Appellant, Skladnev does not teach autolysis and Hansen does not remedy this deficiency and, in fact, teaches away from using acid hydrolysis. *Id.* Specifically, Appellant asserts that Hansen teaches that acid hydrolysis causes detrimental side effects to the amino acids in the medium because hydrolysis destroys amino acids such as glutamine, asparagine, cysteine, and tryptophan and that cell growth is stunted because acid hydrolysis was used to lyse the biomass. *Id.* (citing Hansen 12715). Therefore, according to Appellant, the skilled person would have to look for other ways to lyse the biomass in order to prepare autolysates of the bacteria but would not find that solution in Skladnev because Skladnev teaches to use acid hydrolysis. *Id.*

Having considered Appellant's arguments in support of claim 13, we are not persuaded of any reversible error in the Examiner's rejection of this claim. We find that the Examiner has presented a prima facie case of obviousness and Appellant has not sufficiently rebutted this prima facie case. First, although Appellant asserts that Skladnev does not teach an autolytic

process, as that process is commonly defined in the field of biology, Appellant does not provide any evidence of how this term is defined in the field nor provide evidence that autolysis excludes the use of hydrochloric acid as taught in Skladnev. Without such evidence, Appellant merely relies on attorney argument regarding how the term "autolysis" is used in the field of biology. "[S]tatements of counsel in a brief cannot take the place of evidence." *In re Walters*, 168 F.2d 79, 80 (CCPA 1948).

Next, we agree with the Examiner that Appellant is relying on unclaimed features to establish patentability because claim 13 does not recite a particular pH nor does it exclude the use of hydrochloric acid for autolysing the biomass. "[T]he claims define the invention. . . . [L]imitations from the specification are not to be read into the claims." *Sjolund v. Musland*, 847 F.2d 1573, 1582 (Fed. Cir. 1988). In addition, although Appellant asserts that the Specification indicates that the process of autolysis is not carried out in an acidic pH, the Specification does not explicitly define autolysis and includes an example in which the yeast cells are allowed to autolyze at a pH of 6.5–10.0, which would include acidic pH ranges of 6.5–6.9. *See* Spec. 21–22. Furthermore, these pH ranges are provided in the context of exemplary conditions and there is no explicit definition of autolysis in the Specification which includes particular pH ranges or excludes the use of 0.3 M HCl as taught in Skladnev. "[D]uring examination proceedings, claims are given their broadest reasonable interpretation consistent with the specification." *In re Hyatt*, 211 F.3d 1367, 1372 (Fed. Cir. 2000). Therefore, we find that Skladnev teaches autolysis as recited in claim 13.

We are also not persuaded by Appellant's argument that Hansen teaches away from the invention, which is premised on Appellant's assertion that Skladnev does not disclose autolysis. As discussed above, we find that Skladnev does disclose autolysis and we are not persuaded by Appellant's attorney arguments that the autolysis disclosed in Skladnev, which uses 0.3 M HCl, is the same as the acid hydrolysis disclosed in Hansen, which uses 4 M methanesulfonic acid. Furthermore, one of ordinary skill in the art would not have been dissuaded from autolysing the biomass using the teachings in Skladnev based on the disclosure of Hansen. As discussed by the Examiner, because Hansen teaches that there are known limitations to acid hydrolysis, one of skill in the art would have been motivated to use the autolytic process of Skladnev instead.

Appellant further contends that, even if Skladnev and Hansen were combined, there would be no reasonable expectation of success. Appeal Br. 9. First, Appellant contends that because Skladnev fails to disclose autolysing the biomass of the organism to produce an autolysate and because Hansen does not cure this deficiency, the "reasonable expectation of success" rationale cannot be applied to this situation. *Id.* Second, Appellant contends that, even if the rationale could be applied, there would be no reasonable expectation of success because of the different types of cells used. *Id.* Specifically, according to Appellant, Skladnev teaches the use of the "acid hydrolysed biomass of *M. flagellates* for the preparation of a medium to grow *E. salmosynnemata* to yield ^{13}C and ^{15}N labeled-Zervamycin II" and that "*E. salmosynnemata* is a mycelial fungus." *Id.* (citing Skaldnev Introduction). Appellant further argues:

> Thus Skaldnev teaches a method of incorporating ^{13}C and ^{15}N labeled amino acids into **fungal** cells, i.e. microbial cells. In contrast, claim 13 relates to a method for producing a biomolecule in **mammalian or insect cells**. A skilled person would have no reasonable expectation of success that a method to grow microbial cells would also work to grow mammalian or insect cells since Hansen teaches that *"Mammalian cells [. . .] are more sensitive to toxic substances than bacteria (Thomas, 1990; Eagle, 1955)"* (top right hand column page 12713 of Hansen).

Id. Appellant further argues:

> Hansen further teaches that when algal proteins are enzymatically hydrolyzed, the media prepared with the resulting amino acids was unable to support the growth of mammalian cells. Hansen states *that "endotoxins typically found in algal cell extracts[. . .] are known to be toxic to mammalian cells."* (See first paragraph of the right hand column at page 12715 of Hansen).

Id.

Therefore, Appellant asserts that "one of ordinary skill in the art would have recognized that it is unpredictable whether an autolysate of a particular microorganism can successfully be used as a nutrient for culturing mammalian or insect cells" and "would not have reasonably expected to successfully arrive at the claimed invention by using the autolysate of the *M flagellateus* of [Skladnev] to grow mammalian or insect cells as recited in claim 13." Appeal Br. at 9–10.

We are not persuaded by Appellant's arguments. First, as discussed above, we find that Skladnev does disclose autolysing the biomass of the organism to produce an autolysate. Second, we note that "[o]bviousness does not require absolute predictability of success. . . . For obviousness under § 103, all that is required is a reasonable expectation of success." *In*

re O'Farrell, 853 F.2d 894, 903–04 (Fed. Cir. 1988). None of Appellant's arguments suggests that Skladnev's radiolabeled autolysate from the bacteria, *M. flagellatus*, could not be predictably added to Hansen's cell culture medium, which is also used for radiolabeled amino acids obtained from bacteria (*E. coli*). Therefore, there is no suggestion that the mammalian cells disclosed in Hansen would not be able to take up the radiolabeled carbon and nitrogen atoms from the autolyzed biomass of *M. flagellates* as disclosed in Skladnev as they took up the radiolabeled atoms from hydrolyzed *E. coli*. Also, because Skladnev's radiolabeled autolysate is obtained from the bacteria *M. flagellates*, there is no indication of, and Appellant has not pointed towards any, alleged toxic substance found in the *M. flagellates*' autolysate that could support Applicant's argument of unpredictability. *Id.*

Appellant also contends that claim 13 uses the phrase "consisting of" and, therefore, excludes steps that are not specifically recited in the claim. Appeal Br. 10–11. We agree with Appellant that claim 13 recites the phrase "consisting of" in the preamble such that the claim excludes additional unrecited steps. However, Appellant has not pointed to a specific step from the combination of Hansen and Skladnev that is excluded by the claim. Furthermore, since the claim does not recite the specific conditions for autolysis, Skladnev teaches this step for the reasons discussed above.

For the reasons described herein and those already of record, we sustain the Examiner's rejection of independent claim 13 under 35 U.S.C. § 103(a) as being obvious over Hansen in view of Skladnev. Claims 40 is not argued separately, and, therefore, falls with claim 13. *See* 37 C.F.R. § 41.37(c)(1)(iv).

Rejection of claims 14 and 15 under 35 U.S.C. § 103(a) as obvious over Hansen and Skladnev, and further in view of Werten

The Examiner's findings with respect to Hansen and Skladnev are discussed above. Regarding claims 14 and 15, the Examiner acknowledges that Hansen and Skladnev do not teach expressing a membrane protein but finds that Werten cures this deficiency. Final Act. 8. Specifically, the Examiner finds that "Werten teaches a method of overexpressing the membrane receptor aquaporin-2 using recombinant DNA constructs in insect cells and purification of aquaporin-2." *Id.* (citing Werten Abstract, 201). The Examiner also finds that "[a] person of ordinary skill in the art would have had a reasonable expectation of success in substituting Werten's aquaporin-2 and insect cells for Hansen's urokinase and CHO cells." *Id.* at 9.

In response to this rejection, Appellant states that, "[a]s outlined here above, a person skilled in the art reading Hansen and Skladnev would not arrive at the present invention" and that Werten does not remedy these deficiencies. Appeal Br. 11. Therefore, for the reasons explained above, we also sustain the Examiner's obviousness rejection of claims 14 and 15.

Rejection of claim 39 under 35 U.S.C. § 103(a) as obvious over Hansen and Skladnev, and further in view of Iding and Castro and as evidenced by Chung

The Examiner's findings with respect to Hansen and Skladnev are discussed above. The Examiner also finds that Hansen further teaches culturing Sp2/0 cells in Gibco® Hybridoma-SFM culture media. Final Act. 10 (citing Hansen 12714, Fig. 1). The Examiner also relies on Chung to show that Sp2/0 cells are hybridoma cells and relies on Iding for its teaching of methods of culturing hybridoma cells with media comprising yeast extract

and glucose. *Id.* (citing Chung Abstract; Iding 442). The Examiner also finds that Castro teaches that glucose and glutamine are typical carbon sources in method of mammalian cell culture. *Id.* (citing Castro 36). Regarding claim 39, the Examiner finds that "absence evidence to the contrary the Gibco® Hybridoma-SFM culture media meets the limitations of [this claim]." *Id.*

In response to this rejection, Appellant states that, "[a]s outlined here above, a person skilled in the art reading Hansen and Skladnev would not . . . arrive at the present invention" and that the teachings of Iding and Castro as evidenced by Chung do not remedy these deficiencies. Appeal Br. 11. Therefore, for the reasons explained above, we also sustain the Examiner's obviousness rejection of claim 39.

CONCLUSION

For the reasons described herein and those already of record, we affirm the Examiner's rejection of claims 13–15, 39 and 40.

DECISION SUMMARY

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
13, 40	103	Hansen, Skladnev	13, 40	
14, 15	103	Hansen, Skladnev, Werten	14, 15	
39	103	Hansen, Skladnev, Iding, Castro, Chung	39	
Overall Outcome			**13–15, 39, 40**	

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). *See* 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED