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D E C I S I O N
of 9 March 2000

Case Number: T 0737/96 - 3.3.4

Application Number: 88903778.4

Publication Number: 0367765

IPC: C12N 1/16

Language of the proceedings: EN

Title of invention:

Astaxanthin-producing yeast cells, methods for their
preparation and their use

Patentee:

DSM N.V.

Opponent:

- (1) Burns Philp & Co. Ltd.
- (2) Archer-Daniels-Midland Company
- (3) K I Chemical Industry Co., Ltd.,
Tokyo Office/ Kyowa Hakko Kogyo Co.
- (4) Igene Biotechnology, Inc.

Headword:

Astaxanthin/DSM

Relevant legal provisions:

EPC Art. 56, 83

Keyword:

"Main request - inventive step (no)"
"Auxiliary request - inventive step (yes)"
"Sufficiency of disclosure (yes)"

Decisions cited:

T 0694/92

Catchword:

-



Case Number: T 0737/96 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 9 March 2000

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Decision under appeal: Interlocutory decision of the Opposition Division
of the European Patent Office posted 27 June 1996
concerning maintenance of European patent
No. 0 367 765 in amended form.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: L. Galligani
C. Holtz

Summary of Facts and Submissions

- I. The patentees (appellants I) and three out of the four opponents (opponents 02 to 04; appellants II to IV, respectively) lodged an appeal against the interlocutory decision of the opposition division by which the European patent No. 0 367 765 was maintained in amended form on the basis of the auxiliary request then on file (method claims 1 to 10). The opposition division decided that this request fulfilled the requirements of Articles 56 and 83 EPC.
- II. During the written phase of the appeal procedure, the appellants made a number of submissions, including expert opinions, declarations and new documents. Opponents 01, party as of right under Article 107 EPC, informed the board that they did not wish to receive any further communication in connection with the appeal.
- III. The board issued a communication pursuant to Article 11 of the rules of procedure of the boards of appeal with an outline of the essential points of the case.
- IV. Appellants I, II and III replied to the board's communication. Appellants I and III filed also new documents.
- V. On 2 March 2000, appellants I specified their claim requests as being a main request and five auxiliary requests, and filed the following two additional documents:

(A14) EP-B-0 553 085

(A15) WO-A-94/23594

VI. Oral proceedings took place on 9 March 2000. They were not attended by appellants IV that had informed the board thereof. During the course of the hearing, appellants I filed a **new main request** (claims 1 to 14) and an **auxiliary request** (claims 1 to 14) in replacement of all previous requests.

Claim 1 of the **main request** read as follows:

"1. A method of preparation of a Phaffia rhodozyma yeast cell which, when grown under conditions comprising an oxygen transfer rate of at least 30 mmoles/l/hour on Difco YM medium at 20-22°C for 5 days in 500 ml shake flasks with two baffles containing 50 ml of the medium and subjected to orbital shaking at 150 rpm, the inoculum being 100 µl of a four days old YM culture, produces astaxanthin in an amount of at least 600 µg per g of yeast dry matter, determined by HPLC analysis using pure astaxanthin as a standard on a methanol extract of the yeast prepared by subjecting a suspension of 0.2 g of yeast dry matter in 20 ml of methanol to 5 x 1 minutes of disintegration at intervals of half a minute, the disintegration being performed at a temperature of at the most 20°C in a glass ball mill containing 15 g of glass balls having a diameter of 0.4 mm, the glass ball mill being provided with a cooling jacket with ice water, said method comprising treating a naturally occurring Phaffia rhodozyma yeast cell with a mutagen which is ethylmethane sulphonate or N-methyl-N'-nitro-N-nitrosoguanide."

Claim 1 of the **auxiliary request** read as follows:

"A Phaffia rhodozyma yeast cell which is a yeast cell belonging to the yeast strain deposited under the accession No. 225-87 CBS, or the yeast strain deposited under the accession No. 215-88 CBS, or a mutant or derivative thereof, or a mutant or derivative of the yeast strain deposited under the accession No. 224-87 CBS, which has retained its astaxanthin-producing capability, and when grown under conditions comprising an oxygen transfer rate of at least 30 mmoles/l/hour on Difco YM medium at 20-22°C for 5 days in 500 ml shake flasks with two baffles containing 50 ml of the medium and subjected to orbital shaking at 150 rpm, the inoculum being 100 µl of a four days old YM culture, produces astaxanthin in an amount of at least 600 µg per g of yeast dry matter, determined by HPLC analysis using pure astaxanthin as a standard on a methanol extract of the yeast prepared by subjecting a suspension of 0.2 g of yeast dry matter in 20 ml of methanol to 5 x 1 minutes of disintegration at intervals of half a minute, the disintegration being performed at a temperature of at the most 20°C in a glass ball mill containing 15 g of glass balls having a diameter of 0.4 mm, the glass ball mill being provided with a cooling jacket with ice water."

Claim 2 of the auxiliary request concerned embodiments of the yeast cell according to claim 1 which produced astaxanthin "in an amount of at least 700 µg per g of yeast dry matter, preferably in an amount of at least 1000 µg per g of yeast dry matter, determined by the method stated in claim 1."

Claim 3 of the auxiliary request was directed to a "method for producing astaxanthin-containing Phaffia rhodozyma yeast cells or cell parts or astaxanthin,

comprising cultivating astaxanthin-producing Phaffia rhodozyma yeast cells as claimed in any of claims 1 or 2..."

Claims 4 to 11 of the auxiliary request concerned embodiments of the latter method, while claims 12 and 13 were directed to animal feed comprising Phaffia rhodozyma yeast cells according to claim 1 and claim 14 was directed to a method for feeding animals comprising administering a feed containing Phaffia rhodozyma yeast cells according to claim 1 or 2.

VII. Appellants II and III had no objections under Articles 123 and 54 EPC against any of the above requests. As regards the main request, they essentially objected that the claimed subject-matter either lacked an inventive step or was insufficiently disclosed. In this respect, reference was made to the case law of the boards of appeal, in particular to decision T 694/92 (OJ EPO 1997, 408), and to the following prior art documents:

- (1) Johnson E. A. and M. J. Lewis, J. Gen. Microbiol., 1979, vol. 115, pages 173 to 183;
- (2) Johnson E. A. et al., Aquaculture, 1980, vol. 20, pages 123 to 134;
- (8) Murillo F. J. et al., Appl. Environ. Microbiol., 1978, vol. 36, pages 639 to 642;
- (17) A. L. Demain, in Advances in Applied Microbiology, 1973, Academic Press, London-New York, D. Perlman Ed., pages 177 to 202;

(31) D. A. Hopwood, in METHODS in MICROBIOLOGY, 1970, vol. 3A, Academic Press, London-New York, J. R. Norris and D. W. Ribbons eds., Chapter VI, pages 363 to 433;

(69) G. K. Jacobson, in BIOTECHNOLOGY, 1981, vol. 1, Verlag Chemie, Weinheim, H.-J. Rehm and G. Reed eds., Chapter 5b, pages 280 to 304.

VIII. Appellants I argued essentially that the inventive contribution to the art by the patent in suit was showing for the first time and against any expectation that a combination of mutagenic treatments with EMS and NTG brought about a considerable improvement in astaxanthin production in *Phaffia*. This contribution, which a posteriori rendered much easier the task for the skilled reader of the patent specification, thus justifying the breadth of protection requested, was indeed reflected by claim 1 of the main request.

IX. Appellants I requested that the decision under appeal be set aside and that the European patent be maintained on the basis of either the main or the auxiliary request as submitted in the oral proceedings.

Appellants II to IV requested that the decision under appeal be set aside and that the patent be revoked.

Reasons for the Decision

Late-filed documents

1. Documents (A14) and (A15) were filed by appellants I one week before the oral proceedings in order to show

that appellants II in their later patent applications were seeking claims of the same type as those at issue in the present case. In consideration of the fact that: (i) the new documents were late-filed and (ii) whether or not others are seeking broad protection in pending cases is irrelevant for a decision in the present case, the board decided to disregard them under Article 114(2) EPC.

Main request: Formal admissibility under Article 123 EPC

2. Appellants II and III have no objections under Article 123 EPC. Nor does the board have any objections in this respect, as all amendments introduced in the claims in comparison with the claims as granted are of a restrictive nature and find support in the application as filed. Thus, the requirements of Articles 123(2) and (3) EPC are satisfied.

Novelty

3. Novelty is undisputed by the opposing parties, nor does the board have any objections in this respect. Thus, novelty is acknowledged.

Inventive step

4. The closest prior art is represented by document (2) together with document (1) to which it refers. The two documents relate to *Phaffia rhodozyma* as an astaxanthin producer and to its use as aquacultural feed. The content of this carotenoid pigment is reported to vary depending on the culture conditions (cf abstract of document (1) as well as page 129, third paragraph of document (2)). Document (2) reports variations between

30-800 µg per g of yeast which corresponds to circa 20-529 µg/g when measured according to the method of the patent in suit. Document (2) indicates on page 129 that the yield "may be increased appreciably with genetic manipulations of the yeast" and makes in this respect reference to document (8) which concerns carotene-superproducing strains of *Phycomyces blakesleeanus* in which the yields of β-carotene were increased from 56 to 25 000 µg/g fungus, which amounts approximately to a 450-fold increase. The "genetic manipulations" described in the latter reference were mutagenesis of wild strains with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) followed by the selection of suitable mutants.

5. Starting from this knowledge, the technical problem to be solved can be defined as the provision of *Phaffia rhodozyma* mutants producing increased yields of the carotenoid pigment astaxanthin.
6. As a solution, claim 1 proposes essentially a method of preparation of a *Phaffia rhodozyma* yeast cell which, under defined conditions, produces astaxanthin in an amount of at least 600 µg per g of yeast dry matter, said method comprising treating a naturally occurring *Phaffia rhodozyma* yeast cell with a mutagen which is ethylmethane sulphonate (EMS) **or** N-methyl-N'-nitro-N-nitrosoguanidine (NTG).
7. The patent in suit describes the isolation of a first mutant producing 570 µg/g of the pigment (made available by deposition as CBS 224-87) by EMS treatment of a known *Phaffia rhodozyma* wild strain, and the further development therefrom by treatment with NTG of a mutant (made available by deposition as CBS 225-87) producing 706 µg/g (cf Example 2, Table 2) or 960 µg/g

(cf Example 5, Table 5). Re-isolation of the latter led to a third mutant (made available by deposition as CBS 215-88) producing 880 µg/g under shake flask cultivation (cf Example 6, Table 6) or 1080 µg/g under fed-batch cultivation. Two further non-deposited strains DBT 403 and DBT 406 are reported to produce 2050 µg/g or 1540 µg/g of the pigment, respectively.

8. The relevant question in relation to inventive step is what measures the skilled person faced with the stated technical problem would have considered adopting, in the light of the quoted prior art and common general knowledge, and whether these would have included a method covered by claim 1.

9. As for this question, the appellants' I position is in essence that, in view of the peculiarities of *Phaffia rhodozyma*, which had been shown to be strikingly different from other yeasts, and which was still unknown in many aspects (ploidy, sexual stage, clustering of the genes involved in the production of astaxanthin etc.), the skilled person would not have readily considered applying mutagenesis thereto in order to improve the yields of astaxanthin. In their view, the reference to genetic manipulations in document (8) indicated to a skilled person a number of different alternative ways: not necessarily only mutagenesis, but also the more precise techniques of recombinant DNA or protoplast fusion. The skilled person knew that random mutagenesis was not straightforward and by no means always successful. Even if he or she had come to the idea of applying it to *Phaffia*, this would not have been done with a reasonable expectation to isolate mutants displaying the yields actually achieved by the patent in suit. The

alleged success in the improvement in the yield of β -carotene in strains of *Phycomyces* (cf document (8) as referred to in document (2)) would not have fostered any expectation of success because these fungi were different from *Phaffia*.

10. Contrary to appellants' I position, the board considers for the following reasons that the skilled person faced with the technical problem as stated would have considered applying mutagenesis techniques to known *Phaffia rhodozyma* strains in order to improve the astaxanthin yields:

- (a) Document (2) explicitly invited the skilled person to improve the astaxanthin yields by way of genetic manipulations and, by reference to document (8), pointed to mutagenesis as this was the only technique described in the said reference;
- (b) Mutagenesis techniques were well known in the art and were the prevailing techniques conventionally used in strain improvement programmes, also in view of the production of secondary metabolites (cf eg documents (17), (31) or (69)). The application of these techniques does not presuppose much knowledge of the genetics of the target organism as they are based on a trial and error approach, ie on treating in one or more rounds with a mutagenic agent(s) the organism of which eg improved mutants are sought (eg producing better yields of a metabolite), plating the survivors and simply testing them for the desired improved parameter (eg for product production);

(c) Apart from the suggestion in document (2) via document (8), mutagenesis would have been in any case the route of choice because, precisely for the reason that not much was known about Phaffia, both the proposed alternative routes of genetic engineering and protoplast fusions were not readily practicable.

For these reasons, it was obvious for the skilled person to enter the route of treating a naturally-occurring Phaffia rhodozyma strain with a mutagen like EMS and/or NTG for making mutants producing more astaxanthin. This involved nothing out of the ordinary, but only the persistent application of routine mutation techniques. The board found nothing in the available prior art which would have dissuaded the skilled person from using this widely used approach on Phaffia. As already stated (cf items (b) and (c) above), the incomplete knowledge about Phaffia would not have deterred the skilled person therefrom as mutagenesis is well suited in such technical circumstances.

11. As for the expectation of success, the board is of the opinion that in the present case it is not appropriate to attempt to evaluate the expectation of success of a random technique such as mutagenesis where results depend on chance events. This is because the skilled person knows that, unless a specific selection method can be developed, which is not the case in the patent in suit, perseverance and chance play a key role in the achievement of success, as no form of control can be exerted over the mutation events. Under these circumstances, like eg in a lottery game, the expectation of success always ranges **irrationally** from nil to high, so it cannot be evaluated in a rational

manner based on technical facts. This is at variance with technical situations in which more predictable methods are relied upon to solve a particular problem, such as the methods of genetic engineering like cloning and/or expressing a DNA sequence. In such situations, it is often possible to make rationally predictions about the possibilities of success, and the evaluation of the "reasonable expectation of success" is then a meaningful and reliable tool in the assessment of inventive step (cf T 694/92 supra, see in particular point 28.7 of the reasons).

12. As for the feature "produces astaxanthin in an amount of at least 600 µg per g of yeast dry matter", this cannot per se contribute to inventive step of the broadly formulated method claim 1 for the following reasons:

- (a) While it is true that the inventors were able to isolate by conventional mutagenesis techniques some strains characterised by that feature (cf point 7 above), it is also a fact that they have not contributed a specific mutagenesis method whereby the skilled person can take a naturally occurring *Phaffia rhodozyma* strain and isolate mutants with the same feature without having to rely only on chance events;
- (b) In the context of the claim, the said feature is the generalisation of a characteristic found in relation to specific isolates obtained by chance. In the absence of the description of a fairly reliable method for obtaining *Phaffia rhodozyma* mutants displaying said feature, without having to rely again on perseverance and chance, the said

feature amounts to arbitrarily setting a minimum yield to be achieved by the skilled reader which per se is an obvious desideratum in view of the prior art. In fact, in the light of document (2) with its reference to document (8) and in consideration of the random nature of mutagenesis techniques, the skilled person, having from nil to high expectations, while expecting a large number of failed attempts, wished obviously to find mutants displaying a large increase in astaxanthin yield. The indication in document (1) of the 450-fold increase in *Phycomyces* reported in document (8) would have cherished this wish, in spite of the differences between the two fungi and the two products.

In such technical circumstances, it is the **actual isolation** of a mutant indeed having such characteristics which can be surprising, not the theoretical possibility of achieving one.

13. In summary, for the reasons given above, the board considers that the measures adopted by a skilled person faced with the underlying technical problem would have included a method as covered by claim 1. Thus, the subject-matter of the claim 1 lacks an inventive step. Consequently the main request of which claim 1 is part is not allowable under Article 56 EPC.

Auxiliary request: Formal admissibility under Article 123 EPC

14. No objections under Article 123 EPC are raised by the opposing parties. Nor does the board have any objections in this respect as all amendments introduced in the claims in comparison with the claims as granted

are of a restrictive nature (limitation to the deposited strains or mutants or derivatives thereof) and find support in the application as filed. Thus, the requirements of Articles 123(2) and (3) EPC are met.

Inventive step

15. The claims of this request, the novelty of which is not contested, are limited to the specifically exemplified, deposited strains of *Phaffia* (cf point 7 above) and to their mutants or derivatives retaining the astaxanthin-producing capability of at least 600 µg per g of yeast dry matter.
16. The opposing parties could see that the specific, deposited strains constituted the actual contribution to art by the patent in suit. However, appellants III objected that the patent proprietors were not entitled to claims covering also their mutants or derivatives producing open-ended amounts of astaxanthin (cf feature "at least 600 µg per g of yeast dry matter"), eg producing 2000 or 3000 µg per g of yeast dry matter, because these were not sufficiently disclosed.
17. The board finds that the isolation of **specific** *Phaffia rhodozyma* mutant strains capable of producing astaxanthin with yields above 600 µg per g of yeast dry matter, albeit achieved by conventional mutagenesis techniques, constitutes a contribution to the art which deserves patent protection because it contains elements of surprise which justify the recognition of an inventive step (cf point 12, item b), last sentence above). In this context, it has to be noted that, notwithstanding document (2) stating that the astaxanthin yield could vary in *Phaffia* between 30-

800 µg per g of yeast, which corresponds to circa 20-529 µg/g when measured according to the method of the patent in suit, admittedly the strains of *Phaffia rhodozyma* actually available in the art did not produce more than 286 µg/g of the pigment.

18. As regards the "mutants or derivatives" of the deposited strains, the board does not share the appellants' III view (cf point 16 above) for the following reasons:

- (a) Using as starting material the deposited mutant strains of *Phaffia rhodozyma* which produce amounts of astaxanthin well above 600 µg per g of yeast dry matter (cf point 7 above), the skilled reader can by conventional mutagenesis or re-isolation techniques obtain further mutants or derivatives which retain the ability to produce at least 600 µg of astaxanthin per g of yeast dry matter. The patent in suit itself shows that re-isolation of the mutant CBS 225-87 led to the further strain CBS 215-88 which, depending also on the culture conditions, produced amounts of astaxanthin up to 1080 µg per g of yeast dry matter (cf point 7 above). It would be contrary to the principle of granting a fair protection (cf the principle expressed in the Protocol on the Interpretation of Article 69 EPC to ensure "a fair protection for the patentee with a reasonable degree of certainty for third parties"; cf also T 694/92 supra, in particular point 3 of the reasons) if mutants or derivatives of the deposited strains were not covered by the claims. Their inclusion is a permissible extent of generalisation which, on the one hand, safeguards the rights of the patent

proprietors to cover all obvious modifications, equivalents and variants of the deposited strains and, on the other, provides a reasonable degree of certainty for third parties as it has its starting point in specific mutant strains which have been made publicly available by way of deposition, and which involve an inventive step;

- (b) In any case, none of the claims at issue is specifically directed to mutants or derivatives producing eg 2000 or 3000 µg per g of yeast dry matter for which questions of enablement would have to be discussed. Claim 2 refers to amounts of at least 700 or 1000 µg per g of yeast dry matter which are well within the yields which have been actually achieved. Claims 1 and 2 set the yields typical of the deposited strains and leave open any improvement that can be possibly achieved when using them as a starting material. It would be unfair, and it would additionally raise matters under Article 123(2), to arbitrarily impose an upper limit to such improvements. The fact that selection inventions can still be made using the deposited strains of the patent in suit as a starting material does not mean that the patent proprietors should be deprived for reasons of insufficiency of patent protection for possible mutants or derivatives thereof which retain the characteristic of yielding at least 600 µg of astaxanthin per g of yeast dry matter.

19. Thus, the board concludes that claims 1 and 2 adequately define in their formulation the actual contribution to the art by the patent in suit and can be allowed both under the provisions of Articles 83/84

EPC and Article 56 EPC. The same applies to the remaining claims which all refers to the Phaffia rhodozyma yeast cell of claim 1 or/and 2. The patent can therefore be maintained on the basis of the auxiliary request.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the first instance with the order to maintain the patent on the basis of the auxiliary request submitted by the patentee in the oral proceedings, and a description to be adapted thereto.

The Registrar:

The Chairperson:

M. Beer

U. Kinkeldey