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D E C I S I O N
of 22 February 1994

Case Number: T 0630/92 - 3.3.2

Application Number: 85114855.1

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Language of the proceedings: EN

Title of invention:
Novel non-reverting salmonella live vaccines

Applicant:
The Board of Trustees of the Leland Stanford Junior University

Opponent:
-

Headword:
Salmonella vaccines/LELAND STANFORD JUNIOR UNIVERSITY

Relevant legal norms:
EPC Art. 56, 111(1)

Keyword:
"Problem-invention (no)"
"Inventive step - main and first auxiliary request (no)"
"Remittal for further prosecution on basis of second auxiliary request"

Decisions cited:
T 0002/83, T 0024/81, T 0013/84, T 0015/81, T 0195/84, T 0109/82

Catchword:
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Case Number: T 0630/92 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 22 February 1994

Appellant: The Board of Trustees of the Leland
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Decision under appeal: **Decision of the Examining Division of the European Patent Office dated 3 February 1992 refusing European patent application No. 85 114 855.1 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: A.J. Nuss
Members: L. Galligani
C.E.M. Holtz

Summary of Facts and Submissions

I. European patent application No. 85 114 855.1 published under No. 0 184 086 was refused by the Examining Division on 3 February 1992.

The decision was taken on the basis of Claims 1 to 12 as originally filed.

Claim 1 as refused reads as follows:

" A method for preparing a live non-virulent vaccine from a virulent pathogenic cellular microorganism, which vaccine is substantially incapable of reverting to virulence in a vertebrate host susceptible to said microorganism while providing for a strong immune response, said method comprising:

transducing cells of an immune response producing strain of said microorganism with a first transducing phage having at least a portion of a first gene, which first gene expresses a protein in a first biosynthetic pathway to a first essential metabolite normally unavailable in said vertebrate host, and which gene includes a non-reverting mutation, resulting in a culture of a first auxotrophic non-virulent mutant;

selecting for said auxotrophic mutant by means of said first auxotrophic mutation or other marker introduced by transduction;

transducing said first auxotrophic mutant with a second transducing phage having at least a portion of a second gene, which second gene expresses a protein in a second biosynthetic pathway to a second essential metabolite normally unavailable in said vertebrate host, and which second gene includes a non-reverting mutation, resulting in a culture of a second auxotrophic non-virulent mutant;

selecting for said second auxotrophic mutant by means of said second auxotrophic mutation or other marker introduced by transduction; and

isolating said second auxotrophic non-reverting mutant transductants to provide a living vaccine."

Dependent Claims 2 to 7 related to specific embodiments of the said method.

Claims 8 to 9 and Claim 10 related to a live **Salmonella** cell having a requirement for aromatic metabolites and adenine as a result of non-reverting mutations and to the corresponding vaccine, respectively.

Claims 11 to 12 related to a broader version of the method of Claim 1, in which the mutations in the first and second gene were stated to result more generally from at least one of a deletion or inversion.

II. The Examining Division refused the application under Article 97(1) EPC on the grounds that the subject-matter of Claims 1 to 12 lacked an inventive step within the meaning of Article 56 EPC having regard to the following citations:

(1) DE-A-2 843 295;
(2) Nature, Vol. 291, 21 May 1981, pages 238 to 239;
(3) WO-A- 83/00437.

The main reasons given for the decision were as follows:

(a) the closest available prior art was represented by document (2) which disclosed the isolation by means of transduction with a transducing phage of a non-virulent single auxotrophic mutant of **S.typhimurium** which carried a non-reverting deletion in the *aroA*

gene as well as its successful use as a vaccine in mice.

(b) The problem underlying the present application was the provision of mutant strains with an even lower reversion rate than that of (2).

(c) As a solution thereto, the present application proposed a method for the isolation of a double auxotrophic mutant which carried, in addition to a first deletion mutation, a second deletion mutation.

The said solution was obvious in the light of (1) which disclosed stable auxotrophic mutants of **Salmonella**, isolated by chemical mutagenesis, which had point mutations in a *pur* gene, said mutants being used as a live vaccine. The same document taught that **Salmonella** strains carrying two independent auxotrophic mutations were safer for use as a vaccine than strains carrying only one mutation.

Thus, by combining the teaching of (1) and (2) the skilled person would have realised that improved vaccine strains would have been obtained by introducing a second, *pur* mutation into the *aro* mutant of (2). This involved the use of a standard method with a reasonable expectation of success.

III. The Appellants lodged an appeal against this decision and paid the appeal fee.

With letter dated 21 February 1994 the Appellants filed further observations and a new main request together with three auxiliary requests.

IV. Oral proceedings took place on 22 February 1994.

During oral proceedings a new main request and two new auxiliary requests were filed.

At oral proceedings the Board drew the Appellants' attention to the following additional citation from the European search report which was considered particularly relevant prior art especially in connection with the newly filed requests:

(4) WO-A- 80/02504

The new main request comprised Claims 1 to 12 as originally filed with amendments in Claims 11 and 12 and additional Claims 13 and 14. The amendment in Claim 11 was aimed at covering also the embodiment wherein the second gene expressed a protein in the first biosynthetic pathway. The amendment in Claim 12 was merely of an editing nature. New Claim 13 specifically related to the embodiment of the method of Claim 11 wherein the second gene expressed a protein in the first biosynthetic pathway. New Claim 14 related to a microorganism obtainable according to the method of Claims 11 to 13.

The first auxiliary request corresponded to the third auxiliary request filed with letter dated 21 February 1994. This request was limited to mutations in an *aro* gene and in a *pur* gene in a first and second biosynthetic pathways, respectively.

The second auxiliary request which corresponded to the second auxiliary request filed with letter dated 21 February 1994 was directed to a method for preparing a live non-virulent vaccine from a virulent pathogenic cellular microorganism which comprised the introduction

of a non-reverting mutation in at least two independent genes in one biosynthetic pathway.

V. The Appellants argued essentially as follows:

(a) documents (1) and (2) essentially defined the state of the art in the present case.

Document (1) dealt with the problem of decreasing the high natural reversion rate to pathogenicity in microorganisms which were attenuated based on one point mutation. To this end, document (1) proposed the combination of a chemically-induced and undefined attenuating mutation with a second point mutation in a purine biosynthetic pathway. However, document (1) did not provide a safe and effective vaccine for use in protecting humans because even the combination of two point mutations could not have provided an acceptable reversion rate. Based on the results reported therein, it could be said that a typical human dosage rate of 10^{10}-10^{11} cells would have contained enough virulent cells to cause disease in humans.

Document (2) disclosed a fully attenuated **Salmonella** which contained a deletion of a portion of the *aroA* gene. The reversion rate of such a deletion was at the limit of the detection assay, i.e. $<10^{-11}$. However, the skilled person would have expected a deletion mutant to have an actual reversion rate approaching "0" since there was no naturally occurring mechanism for the organism to correct such a mutation when grown in a pure culture. Thus, the skilled person would have regarded the attenuated microorganism of (2) as an improvement over that of (1).

(b) Given the state of the art, especially in view of the fact that document (2) had provided mutant strains which "were virtually non-virulent" and which "as live vaccine conferred excellent protection against challenge with a virulent strain", the skilled person could not be confronted with the problem of preparing mutant strains with an even lower reversion rate. The disclosure of (2) was at that time considered a major achievement and the said mutant strains were distributed to several laboratories around the world and proved to be a safe and effective vaccine. For these reasons, the skilled person had no necessity to attempt their further improvement and, thus, no motivation to combine the teaching of (2) with that of (1).

(c) The present inventor appreciated for the first time that the oral route of administration could have posed the potential but significant problem of the correction of the deletion in an *aroA* gene in the vertebrate digestive systems by means of transfer of DNA encoding an *aroA* gene from an enteric bacterium (see present description, page 8, lines 1 to 3).

Thus, the inventiveness of the claimed subject-matter lay in the appreciation of a problem not previously identified in the prior art ("problem invention"). In this respect reference was made to decision T 2/83, OJ EPO, 1984, 265. The proposed solution could perhaps appear simple and obvious **after** the problem had been stated, certainly **not before**.

(d) The contents of document (4) were not considered of relevance for the present case.

VI. The Appellants requested that the decision be set aside and a patent be granted on the basis of one of the above requests in the order.

Reasons for the Decision

1. The appeal is admissible.

2. *Formal admissibility (Article 123(2) EPC)*

There are no objections in respect of the formal admissibility under Article 123(2) EPC of any of the above requests because none of them relates to subject-matter which extends beyond the contents of the application as filed.

In particular, the amendments in the original Claims 11 and 12 as well as the additional Claims 13 to 14 find formal support on page 15, lines 22 to 26, on page 5, lines 28 to 35 and on page 11, lines 22 to 27 of the description as filed.

The first auxiliary request is supported by the working examples and by the original claims.

Formal support for the second auxiliary request can be found on page 15, lines 22 to 26.

3. *Main request and first auxiliary request*

3.1 Novelty (Article 54 EPC)

Novelty was not contested by the Examining Division with respect to Claims 1 to 12 as originally filed.

In the Board's view, no novelty objection applies to any of the claims of the main and first auxiliary request *vis-à-vis* the available prior art documents, in particular *vis-à-vis* documents (1), (2) and (4).

3.2 Inventive step (Article 56 EPC)

(a) Background art

The use of live attenuated microorganisms as vaccines was known in the art before the relevant filing date of the present application (see the introductory part of the present application on pages 1 to 5). One of the known techniques for attenuating the virulence of live microorganisms while allowing them to retain their immunogenic potential was the development of non-virulent or slow-growing strains or mutants incapable of sustained replication in the host. In particular, the preparation of live vaccines containing auxotrophic mutants of a pathogenic organism which either grew too slowly or did not grow at all in the host because of their dependency upon the presence of metabolites not normally found in the said host [see documents (1), (2) and (4)] was known.

A known major concern when providing such live vaccines was the possible reversion of the mutated organism to a virulent wild strain. The skilled person was, therefore, aware of the necessity to ensure the lowest possible reversion rate.

To this end, a number of different solutions were proposed in the art, *inter alia* the combination of two or more independent mutations [see (1) and (4)]

and the introduction of a deletion or inversion mutation [see (2)].

(b) The closest prior art

Document (2) represents the closest prior art in the present case.

This document described a live non-virulent vaccine consisting of auxotrophic non-reverting mutants of **Salmonella typhimurium** which were prepared by transducing cells of the pathogen with a phage containing the transposon aroA554::Tn10 so as to cause - by deletion or inversion - a non-reverting auxotrophic mutation at the level of the *aro* pathway.

The resulting selected strains did not revert to *aro*$^-$ at detectable frequency ($<10^{-11}$ per bacterium per generation).

The said mutants were stated to be "virtually non-virulent" and to confer "excellent protection against challenge with a virulent strain" (see abstract, last sentence).

According to the tests reported in Table 1 the vaccine so prepared did indeed confer protection against challenge with virulent **S.typhimurium**.

(c) The difference between (2) and the present application

The present application differs from the disclosure in (2) essentially in that a second auxotrophic non-reverting mutation in a second gene is produced

in a microorganism which already bears an auxotrophic non-reverting mutation in a first gene.

(d) The technical problem

In view of the Appellants' arguments as set out in Section V, paragraph c, the Board would like to stress that in accordance with the "problem-and-solution approach" developed in the jurisprudence of the European Patent Office, the problem underlying a patent application must be **objectively** defined *vis-à-vis* the closest prior art, taking into account the claimed invention and its effect. When assessing inventive step, the subjective achievement of the inventor is not the point (see, for example, T 24/81, OJ EPO 1983, 133, see items 4 and 14, and T 13/84, OJ EPO 1986, 253, see items 10 and 11).

Thus, in the present case, in the light of (2) the underlying technical problem is to be seen **in the provision of auxotrophic mutants with even lower reversion rates to be used as vaccine**.

(e) The solution proposed

The claims in accordance with the Appellants' main and first auxiliary request essentially propose the introduction in the pathogenic organism of two independent auxotrophic mutations by producing a deletion or inversion in two different genes, in particular in the *aro* and *pur* genes, *inter alia* by means of two transduction exercises with transducing phages.

The preparation of live *aro*$^-$, *pur*$^-$ **Salmonella** strains is exemplified in the present application.

Although no specific comparison is made with single mutant strains, the results of the vaccination tests with volunteers are stated to be "generally satisfactory" (see description, page 35). Accordingly, the Board is satisfied that the underlying technical problem has been solved.

(f) Assessment of inventive step

(1) The contribution of the problem to the inventive step

The Appellants maintained that in the present case the invention lay in the discovery of the unrecognised problem of the possible correction of the deletion or inversion mutation in the *aro* gene of the live auxotrophic microorganism which could have taken place in the digestive system of the host vertebrate. In their submissions, the skilled person prior to the present application had no reasons to attempt to improve the live vaccine of (2) because he would have expected it to be completely safe and effective in view of the nature of the mutation (deletion or inversion) and of the reported extremely low reversion rate ($< 10^{-11}$). Thus, in accordance with decision T 2/83 (OJ EPO 1984, 265), even if, retrospectively, the proposed solution was to be considered trivial, it still had to be regarded as non-obvious since its merit in terms of an inventive contribution to the art had to be seen in conjunction with the perception of the unrecognised problem ("problem invention").

In the Board's view, this line of argumentation is not realistic because it is the normal task of the skilled person to be constantly occupied with the elimination of deficiencies, with the overcoming of

drawbacks and with the achievement of improvements of known devices and/or products (in this respect see, for example, decisions T 15/81 OJ EPO 1982, 2, point 3 of the Reasons and T 195/84, OJ EPO 1986, 121, point 8.1 of the Reasons).

The fact that the auxotrophic mutant strains of (2) were described as "virtually non-virulent", i.e. safe and effective for use as vaccine in humans, cannot necessarily lead to the conclusion that the skilled person did not perceive potential problems connected with their administration.

In the absence of strong indications to the contrary in the state of the art, it is not credible that a skilled person working in the field of vaccines would have regarded the live vaccine of (2) as the ultimate solution to the problem of a possible reversion of attenuated strains to virulent wild strains.

Consequently, in spite of the good results reported in (2) (detected reversion frequency $<10^{-11}$), the skilled person would not have excluded *a priori* the possibility of further reducing the reversion frequency of the mutants strains to even lower levels.

In this respect, it is observed that according to document (4), page 11, lines 1 to 3, a reversion frequency $<10^{-20}$ was actually considered to be negligible. In the Board's view, this information alone would have given to the skilled person sufficient motivation to attempt a further improvement of the known vaccine.

Thus, in the present case, the posing of the problem of providing auxotrophic mutants with even lower reversion rates than those of (2) cannot confer any inventive merit to the claimed subject-matter (see decision T 109/82, OJ EPO 1984, 473, in particular item 5.1).

(2) The step to the solution

(a) It therefore remains to be established whether the skilled person, in order to solve the underlying technical problem, i.e. to further decrease the reversion frequency of the non-virulent strain of (2), would have **in a straightforward manner** considered the repetition of the procedure already described in (2) with respect to a second independent gene (for example, a *pur* gene) thereby arriving at something falling within the terms of the present claims.

(b) The Board answers this question in the affirmative for the reasons outlined below.

When attempting to prepare auxotrophic mutants with even lower reversion rates of those of document (2) to be used as vaccine, the skilled person would have directed his attention to documents dealing with the problem of reducing the revertant frequency. The skilled person would, therefore, certainly have considered the contents of documents (1) and (4) which, at this end, proposed the combination of two mutations either in two different pathways or in the same pathway. Both documents were based on the rationale that, since the reversion rate of a strain

containing multiple mutations was the product of the reversion frequencies of each individual mutation, revertant frequency would have been reduced by introducing more than one mutation. This was, consequently, expected to reduce the risk of infection (see, for example, document (1), page 8, lines 17 to 23 and 31 to 34 and document (4), page 11, lines 1 to 15).

In particular, document (1) disclosed the introduction of a second auxotrophic mutation in the *pur* pathway in an auxotrophic mutant which carried a first mutation in a first metabolic pathway . Document (1) reported for the double mutants **theoretical** reversion frequencies in the order of $<10^{-14}$-10^{-16} (see Example 2). The actual experiments on test animals showed a lower toxicity (increase in the LD_{50}) of double mutants in comparison with single mutants (see, for example, Table 9) and, unexpectedly, no reduction of the immunogenicity following the introduction of the second *pur* mutation (see page 12, first paragraph). At least in a study with a *pur ade*$^-$ *thia*$^-$ double mutant (Example 2a) no *in vivo* reversion was reported to have occurred (see page 22, lines 30 to 31).

In the light of the indications given in (1) and (4), the skilled person would therefore have considered the introduction of a mutation in a second gene as being the most appropriate measure in order to further decrease the reversion frequency of the non-virulent strain of (2). The *pur* phenotype would have been the

obvious candidate for a second auxotrophic mutation in view of the teaching of (1).

(c) The arguments put forward by the Appellants that:

(i) the skilled person would not have considered document (1) because the mutations therein were point mutations produced by chemical means, not deletion or inversion mutations as in the present case;

(ii) reference (1) did not teach the use of two auxotrophic mutations in the sense of the present application;

(iii) the mutated genes in the metabolic pathways were not clearly identified;

(iv) document (1) did not provide a safe and effective vaccine for use in protecting humans

are not considered relevant in the particular circumstances of this case. This is because the Board considers that the relevant teaching that the skilled person would have derived from document (1) [but also from document (2)] was the indication that two mutations in two different pathways provided extra safety to a live vaccine in comparison with one mutation in one pathway.

When deciding to introduce a mutation in a second independent gene, the skilled person had indeed good reasons to repeat a second

time the deletion or inversion mutation exercise according to the teaching of (2) because one mutation of this type had already shown to produce a low reversion frequency going even beyond that of a point mutation. Since nothing in the art discouraged the skilled person from trying this approach, he would have reasonably expected the reversion frequency of the combination of two such mutations to be the product of each individual mutation, namely $<10^{-22}$.

Thus, in the Board's opinion, the skilled person not only had the motivation further to improve the existing live vaccine by introducing an additional mutation in a second independent gene, but he would have done it by using the known technique of (2) with a reasonable expectation of success.

(d) For these reasons, the Board considers that the subject-matter of both the main and first auxiliary request lacks an inventive step.

4. *Second auxiliary request*

The second auxiliary request is centred on a method for preparing a live non-virulent vaccine from a virulent pathogenic cellular microorganism which comprises the introduction of a non-reverting mutation in at least two independent genes in **one** biosynthetic pathway.

Since the original Claims 1 to 12 which were refused by the Examining Division were centred on a method in which the two independent genes were in **two** different biosynthetic pathways, it is clear that this aspect of the invention has not yet been examined by the Examining

Division for its compliance with the EPC. The Board also observes that **after** the issue of the decision of refusal, the Appellants submitted with letter dated 4 February 1992 a report in respect of this aspect of the invention for which no working examples are available in the description. This report could, therefore, not be considered by the Examining Division.

Thus, in order to guarantee such examination without loss of instance, the Board considers it appropriate to make use of the power granted to it under Article 111(1) EPC and to remit the case to the Examining Division for further prosecution.

Order

For these reasons, it is decided that:

1. The decision under appeal is set aside.

2. The main and first auxiliary requests are refused.

3. The case is remitted to the Examining Division for further prosecution on the basis of Claims 1 to 16 of 21 February 1994 (second auxiliary request).

The Registrar: The Chairman:

P. Martorana A. Nuss

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