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Barriers to application of genetically modified lactic acid bacteria

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Abstract

To increase the acceptability of food products containing genetically modified microorganisms it is necessary to provide in an early stage to the consumers that the product is safe and that the product provide a clear benefit to the consumer. To comply with the first requirement a systematic approach to analyze the probability that genetically modified lactic acid bacteria will transform other inhabitants of the gastro- intestinal (G/I) tract or that these lactic acid bacteria will pick up genetic information of these inhabitants has been proposed and worked out to some degree. From this analysis it is clear that reliable data are still missing to carry out complete risk assessment. However, on the basis of present knowledge, lactic acid bacteria containing conjugative plasmids should be avoided. Various studies show that consumers in developed countries will accept these products when they offer to them health or taste benefits or a better keepability. For the developing countries the biggest challenge for scientists is most likely to make indigenous fermented food products with strongly improved microbiological stability due to broad spectra bacteriocins produced by lactic acid bacteria. Moreover, these lactic acid bacteria may contribute to health.

Introduction

In the mid-seventies, in spite of the efforts of scientists to evaluate the consequences of the rDNA technology before starting to develop this technology, public opinion about this technology was quite negative. For a considerable part, this was due to poor communication. Instead of addressing the public in an understandable language, scientists discussed the complex aspects of this emerging technology in their own jargon. Moreover, new discoveries in science pose more questions than provide answers (Campbell, 1990). These questions and uncertainties were communicated by the media and sometimes even lead to fear by the public. This fear was further increased by books like 'Playing God' or 'The Boys of Brazil'. It is understandable that the majority of the public rejected this technology which resulted in unprecedented constraints to carry out research in biosciences.

However, the rejection of rDNA technology is not unique in the history of science and technology. Most scientific breakthroughs, like Copernicus theory of the universe, Darwin's evolution theory, or Mendel's laws and even Fleming's penicillin were received with disbelieve or fear. Even scientists are sometimes dogmatic and reject new discoveries as was shown with Temin's discovery of reverse transcriptase, one of the crucial elements in rDNA technology. Technological developments like printing, steam engines, bicycles, or airplanes were received also by the public with mistrust and often fear. Negative attitudes towards technology are much more pronounced in Northern European countries and parts of the US where immigrants from Northern Europe live, than in Southern European, Asian, or South American countries. A clear example of positive perception in Southern Europe of scientific discoveries were those made by Louis Pasteur, who was considered by the French people as a hero. However, we also have to remind Galileo Galilei, who was banned by the church of Rome because of his revolutionary ideas.

Beside the fact that rDNA technology is really a new technology, the public perception of this technology was negatively influenced by the lack of under-

standing of biology and genetics. However, it was understood that the rDNA technology changes the key molecule of living systems and this caused emotional reactions and concern by a considerable amount of people in Europe and the US. They were under the impression that some of these products may affect their health adversely. Scientists, representatives of governments, and the private industry have communicated this technology to the consumer in such an unstructured and complicated way, that it offered opponents of this technology plenty of opportunities to attack. Figure 1 shows a simple division of the various potential applications of rDNA technology which proved to be very helpful to keep the discussion focused, and free of emotional or ethical aspects.

The acceptance of rDNA food products by consumers depends on a number of factors. The main factors are:

1. Absence of any (perceived) risk of foods containing rDNA;
2. Benefits to the consumers;
3. Clarity and timing of communication to the consumer and environmental organizations on why and how the genetic modification was performed;
4. Positive effects on the environment.

The logic behind the order of these factors is that if, according to the best possible risk assessment, the manufacturer of the food product cannot provide information to the authorities that show that the risk is 'absent', there is no way that such a product will enter the market. However, when the consumer does not see a clear personal benefit, either in terms of quality, health, convenience, shelf life, or price, it is very unlikely that the he or she will buy this product. Assuming that the first two criteria are met, then it becomes the job of the manufacturer and the retailer to communicate why this new product has been developed, how it was done, why it is safe, and what the benefits are to the various organizations and subsequently to the consumer. This communication should take place in an early stage of product development. It is very important in discussions with authorities and organizations that when claims are made that the new process or product contributes to a better environment, such statements are supported by a life cycle analysis of the old process/product versus the new process/product. At least in one case, the addition of phytase to animal feed to reduce the manure production, the environmental benefit was so clear that acceptance by consumer and environmental organizations was not a problem.

Risk of foods containing rDNA products or organisms

It is essential to define the terms, hazard, and risk before starting any discussion on this subject. *Hazard* is defined as the potential (toxicological or ecotoxicological) harmful intrinsic property of the product encoded by the newly constructed genetic material or by the host carrying this genetic information. *Risk* is the probability of *hazard* occurring.

To prove the absence of *risk* is impossible, and therefore 'absence' has to be defined in clear figures. Fortunately, in the food industry a number of risk calculations have been made for various types of products, and it is therefore possible to quantify risks that have proven to be acceptable to authorities, consumer organizations, as well as the public. Even more important is, that based on these risk assessments, the food industry managed to obtain and maintain an extremely good record of safety, in spite of some incidents. For an *extremely hazardous* microorganism as *Clostridium botulinum*, heat treatments for non-microbiologically stable, non-chilled distributed foods have been developed, and are now described in various codes of practice. The prescribed heat treatment will result in a destruction of the most heat resistant *Clostridium botulinum* spores with a factor 10^{12}. Such a heat treatment normally guarantees that statistically one in 10^{12} cans will be contaminated with one spore of *Clostridium botulinum* (Smelt, 1980). For food products that may support growth of the less *hazardous Salmonella* species, heat treatments or other physical decontamination methods are applied resulting in the probability that less than one in 10^8 product units will be contaminated. Clear criteria for spoilage of microorganisms are not present. However, in the dairy industry it is generally accepted that for chilled distributed fresh dairy products, a probability of spoilage by moulds or yeast during the limited shelf life should be less than one in 10^4 product units. Although as such, the risk assessment for these microorganisms look straight forward, this is not the real situation. Lack of reliable data on the contamination of the product before heat treatment, or the probability of contamination during the filling procedure during manufacturing of fresh dairy products, result in uncertainties. Often this leads to an even more severe heat treatment of the product, or extreme decontamination processes for air and packaging material in filling procedures. However, the hazards of toxigenic and pathogenic microorganism are clear. A risk can be calculated and we guess that the calculated risk is

between 1 and 1000 times the actual risk. The figures given above will be used as yardsticks for risk assessments of genetically modified microorganisms in food products, assuming that a hazardous situation can be created by transfer of genetic information from the original host to a recipient microorganism or vice versa.

The situation for rDNA modified lactic acid bacteria is quite different from toxigenic or pathogenic bacteria as discussed before. Lactic acid bacteria have been used for centuries in human food products, and consequently these bacteria are generally recognized as safe (so called GRAS status), so the *hazard* is zero. Tables 1A and 1B summarize the application of lactic acid bacteria in European and Indigenous Fermented Foods respectively. Recently, a workshop on the 'Safety of Lactic Acid Bacteria' has been organized by the Lactic Acid Bacteria Industrial Platform of the EU. The main point of discussion was whether certain members of lactic acid bacteria are involved in human infections, such as endocarditis. In particular *Lactobacillus rhamnosus* has been isolated from clinical cases (Klein et al., 1992; Gasser, 1994). As *L. rhamnosus* is a functional microorganism in cheese manufacturing, and a common inhabitant of the oral cavity, it is very difficult to trace the origin of the *L. rhamnosus* species involved in endocarditis. Although the participants of the meeting concluded that it is very unlikely that *L. rhamnosus* is the causative microorganism (Adams & Marteau, 1995), this microorganism has been placed by the 'Berufsgenossenschaft der Chemischen Industrie' in group II (small risk) and not in group I as the other lactic acid bacteria. Also, the involvement in human diseases of *Enterococcus faecalis* and *E. faecium* is under discussion (Jett et al., 1994) although during the workshop it has been concluded that foods containing *Enterococci* have a long history of use without established risk and that no cases of infection have been linked to the consumption of fermented foods or probiotics. Nevertheless the idea that microorganisms found or used in the production of food products are always safe should not be applied any more. However, for the vast majority of lactic acid bacteria there is no doubt about their safety for human consumption.

The consequence of the use of a GRAS organism is that such host cell can be considered as intrinsically free of any *hazard*. The next questions to be answered are:

(1) Does the newly introduced gene codes directly or indirectly for a hazardous property in the intrinsically safe host organism, if not,

(2) do the lactic acid bacteria serve as host cell for production of a single rDNA gene product, which gene product or metabolites produced with this gene product will be isolated and added to a food product or,

(3) do the recombinant organism encoding a new gene product and metabolites made by this gene product remain in the food product. Will the food product be pasteurized or otherwise treated in such a way that the host lactic acid bacterium is killed with an efficiency comparable to a pasteurization process of 15 seconds at 80 °C or,

(4) do the recombinant organism encoding a new gene product and metabolites made by this gene product remain in the food product.

(1) Newly introduced gene codes for a hazardous product

It is obvious, that when the answer on question (1) is positive, the development of food products based on this genetically modified microorganism (GMO) should be stopped immediately. Consequently question (1) will not be further discussed. What will be discussed in some detail are the spheres A 1–3, B 1–3 and C 1–3 of Figure 1 that represent products defined under well defined single food components, well defined food products that contain inactivated rDNA modified lactic acid bacteria and food products containing living lactic acid bacteria respectively.

(2) Well defined single food components

This type of rDNA products, like chymosin (Maat et al., 1981; Teuber, 1990; van den Berg et al., 1990), α-galactosidase (Overbeeke, 1989, Giuseppin et al., 1993) and, endoxylanase (Maat et al., 1992) are on the market. The host for these products are GRAS organisms, although not lactic acid bacteria. Many protocols for the approval of this type of products are applied in different countries. The scheme used in The Netherlands for single food components (Figure 2) proved to be suitable to obtain approval in other European countries as well. Although it is not absolutely required, it is useful to determine first whether the product belongs to sphere A1, A2 or A3 of Figure 1 before walking through the decision tree depicted in Figure 2. Especially whether the vector used to transform the host cell is a self replicating vector, or is integrated at a defined locus of the host chromosome, and/or if the vector is free of any resistance marker or non-essential

Table 1A. Main functional lactic acid bacteria in European Fermented Foods[1]

Product name	Substrate	Main lactic acid bacteria
Baked Goods	Wheat	*Lactobacillus plantarum*
		acidophilus
		delbrueckii
		brevis
		buchneri
		fermentum
		s.francisco
Wine & Brandy	Grapes	*Leuconostoc gracile*
		oenos
		Lactobacillus plantarum
		casei
		fructivorans
		hilgardii
		brevis
		Pediococcus cerevisiae
Cheese & Dairy Products	Milk	*Brevibacterium linens*
		Lactococcus lactis
		cremoris
		Lactobacillus casei
		helveticus
		bulgaricus
		plantarum
		Leuconostoc cremoris
		Pediococcus acidilactici
		pentosaceus
		Streptococcus thermophilus
		Enterococcus faecium
Fermented vegetables/fruits	Cabbage & Cucumbers	*Lactobacillus brevis*
		plantarum
		Leuconostoc mesenteroides
		Pediococcus cerevisiae
	Olives	*Lactobacillus plantarum*
		paracasei
		brevis
		delbrueckii
		Streptococcus sp.
		Pediococcus sp.
		Leuconostoc sp.
Sausages	Meat	*Lactobacillus curvatus*
		lactis
		plantarum
		sake
		Pediococcus acidilactici
		pentosaceus
		Micrococcus caseolyticus

[1] Deducted from Biotechnology Vol.5, Chapter 1–8.

Table 1B. Main functional lactic acid bacteria in Indigenous Fermented Foods[2]

Product name (Country)	Substrates	Main lactic acid bacteria
Banku (Ghana)	Maize, cassava	Lactic acid bacteria
Burukutu (Nigeria)	Sorghum, cassava	Lactic acid bacteria
Busa (Egypt)	Rice, millet	*Lactobacillus* sp.
Dawadawa (Nigeria)	Locust bean	Lactic acid bacteria
Dosai (India)	Black gram and rice	*Leuconostoc mesenteroides*
Hamanatto (Japan)	Whole soybean, wheat flour	*Streptococcus* sp.
		Pediococcus sp.
Idli (India)	Rice, black gram	*Leuconostoc mesenteroides*
Kecap (Indonesia)	Soybean, wheat	*Lactobacillus* sp.
Kimchi (Korea)	Vegetables (seafood, nuts)	Lactic acid bacteria
Kishk (Egypt)	Wheat, milk	Lactic acid bacteria
Mahewu (S. Africa)	Maize	*Lactobacillus delbrueckii*
Miso (China, Japan)	Rice and soybean	*Lactobacillus* sp.
	Rice and cereals	*Lactobacillus* sp.
Ogi (Nigeria)	Maize	Lactic acid bacteria
Puto (Philippines)	Rice	Lactic acid bacteria
Sorghum beer (S.Africa)	Sorghum, maize	Lactic acid bacteria
Soybean milk (Asia)	Soybean	Lactic acid bacteria
Soy sauce (Asia)	Soybean and wheat	*Lactobacillus* sp.
		Pediococcus sp.
Tarhana (Turkey)	Wheat and milk	Lactic acid bacteria

[2]Deducted from Biotechnology Vol.5, Chapter 1–8.

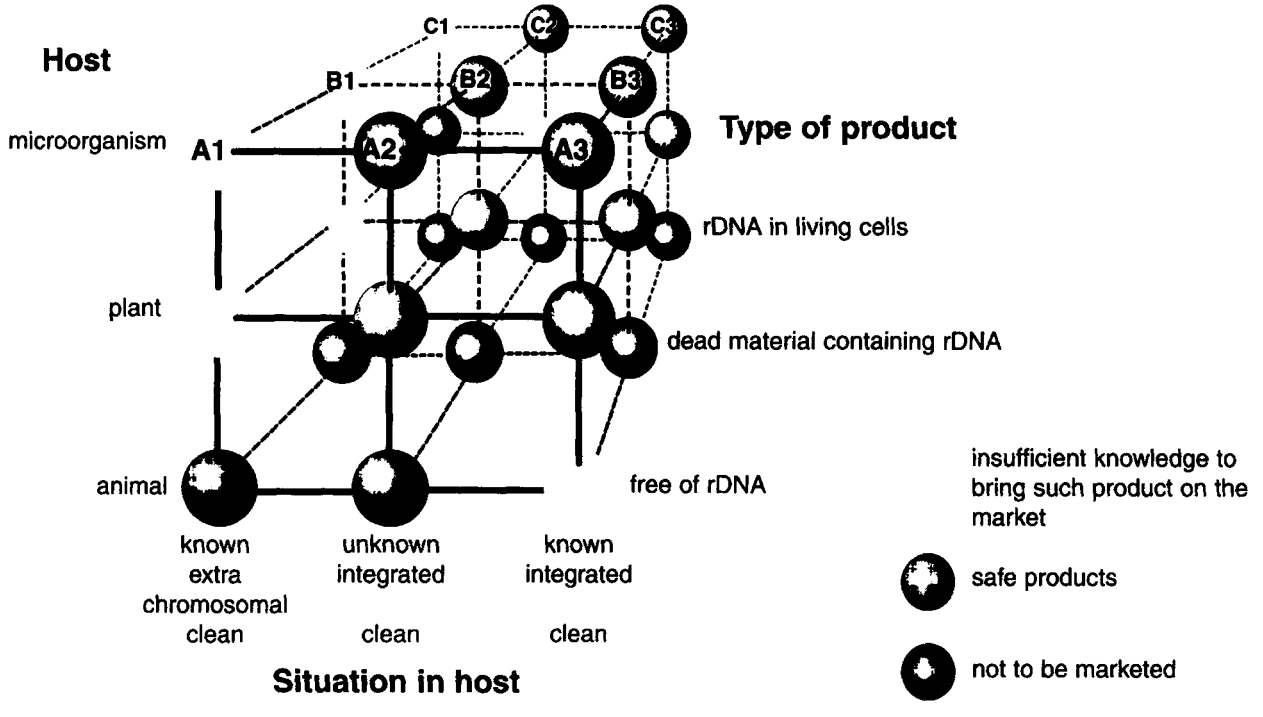

Figure 1. Matrix for the first evaluation of the risk of rDNA products on basis of three criteria: x-axis:Type of vector (epichromosomal, known and unknown integrated in chromosome) y-axis:Type of host (animal, plant or microorganism) z-axis:Type of end product (free of rDNA, contains inactivated rDNA or intact rDNA).

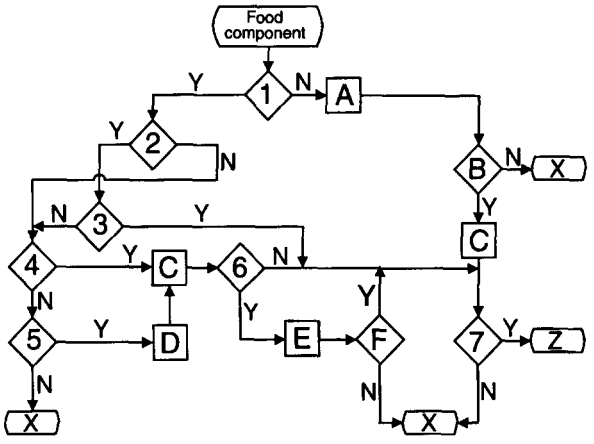

Figure 2. Decision scheme for single food components make by rDNA technology (see also text).

Actions and Results

C. This product should be analyzed as a novel food

X. It is not allowed to bring food products containing ingredients produced with this GMO on the market

Z. Food products containing ingredients produced with this GMO are approved

Questions

1. Does the unmodified microorganism have a record of safe use in food products?
2. Can on the basis of feeding and/or toxicological studies the unmodified microorganism be considered as safe in food products?
3. Is there sufficient knowledge and documentation that the new genetic material codes for (a) product(s) that is (are) acceptable in food products?
4. Does the GMO or an inherent part of it or the product(s) encoded by the new genetic material remain in the food product?
5. It is intended that the modified microorganism fulfills a functional role in the gastrointestinal tract of the consumer?
6. Has the intended functionality been demonstrated?
7. Is the modified microorganism free of genes encoding antibiotic resistance?
8. May the consumption of the food, in particular the GMO or an inherent part of it, or the product(s) encoded by the new genetic material in the intended or expected consumed quantities result in any negative aspect on the health of the consumer?
9. Is it possible to reduce the quantity of the GMO or an inherent part of it or the product(s) encoded by the new genetic material to an acceptable level?
10. Is the physical state or the integration into the chromosome of the host of the new genetic material fully known?
11. Does the integration of the new genetic material disturb the metabolism of the host in such a way that hazardous products may be formed?
12. Does a 90-day feeding trial with the food product containing the GMO or an inherent part of it show that the introduction of the new genetic material into the host does not have an effect on the metabolism of the host cell resulting in (a) hazardous compound(s)?

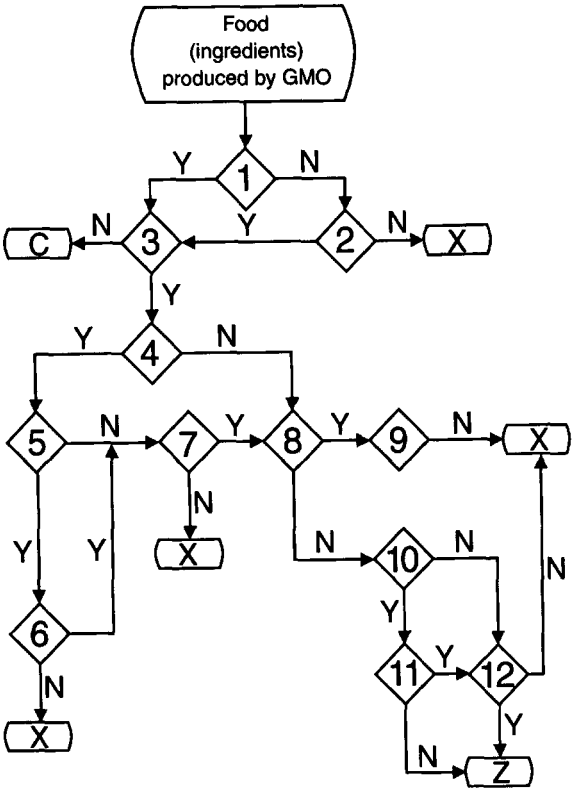

Figure 3. Decision scheme for food products made by genetically modified organisms (see also text).

Entry, Actions and Results

A. Carry out evaluation studies to determine the safety of the product and make specifications

C. Make new specifications

D. Apply a process to reduce the level of undesirable components

E. Carry out a 90-day feeding trial

X. It is not allowed to bring the component on the market

Z. The component is approved for use in foods

Questions

1. Is the use of the *component* in foods allowed at this moment?
2. Does the *component* comply with existing specifications on identity and purity?
3. Are the existing specifications sufficient to control the presence of undesirable site components or too high levels of the intended *component*?
4. Are the levels of known components within the safety specifications?
5. Is it possible to reduce the level of undesirable components during processing in order to comply with the existing specifications?
6. Is the possible that the product contains unknown components?
7. If the intended or assumed consumption of the *component* results in a change in eating habits will the new habit still be considered as safe?

B. Does the evaluation show that the *component* is safe?

F. Does the 90-day feeding trial show that the *component* is safe?

Note: Questions B and F are not (yet) included in the Dutch decision trees as separate questions but form part of *action A and question 5*, respectively.

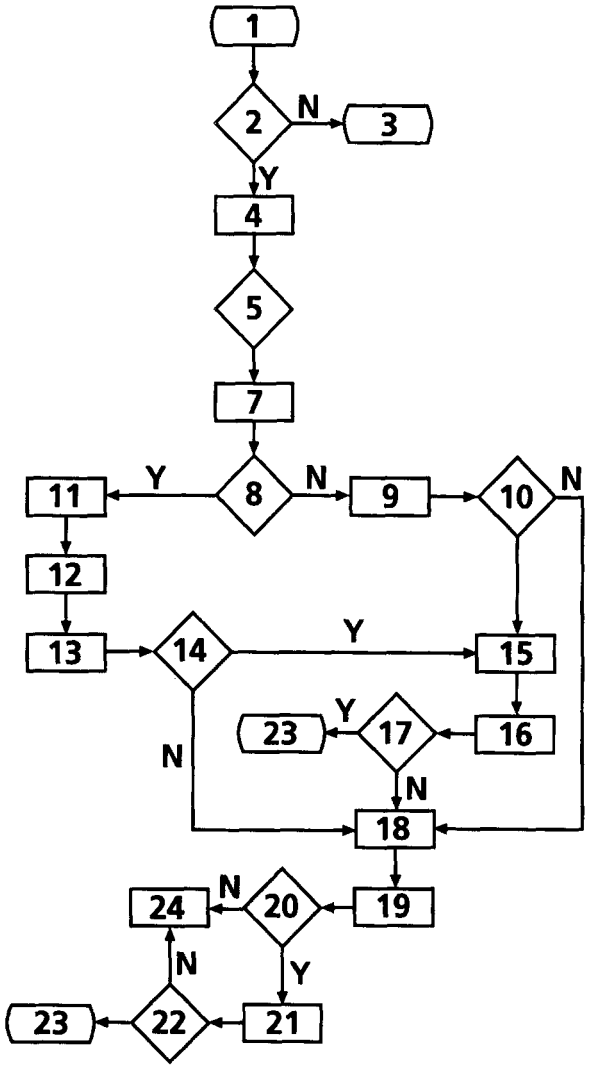

Figure 4. Decision scheme for the evaluation of the potential risk of rDNA lactic acid bacteria in food products. (I) Evaluation of events in the gastro-intestinal tract (see Table 2).

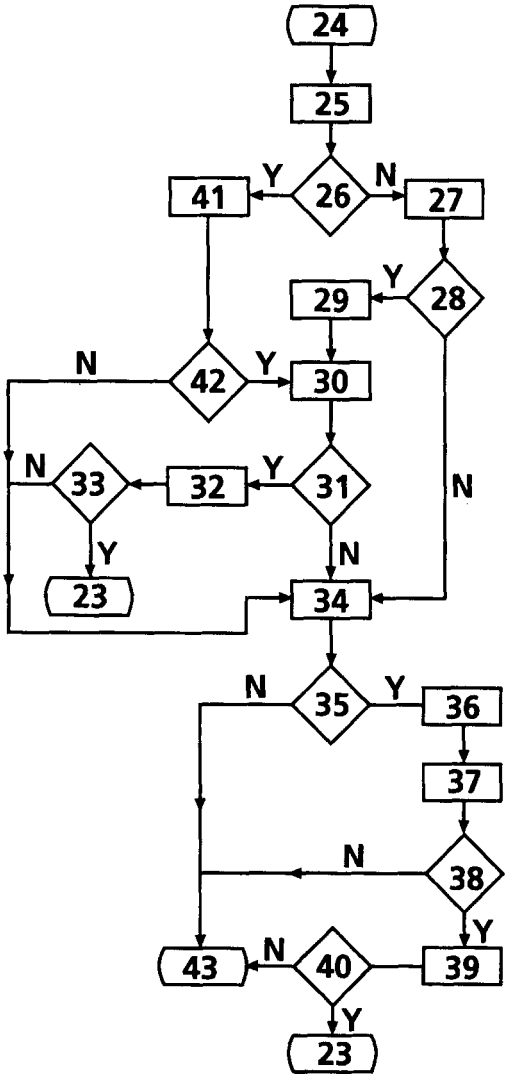

Figure 5. Decision scheme for the evaluation of the potential risk of rDNA lactic acid bacteria in food products. (II) Evaluation of events in the sewage and soil systems (see Table 2).

foreign DNA, are important issues in discussions with authorities and consumer organizations.

(3) Well defined rDNA killed microorganisms in food products

As far as known to the authors this type of products are not (yet) on the market, although Gist-brocades obtained approval in the UK for a Baker's yeast that was modified in such a way that two enzymes in the degradation of maltose (maltose permease and maltose hydrolase) were placed under constitutive promoters to shorten the proofing time of dough (Osinga et al., 1988). For this type of products no clear decision models are available. However, a modification of the model applied in The Netherlands for food ingredients produced by GMOs (Figure 3) can be used. In principle, also the model applied by the FDA for approval of 'Food derived from new plant varieties' (Verrips, 1995) can be applied, if some words are changed.

Before discussing the decision model presented in Figure 3 it is essential to determine whether the product belongs to sphere B1 or B2 or B3 of Figure 1 that represent respectively dead microorganisms containing extrachromosomal rDNA and integrated rDNA either

Table 2. A proposal for a structured assessment of the risk related to the introduction of genetically modified lactic acid bacteria in (or as) food products (Figs. 4 and 5)

Entry, Questions, Actions and Results:	
FOOD PRODUCTS	
1 (E).	Genetically modified lactic acid bacterium (GMO);
2 (Q).	Has the product containing the GMO formal clearance based on animal feeding trials ?
3 (R).	The release of this GMO cannot be evaluated before it has this formal clearance.
4 (A).	Determine the amount of product that comes in the environment (into the sewage system either from factories or households or directly in the soil) before consumption (= spilled product).
5 (Q).	Is *V (spilled)* > *a V (produced)*?
6 (A).	Go to *action 24* to evaluate the behaviour of g.m.o. (and if appropriate the s.t.o.) in spilled products and continue with *action 7* for consumed product.
7 (A).	Determine the distribution of the residence times of GMO in the gastro/intestinal (g/i) tract of the consumers. Take the time corresponding with 95 % of this distribution curve as *t(r)*.
8 (Q).	Will the GMO lyse with *P(b)* > *b* in the G/I-tract ?
9 (A).	Determine the probability *P(c)* that intact cells of the GMO transfer genetic information to normal inhabitants of the G/I-tract. Use in these studies *t(r)* as contact time and the conditions of the G/I-tract.
10 (Q).	Is *P(c)* > *c* ?
11 (A).	Although the correct procedure will be the determination where lysis will occur and the determination of the distribution of the time of intact cells in the G/I-tract, a worst-case scenario is used assuming the concentration of intact cells is not changed by the lysis and that the intact cells can transfer their genetic information during *t(r)* to other microorganisms in the G/I tract.
12 (A).	As described in *action 11* a worst-case scenario is used to determine the probability that DNA of lysed GMO cells transform other microorganisms of the G/I tract. Use for these studies *t(r)* as contact time and the G/I-tract conditions.
13 (A).	Determine the probability *P(f)* that DNA originating from lysed GMO transforms normal inhabitants of the G/I tract (resulting in transformed inhabitant).
14 (Q).	Is *P(f)* > *f*?
15 (A).	Determine whether the transformed inhabitants obtain an advantage over untransformed inhabitants in the G/I-tract: *A(i)*. Define *A(i)* in either faster growth rates *t'(g)*; better adhesion *h'*; or higher production of certain metabolites *p(x)'*, x = 1, ...
16 (A).	Determine the probability *P(e)* that any of the events described under *action 14* will result in the formation of a hazardous (= transformed inhabitant produce toxins or that will replace beneficial microorganism in the G/I tract) microorganisms.
17 (Q).	Is {*P(c)* + *P(f)* } * *P(e)* > *e* ?
18 (A).	Determine also the probability *P(d)* that the GMO will be transformed by genetic material originating from the common G/I tract microorganisms (= modified GMO).
19 (A).	Determine whether the modified GMO gains an advantage over untransformed GMO: *B(i)*. Define *B(i)* in either faster growth rates *t''(g)*; better adhesion *h''*; or higher production of certain metabolites $\{p(\chi)'', \times = 1.. . . .\}$.
20 (Q).	Is *P(d)* > *d* ?
21 (A).	Determine the probability *P(g)* that any of the events described under *action 21* will result in the formation of an hazardous GMO
22 (Q).	Is *P(d)* * *P(g)* > *g* ?
23 (A).	This Risk is unacceptable and the GMO should not be released.
24	THIS IS THE END OF THE RISK ASSESSMENT IN THE G/I TRACT. THE NEXT PHASE WILL BE THE RISK ASSESSMENT OF GMOs IN THE ENVIRONMENT (Figure 5).
25 (A).	Determine the average residence times of the GMO or modified GMO in sewage (*t(r2) and t(r3)*)
26 (Q).	Will the GMO lyse with *P(h)* > *h* in the 'sewage and soil system' (S-system)?
27 (A).	Determine the probability *P(i)* that the GMO will transfer its genetic information to other inhabitants of the S-system. Use in these studies *t(r2)* and *t(r3)* respectively as contact time and the various S-system conditions to determine *P(i)*.
28 (Q).	Is *P(i)* > *i* ?

Table 2. continued

29 (A).	Determine whether the transformed inhabitants gain an advantage (or new property) over untransformed inhabitants of the s-system: *C(i).*
	Define *C(i)* in either faster growth rates $t'''(g)$; better survival s''' or higher production of certain metabolites $\{p(\chi)'''\ , \times = 1. \ldots\}$.
30 (A).	Determine the probability *P(j)* that any of the events described under *action 29* will result in the formation of a hazardous microorganism.
31 (Q).	Is $P(i) + P(k) * P(j) > j$?
32 (A).	Determine the probability *P(l)* that a transformed hazardous microorganism in the s-system ((secondary transformed organism (STO)) will enter the food chain.
33 (Q).	Is $P(i) + P(k) * P(j) * P(l) > l$?
34 (A).	Determine the probability *P(m)* that the GMO will be transformed by DNA of the normal inhabitants of the S-system.
35 (Q).	Is $P(m) > m$?
36 (A).	Determine whether the transformed GMO gains an advantage or new property over untransformed GMO in the s-system: *D(i).*
	Define *D(i)* in either faster growth rates : $t''''(g)$; better survival: s'''': or higher production of certain metabolites $\{p(\chi)''''\ , \times = 1. \ldots\}$.
37 (A).	Determine the probability *P(n)* that any of the events described under *action 37* will result in the formation of a hazardous modified GMO.
38 (Q).	Is $P(m) * P(n) > n$?
39 (A).	Determine the probability *P(q)* that the modified GMO will enter the food chain.
40 (Q).	Is $P(m) * P(n) * P(q) > q$?
41 (A).	Determine the probability *P(k)* that the DNA originating from lysed GMO transforms the normal inhabitants of the s-system.
42 (Q).	Is $P(k > k$?
43 (R).	Provided that the new genetic information of the (surviving) GMO still has its original configuration, the GMO can be released.

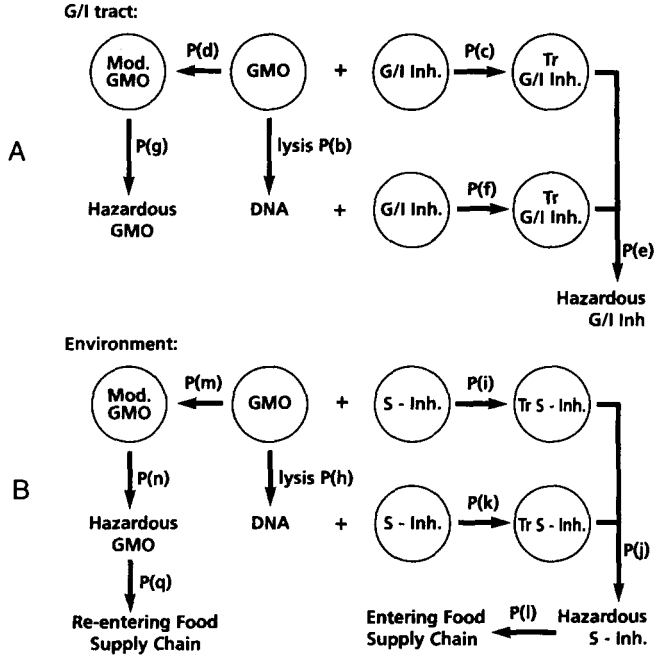

Figure 6. A. Schematic representation of the major events that may occur in the G/I tract and may result in modification of the GMO used or modification of the inhabitants of the G/I tract by conjugation and transformation processes. B. Schematic representation of the major events that may occur in the S-system and may result in modification of the GMO used or modification of the inhabitants of the S-system by conjugation and transformation processes.

Table 3. Estimates of probabilities of transfer of genetic information by/to lactic acid bacteria

Biological process	Probability	Probability function	References values
Lysis of GMO in G/I tract	P(b)	0.75 – 0.99	Marteau & Rambaud (1993)
Conjugative transfer DNA from GMO to lactic acid bacterium of G/I tract	$P(c_1)$	$10^{-3} - 10^{-10}$	El Alami et al. (1992)
Conjugative transfer DNA from GMO to non lactic acid bacterium of G/I tract	$P(c_2)$	$10^{-7} - 10^{-10}$	Langella et al. (1993)
Conjugative transfer DNA from lactic acid bacterium G/I tract to GMO	$P(d_1)$	$10^{-3} - 10^{-10}$	Langella et al. (1993), El Alami et al. (1992)
Conjugative transfer DNA from non lactic acid bacterium G/I tract to GMO	$P(d_2)$		Rood & Cole (1991)
Transformation of GMO by DNA of lactic acid bacteria of the G/I tract	$P(d_3)$	$10^{-8} - 10^{-10}$	El Alami et al. (1992)
Transformation of GMO by DNA of inhabitant G/I tract	$P(d_4)$	$10^{-8} - 10^{-10}$	
Formation hazardous inhabitant G/I tract	P(e)	$< 10^{-8}$	Guinee (1977), Israel et al. (1979) Isberg & Falkow (1985)
Transformation of lactic acid bacterium of G/I tract with DNA of GMO	$P(f_1)$	$<< 6.5\ 10^4/\mu g$	Derived from Tannock et al. (1994)
Transformation of lactic acid bacterium of G/I tract with DNA of GMO	$P(f_2)$	$< 10^{-8}$	Derived from Tannock et al. (1994)
Formation hazardous GMO	P(g)	$< 10^{-10}$	Guinee (1977), Israel et al. (1979)

on a known or unknown chromosomal locus. Bringing a food product on the market made by rDNA lactic acid bacteria that contain resistance markers is (even if the microorganism is killed) a difficult issue to discuss with consumer organizations and in fact they are right. Although the probability of the spread of genes encoding for antibiotic resistance is extremely low, one simply should not take any risk, just because the use of a resistance marker is convenient for scientists to select the transformed microorganism. So situation B1 and preferably also situation B2 of Figure 1 should be avoided. Moreover, in all cases the absence of antibiotic markers is strongly recommended.

(4) Food products containing living rDNA microorganisms

The authors are not aware that a product containing living rDNA microorganisms has been approved. This type of products is different from foods derived from new plant varieties, although the well known transgenic tomato Flavr Savr™, which is on the market, contains living cells. However, the probability that these living tomato cells will grow in the environment is extremely small. This is completely different for food products containing living lactic acid bacteria. A quite extensive decision scheme to cope with this type of products has been proposed, even taking into account the potential transfer of genetic information of the lactic acid bacteria to other microorganisms either in the gastro-intestinal tract (G/I tract) or in the environment (S-system) as described by Verrips (1995). There are two key questions: (i) what is the probability of transfer of rDNA encoded information to human cells; (ii) what is the probability of transfer of rDNA encoded information to normal inhabitants of the G/I tract or the probability that the G.M.O. will be transformed by DNA of inhabitants of the G/I tract.

(i). In these schemes it is assumed that the probability that genetic material encoding functional properties will be transferred from a genetically modified lactic acid bacteria to human cells is zero. This is based on the convincing experiments of Israel et al. (1979) and Chan et al. (1979). In these experiments the complete genomes of the Polyoma virus on either a plasmid or a lambda phage were transferred to *E. coli* K12 and 5×10^9 transformed cells were injected into mice. No transformed mice cells could be detected and the conclusion was that the probability of transfer of polyoma DNA present in *E. coli* was 10^{10} times less than

the transfer of viral polyoma DNA. Also feeding trials with 100 mice were performed and these studies showed clearly that the probability of polyoma DNA transfer was less than 5×10^{-14}. Such a figure is even less than the probability of botulism in canned products and for that reason the probability is considered to be zero. Simplified forms of the original decision schemes are presented in Figures 4, 5 and Table 2.

(ii). These decision schemes also deal with the probability that living rDNA modified lactic acid bacteria (GMO) transform inhabitants of the intestine or that this GMO is transformed with genetic material from inhabitants of the G/I tract. The probability that the GMO enters the S- system and transforms microorganisms there (Klijn et al., 1995b), or that GMOs are transformed by soil and sewage microorganisms and re-enter the foods chain, are also part of these decision schemes. Figure 6 presents these events systematically. From this figure it can be concluded that a number of probabilities should be determined to assess in detail the risks involved with the introduction of living rDNA lactic acid bacteria in food products. Only for a few cases sufficient data and knowledge are available to carry out the risk assessment properly. Although extremely unlikely, here it is assumed that the intrinsically safe lactic acid bacteria, can by the uptake of genetic information from other inhabitants in the G/I tract, change into a hazardous one. On the other hand an inhabitant either of the G/I tract or S-system can pick up genetic information of lysed rDNA lactic acid bacteria and acquire new properties. A considerable research programme in order to answer quantitatively these questions is needed. Fortunately, some studies with lactic acid bacteria have been carried out and there are some data on genetic transfer of other microorganisms. In Table 3 a guestimate is presented of the probabilities of the events that may occur in the G/I tract. A number of comments on these figures have to be made:

During the workshop on 'Safety of Lactic Acid Bacteria' some calculations have been made that are helpful for quantification of the probabilities of Table 3. It is reasonable to assume that a person in Europe consumes daily 200 g of a naturally fermented food product. This means a daily intake of $1\text{–}10 \times 10^9$ living lactic acid bacteria and this number can be used as starting point for all the estimations of the probabilities. The most important assumption is that a probability that transformation of a GMO in the G/I tract occurs is less than 10^{-4} (compare this figure with the acceptability that a food product contains spoilage organism). Starting with $1\text{–}10 \times 10^9$ donor cells such event should be less than one donor cell 10^{13} to 10^{14} cells. It is important to define precisely the donor cell as this cell is not always the original GMO. El Alami et al. (1992) and Vogel et al. (1992) have demonstrated that plasmid transfer can take place in milk and fermenting sausages respectively. Very useful as model for events in the G/I tract are the batch fermentation studies of El Alami et al. (1992) on the transfer of plasmids between donor *Lc. lactis* subsp. *lactis* strains IL 2674, IL 2682 and IL 2683 and the recipient strain *Lc. lactis* subsp. lactis strain CNRZ 268M3. In stirred reactors non-self transmissible plasmids were not transferred (Probability $< 10^{-10}$) and self transmissible plasmids were transferred with frequencies between 10^{-7} to 10^{-8} in 12 hours. In static reactors this frequency is in the order of 2×10^{-4} to 2×10^{-6}. Vogel et al. (1992) studied the transfer of plasmids in sausage fermentations and concluded that in fermenting sausages the transfer rate of a conjugative plasmid from *Lactobacillus curvatus* (pAMβ1) to *L. curvatus* (pNZ12) was about 10^{-6}, a figure close to that in model systems. Therefore it is necessary to determine the probabilities of these events in more detail before introducing any GMO-containing food product (Figure 6 a,b).

P(b). It is assumed that lactic acid bacteria pass the stomach without any reduction in number, although more realistic is to take into account the lysis of lactic acid bacteria in the stomach (Marteau & Rambaud, 1993). In the G/I tract two scenarios have been worked out, one in which all lactic acid bacteria stay alive, and one in which all cells are lysed and that DNA is liberated in the G/I tract and that this DNA has a size that can transform other bacteria. The most realistic scenario should take into account both transformation of naked DNA and conjugation processes. From the studies of Marteau & Rambaud (1993) it can be concluded that about 1 % or less of *L. bulgaricus, L. acidophilus* and *S. thermophilus* survive these harsh conditions but about 25% of *Bifidobacterium* sp. survive. So the probability of lysis in or before the G/I tract, *P(b)*, is between 0.75 and 0.99. In the subsequent steps of the risk assessment the growth of the survivors as reviewed by Marteau & Rambaud (1993) should be taken into account. The volume of the G/I tract is about 7 litre. However, in the estimations of gene transfer events only the caecum (about 4 litre) is considered to be important as in the caecum rather high numbers of bacteria are present. The estimated number of inhabitants of the caecum are *Bacteroides* and *Eubacteria* 10^{11}/g; (Anaerobe) *Streptococci* and *Bifidobacterium* 10^{10}/g, *Escherichia* 10^7/g, *Lactobacilli* 10^6/g, *Veillonella* and *Clostridium* each

10^3/g (Tannock 1990). Accepting a probability of 10^{-4} that an inhabitant of the G/I tract will be transformed by a GMO the transfer frequency should be less than $10^{-4} : (4 \times 10^6 \times 10^{11})$ for *Bacteroides* and less than $10^{-4} : (4 \times 10^6 \times 10^3)$ for *Clostridia*. Important is that most bacteria in the G/I tract are not in a competent state. Taking that only one in 10^6 cells is in a competent state the estimated frequencies should be $< 10^{-15}$ for *Bacteroides* and $< 10^{-7}$ for *Clostridia*.

P(c$_1$) and P(d$_1$). As described above the exchange of genetic information between various lactic acid bacteria has been studied both in batch and continuous cultures by several groups. These data give also some indication on the probability of these events in the G/I tract and S-system. Moreover El Alami et al. (1992) determined that on solid media the transfer frequency is in the order of 10^{-2} to 10^{-3}. In the above described sausage fermentation studies a probability of transfer of a conjugative plasmid of 10^{-6} has been found. The most convincing data of transfer of plasmids in the G/I tract has been described by Brockmann et al. (1996). In germ-free rat feeding trials the probability of transfer from the conjugative plasmid pAMβ1 from a donor *Lactococcus lactis* (about 10^7 cells/g) to a recipient *L. lactis* (about 10^8 cells/g) is in the order of 10^{-3} to 10^{-4} taking the figures determined from samples of caecum, colon and faeces. From all these date it seems reasonable to assume a probability in the gut system of 10^{--5}. This is far beyond the acceptable probability and therefore food products containing lactic acid bacteria with conjugative plasmids should not be marketed before more evidence on their safety is provided. Brockmann et al. (1996) carried out similar studies with the non-conjugative plasmid (pLMP1). Using the same cell densities no transfer has been found, so the probability is $< 10^{-8}$. The probability in the actual G/I tract will be much less, as the fate of the donor cells is quite high. Most probably the transfer frequency will be below 10^{-12}, which is close to the acceptable limit. More studies as carried out by Brockmann will result in a more precise figure, either below or above the acceptable limit.

P(c$_2$) Langella et al. (1993) describe a streptococcal conjugative plasmid pIP501 that encodes transfer functions which allow its transmission into a wide variety of gram positive bacteria. The frequency can be estimated between 10^{-7} to 10^{-10} transconjugants/recipient strain. Also these probabilities are too high. Brockmann et al. (1992) have found that in non-germ free rats the conjugative plasmid pAMβ1 can be transferred from *L. lactis* to *Enterococcus faecalis*. From this result in can be concluded that the probability of transfer of a conjugative plasmid from a Gram positive can occur with a probability of about 10^{-10} donor cells, a figure clearly above the proposed acceptable limit.

P(d$_2$). Clostridium perfringens is commonly found in the G/I tract of humans as well in sewage and soil. In a number of different strains of *C. perfringens* conjugative plasmids have been found, often these plasmids contain tetracyclin or chloramphenicol resistance (Rood & Cole, 1990). Unfortunately, clear data on the transfer of these plasmids via conjugation to lactic acid bacteria are lacking.

P(d$_3$). The importance of the stability of integrated rDNA on the probability of transfer of this rDNA to other cells is not known. The stability of integrated rDNA in lactic acid bacteria is very high. Without any selection pressure even a single copy integrant proved to be stable for more than 100 generations. This means a probability of loss of this gene is less than 10^{-13}. However, the integrated sequence should be free of a replicon (Leenhouts et al., 1990). From their work it can be estimated that the loss of erythromycin resistance was much less than one in 10^{13} cells. Consequently, the probability *P(d$_3$)* that other inhabitants of the G/I tract are transformed by chromosomal DNA originating from the GMO is $<10^{-13}$.

P(d$_4$). Rood & Cole (1991) constructed a *C. perfringens/E. coli* shuttle vector and this vector could electrotransform *C. perfringens*. On this basis we assume that the probability will be in the order of 10^{-8} to 10^{-10} transformed GMO/μg DNA. The frequency of electroporation is most likely 10^4 times higher than normal transformation, so it is unlikely that the proposed acceptable probability will be exceeded.

P(e). An important issue is the probability that harmless inhabitants of the G/I tract or the sewage and soil system become pathogenic. Various groups have tried to reconstruct the pathogenicity of *E. coli* K12, a strain isolated from the gut, that lost four of the five essential properties of the pathogenicity of the original strain, being transferred on rich media for many generations. This concerns notably the property of adhesion in the gut, the production of enterotoxins, and resistance against phagocytosis. Studies of Guinee (1977) proved that the probability of reconstruction of the pathogenicity will be less than 10^{-10}. On the other hand, Isberg & Falkow (1985) were able to render *E. coli* K12 into a for cultured animal cells invasive microorganism by transferring a 3.2 Kb plasmid encoding *virulence* genes from *Yersinia pseudoturberculosis*. From these studies a probability fac-

tor for invasion between 9×10^{-2} to 5×10^{-5} can be deducted. However, these experiments were done with genetic material of which the gene products were directly involved in the pathogenicity (so hazard 100% certain), whereas in GMO discussed here, lactic acid bacteria, are intrinsically safe. Therefore, the probability of the creation of a hazardous inhabitant of the G/I tract by transfer of genetic material from the GMO to these inhabitants *P(e)* can be considered as very small, certainly less than 10^{-10}. The probability that a hazardous GMO will be created can not be deduced from these experiments, but the guess that *P(g)* will be considerable less than 10^{-10} seems reasonable, but studies are necessary to get realistic data.

P(f_1). A broad host range plasmid isolated from *L. reuteri* (Tannock et al., 1994) could transform *Bacillus subtilis, Streptococcus sanguis, Staphylococcus aureus, Enterococcus faecalis* and several *Lactobacillus* spp. Remarkable was that the transformation into competent cells of *Streptococcus sanguis* was $6.5 \times 10^4/\mu$g DNA. This high number demonstrates that transformation of plasmid DNA from GMOs to other (potential) inhabitants can not be ruled out. However, these studies were conducted with competent cells. It is unlikely that cells in the G/I tract will have that physiological state. The probability *P(f_1)* can be estimated as less than 10^{-8}, which probability might be just acceptable or just too high.

P(f_2). On the other hand Tannock et al. (1994) showed that even using electrotransformation, only 15 transformed *Enterococcus faecalis*/μg DNA could be obtained. Although it is not allowed to extrapolate data from electrotransformation to normal transformation processes, it is extremely likely that the probability of the latter is much lower. Taking into account the amount of free DNA in G/I tract that probability will be much less than 10^{-8}.

The survival of lactic acid bacteria in the environment is not very well studied. For the detection of *Lactococcus* species, Klijn et al. (1991) developed a sensitive method based on analysis of the hypervariable region of 16S rRNA using PCR and specific DNA probes. This method was used to study the survival of *Lactococcus* species in the waste flow of a cheese factory and in the environment of cattle (Klijn et al., 1995a, c). However, studies do not provide quantitative data to calculate *P(h)*. Therefore, the two extreme scenarios of 100% intact cells and 100% lysis have to be worked out in the second part of the risk assessment. Similar to the considerations for *P(e)* the probability of *P(j)* can be estimated as 10^{-10}. The probability *P(n)* will just as *P(j)* be also in the order of 10^{-10}. An important difference between the risk assessment in the G/I tract and in the S-system is that in the latter system the concentration of recipient cells is very low. However, the probability that the modified GMO's or the modified inhabitants of the S-systems re-enter the Food chain should be included. From our long experience in food factories we estimate the probability that (modified) lactic acid bacteria re-enter the food chain between 10^{-6} to 10^{-10} (Verrips, unpublished results). The probability of (modified) inhabitants of the S-system to re-enter the food chain is for canned foods in non chlorinated water in the order of 10^{-4}. If the cooling water is properly treated this probability is at least 10^4 times less.

To summarize the present situation, it is still not possible to carry out a complete and reliable risk assessment, and that is certainly a big problem to bring these products on the market. Especially the use of (conjugative) plasmids should be avoided. When a new gene (that does not code for a hazardous property) is integrated stably in the chromosome the data that are available show that with a very high probability products containing such GMO will get permission to enter the market.

Benefits to the consumer

The selection of food products by consumers depends on factors such as quality, functionality (including health and nutritional functionality), natural or 'Green', and convenience (Verrips, 1991). These factors should be improved to offer benefits to the consumers. At present it will not be easy to convince the consumer that rDNA modified lactic acid bacteria will provide any benefit to them. Most of the present targets are related to the improvement of phage resistance, controlled and/or accelerated proteolysis, stabilization of the proteolytic capability, introduction of genes encoding for hydrolytic enzymes to improve fermentation capacity or the usage of normally non-fermentable sugars, etc (McKay & Baldwin, 1990). Achievement of these targets will result in benefits to the manufacturer. The majority of studies, directed to fulfil the demands of the consumer, is related to improved processing and at the best this will result in a marginally lower price of the product. According to consumer studies carried out at Unilever Research Vlaardingen, this is not a major factor in consumer habits. However, in at least four areas significant ben-

Table 4. Potential health benefits of foods prepared with lactic acid bacteria

1.	Improved nutritional value, in particular higher levels of B vitamins and certain amino acids like methionine, lysine and tryptophan
2.	Antagonistic action towards enteric pathogens in the intestines, especially important for patents suffering from disorders such as functional diarrhoea, mucous colitis, ulcerated colitis, diverticulitis and antibiotic colitis
3.	Improved lactose utilization, which is of importance to negroes and some caucasians;
4.	Conversion of potential pre-carcinogens into less harmful compounds
5.	Inhibitory action towards some types of cancer, in particular cancers of the g/i tract by degradation of pre-carcinogens and simulation of the immune system
6.	Hypercholesterolemic action of lactic acid bacteria
7.	Naturally occurring or rDNA vaccinal epitopes

Table 5. Attitudes to applications of genetic manipulation (adapted from (A) Martin & Tait (1992) & (B) Heijs et al. (1993)

		Comfortable	Neutral	Uncomfortable
Microbial Production of bioplastics	(B)	91	6	3
Cell fusion to improve crops	(B)	81	10	10
Extension shelf life tomatoes	(B)	71	11	19
Anti blood clotting enzymes produced by rats	(B)	65	14	22
Medical research	(A)	59	23	15
Making medicines	(A)	57	26	13
Making crops to grow in the Third World	(A)	54	25	19
Mastitis resistant cows by genetic modification of cows	(B)	52	16	31
Producing disease resistant crops	(A)	46	29	23
Chymosin production by yeast	(B)	43	30	27
Improving crop yields	(A)	39	31	29
Using viruses to attack crop pests	(A)	23	26	49
Improving milk yields	(A)	22	30	47
Cloning prize cattle	(A)	7.2	18	72
Changing human physical appearance	(A)	4.5	9.5	84
Producing hybrid animals	(A)	4.5	12	82
Biological warfare	(A)	1.9	2.7	95

efits to consumers and sometimes also to the retailers can be delivered:

(a) Improved and more constant flavour profile of the products, and improved keepability of the organoleptic and physical appearance during retail as well during storage at home. This is important as shown by the acceptance by the consumer of the genetically modified tomato Flavr Savr™. This tomato contains anti-sense polygalacturonase and this results in a slower degradation of pectins in the cell wall of the tomato. Consequently the ripening and decay of the tomato is delayed which was the main reason for consumers to buy these tomatoes, as the keepability at home of the rDNA tomato is increased from 3–5 days to about 10 days.

(b) Better microbiological keepability using natural isolates of lactic acid bacteria that produce inhibitors of other microorganisms (Geis et al., 1983; Van den Berg et al., 1993). Alternatively rDNA lactic acid bacteria can be used to overproduce these bacteriocins or produce bacteriocins that have a wider range of target organisms the activity of bacteriocins (Vandenbergh, 1993). The development of bacteriocins against Gram(-) bacteria will contribute substantially to the keepability of a lot of fermented foods. Especially for products used in developing countries improved microbiological keepability can be of great importance to ensure the food supply for a rapidly growing population.

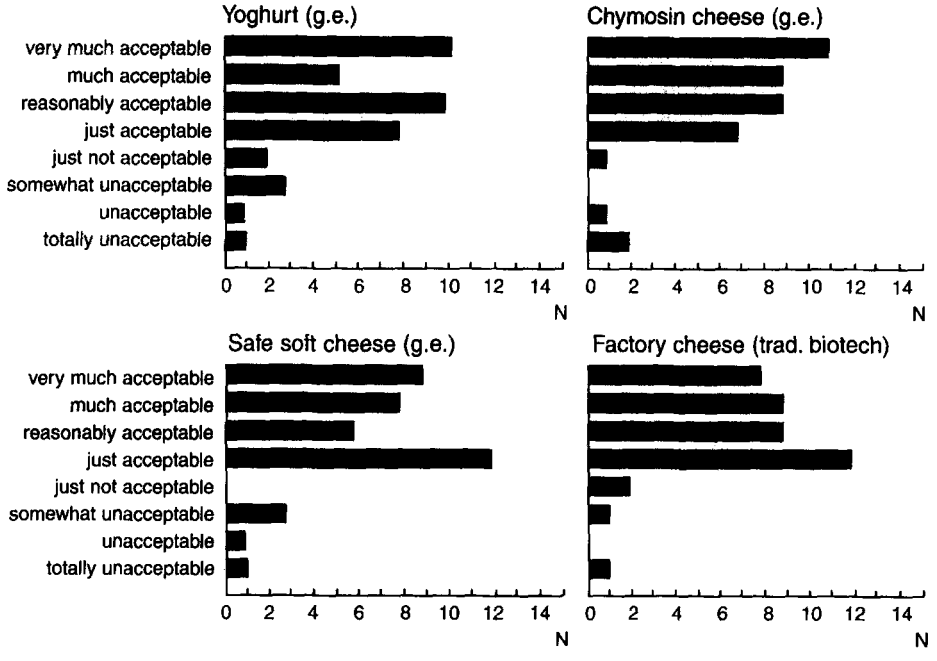

Figure 7. Acceptation of dairy products containing rDNA derived enzymes, GMO's or traditional biotechnological products.

(c) An other interesting option is the use of lactic acid bacteria to produce the right consistency of the product. Especially isolates from meat and olives are able to produce a range of extracellular polysaccharides that improve the viscosity of products (Van den Berg et al., 1993). In this way products with the right consistency can be made without addition of thickeners.

(d) Nutritional aspects of lactic acid bacteria have received a large amount of attention and in the literature various claims are made. Gilliland (1990) and Marteau & Rambaud (1993) summarized the literature on potential beneficial health aspects of lactic acid bacteria (Table 4). In spite of many studies on the nutritional aspects of lactic acid bacteria, no studies have been reported that were designed in such a manner that conclusions on beneficial health aspects could be drawn beyond any reasonable doubt. At the Dutch Institute for Dairy Research (NIZO) and at Unilever Research Vlaardingen quite some attention has been paid on lactic acid bacteria with increased bile salt hydrolase activity. As was proposed by Gilliland (1985), this property would result in a lowering of the blood cholesterol level. We proved that Gilliland's conclusion on the positive action of these lactic acid bacteria was incorrect (Fletcher, personal communication). This confirms findings of Klaver & Van de Meer (1993) on the same topic. Also for the other beneficial aspects given in Table 4, no solid support can be found. It is clear that much better designed experiments are required to change the presently soft, but potentially very important claims into scientifically solid claims about the beneficial health aspects of lactic acid bacteria. If anything can contribute to the acceptance of rDNA lactic acid bacteria in European and American food products, it will be solid health claims.

Clarity and timing of communication to the consumer and environmental organizations and consumer acceptance of rDNA technology

Many studies have been carried out to determine the acceptance of consumers of rDNA technology. Some of the results of these studies are summarized in Table 5, whereas Figure 7 gives specific information on some product containing lactic acid bacteria. In most of these studies it turned out that clarity and timing of communication to consumer- and environmental organizations is a key factor in the acceptance of products made by rDNA technology. SWOKA, the Dutch institute that studied the relation between science and

technology and consumer aspects, developed a nice model on the factors that influence consumer acceptance of new products (Figure 8). From another report of SWOKA it is clear that the knowledge of Dutch consumers on biotechnology is quite low as illustrated by the fact that only 58 and 55 % of the consumers know that yoghurt, respectively penicillin, are biotechnological products (Hamstra & Feenstra, 1989). There are several reports that in other countries the situation is similar. In the investigations of Martin & Tait (1992) and Heijs et al. (1993) information was collected on the probability that consumers will buy at a certain moment rDNA-containing products. Table 6 clearly shows that the number of consumers that do not yet know whether they will buy or reject these products is very large. So the information on benefits and perceived or real risks have to be supplied in a clear and structured way to environmental and consumer organizations. Moreover, the timing of supplying information is very important. The development of a new biotechnological product will take somewhere between 4 and 10 years and is characterized in discrete steps of this innovation process (Verrips, 1995). Normally about two years before introduction into the market, sufficient knowledge on the new product is available to communicate the innovation to various organizations. When planned properly, that will not interfere at all with proprietary right on that product, as nearly always patents are filed in an earlier state. Information on the way a product is made will not always result in a more positive attitude of the consumer. Studies of Smink & Hamstra (1995) found that information of production methods for food products (not including transgenic animals) have different effects on different groups of consumers. Consumers with an quite positive attitude are consumers interested in (bio)technology and they like to have information because of this interest. Consumers with a neutral attitude towards biotechnology are not interested in information on the label, whereas consumers with a negative attitude want to have information on the label, because they will use that information to decide whether they will buy the product or not (Figure 9). On the other hand information on production methods including transgenic animals decrease the acceptance. On the question 'I think it is acceptable that this product is made via biotechnology', the two applications of this technology that were rejected by the consumers involved transgenic animals, whereas products like yoghurt, cheese and tomatoes were quite well accepted (Figure 10). This

Table 6. Intentions to buy or reject products made with rDNA technology

	Intention to buy	Intention to protest
Certainly yes	7%	4%
Probably yes	50%	15%
Probably no	34%	46%
Certainly no	9%	36%

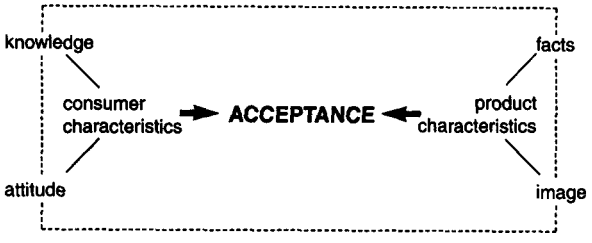

Figure 8. Factors that influence acceptance of new food products by consumers.

illustrates again the importance of a clear division of food products as pointed out in Figure 1.

Conclusions

The introduction of food products made with genetically modified lactic acid bacteria will be accepted most likely in developing countries, Japan and the USA, provided that integrative systems are used and that the benefits to the consumer are clear. In Europe the situation is more complex. If the product does not contain living cells with rDNA, most likely these products with clear consumer benefits will be accepted. For products containing living lactic acid bacteria, which is the majority of fermented food products, the situation is promising provided that consumer benefits are clear. The emphasis of the usage of rDNA technology for lactic acid bacteria should be on the development of clear nutritional and health benefits to the consumers, and on the production of better and more consistent quality of these products. For developing countries, besides the factors mentioned above, also the usage of rDNA technology to extend the microbiological keepability of food products should be investigated. Better and more consumer directed communication is also necessary to get acceptance of the majority of these products by consumers. Finally research should be conducted to quantify the probabilities necessary to carry out proper risk assessments, because as long as

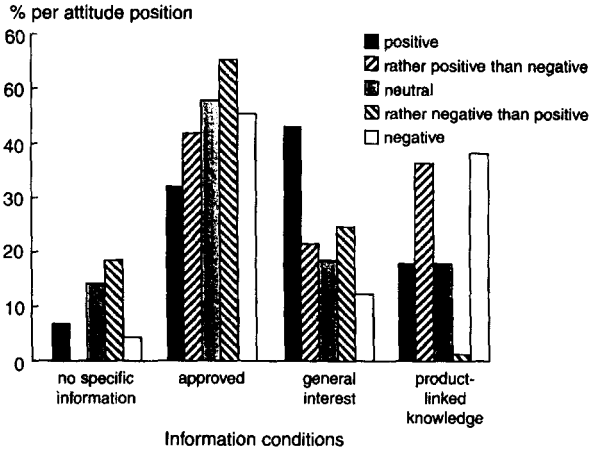

Figure 9. Effect of information and knowledge about food products and acceptance by consumers.

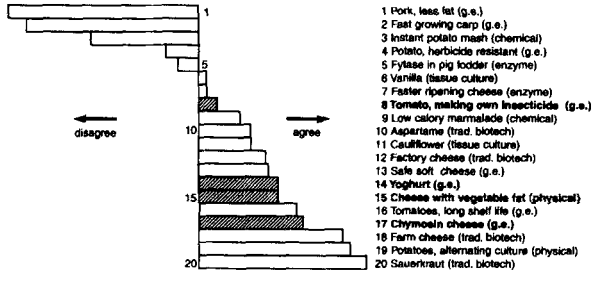

Figure 10. Weighted answers of consumers on the question 'I think it is acceptable that this product is made via biotechnology'.

uncertainties on the safety of certain rDNA products remains these products will not enter the market. Especially the probability of transfer of genetic information via conjugation in the G/I tract *P(c)* should be studied. Until better data are available the use of rDNA lactic acid bacteria containing (conjugative) plasmids is not recommended. Clear, realistic and uniform legislation for the whole European Union is necessary to remain competitive with USA, Japan and some emerging technologically advanced countries.

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