FOOD MICROBIOLOGY : A MULTIFACETED APPROACH

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Good morning !

It is my great pleasure to be here today and I would like to thank the organisers , Josep and Marta, for inviting me so kindly to present the introductive paper to the XI symposium on Rapid methods and automation in Food microbiology .

Food microbiology is somewhat recent ; it is not really well known and I must say that , when I began to work, I was far to imagine the different paths on which I would walk when trying to answer to different questions .

That is the reason why I thought it would be interesting to share with you some aspects of that field and I proposed the following title :" Food Microbiology : a multifaceted approach ".

Nothing really new ! maybe only an opportunity to think of Food microbiology in another way than usual...

*In a first part, we shall try to define the meaning of Food microbiology

*In the second part, we shall examine how it is connected with various fields of Microbiology .

*The third part will be devoted to the evolution of the methods of investigation .

* In the fourth part , before a few words of conclusion, we shall have a look to the environment in which Food microbiologists may find some support .

I- What is Food microbiology ?

The term **" Food microbiology"** includes two words : Food and Microbiology , i.e microbes in foods ; it is very easy to understand it may be very hard to have even a basic knowledge of that field if we think to the variety of foods and also the multiplicity of microbes susceptible to contaminate the foods and, finally, the multiplicity of incidences of microbes in foods . .

What is **food** ? , " officially"? . According to the European regulation EC n° 178/2002, 28 January 2002, giving the general prescriptions on which EFSA has been based, a food is " every substance or product , whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans.

"Food" includes drink, chewing-gum and any substance, including water, intentionally incorporated into the food during its manufacture, preparation or treatment .It includes water after the point of compliance as defined in Article 6 of Directive 98/83/EC and without prejudice to the requirements of Directives 80/778/EEC and 98/83/EC .

"Food" shall not include :

(a) Feed;
(b) Live animals unless they are prepared for placing on the market for the human consumption;
(c) Plants prior to harvesting;
(d) Medicinal products within the meaning of Council Directives 65/65/EEC(21) and 92/73/EEC(22)
(e) Cosmetics within the meaning of Council Directives 89/622/EEC(24);
(f) Tobacco and tobacco products within the meaning of Council Directive 89/622/EEC(24);
(g) Narcotic or psychotropic substances within the meaning of the United Nations Single Convention on Narcotic Drugs , 1961, and the United Nations Convention on Psychotropic Substances , 1971;
(h) Residues and contaminants.

Looking at the multiplicity of types of foods, the multiplicity of combinations food-microbe, we understand immediately that it is far more complex than a few centuries ago when bread and meat were the main foods for humans

Microbes , including bacteria , appeared on earth a very long time , about 3 billion years ago We all know the world of microbes is a fascinating one : it is a real world with a lot of individuals having special ways of living , important possibilities of adaptation and also genetic changes .. The existence of microbes is known especially from the 17th century , due to the development of optical devices .From that time, different approaches of microbiology have been developed in different fields and their knowledge has been improved.

In fact, if we summarize, three groups are usually recognized among a lot of microbes , and especially bacteria, present in the environment and in foods:

-the first group concerns microorganisms implicated in Food safety .

- the second group concerns microorganisms leading to Food spoilage
-the third group concerns microorganisms used for biotechnologies , fermentations ...

Whichever the type of contaminant and the type of food , we can make some general comments :

- The microbes are placed in the food environment and their possibilities of growth or inhibition depends ,as you know, on different factors : disponibility in oxygen, pH, activity of water, but also nutrient agents , inhibitors ...

And the food itself may also be placed in a special environment and at different temperatures which may facilitate or reduce the possibilities of growth of a given microorganism according to its nature itself . Food technology " plays" with the food and microbes susceptible to find there some possibilities of growth .
Important changes have been observed somewhat recently :during the past decades , the conditions of food production have been submitted to tremendous changes , leading to more and more complexity and giving to microorganisms new opportunities to emerge . I will tell a few words about food production , then about changes in the landscape of food microbes ...

- The contamination of food we eat nowadays can be considered as the consequence of what happens at different steps of the food chain in a context which differs considerably from one century ago : for example, noboby can ignore the intensification of all types of productions ; but this intensification has enhanced the risk of microbial contamination and their transmission from one animal to another ; following the rearing stage, the conditions of slaughtering lead to a contamination of the meat through faeces found on animal skin and we must not forget the contamination of the environment of processing and further-processing plants .

- While the food chain was changing and beginning more and more complex, new microorganisms have emerged , and , when they emerge , those pathogens are unknown dangers; we can mention that the new situation will necessitate new duties for the laboratories (in terms of methods , but they concern also the scientific and technical team, equipment ..)We must also add that the types of foods we eat are very different of that we ate 50 years ago but there are also large changes concerning the origin (different types of meats or vegetables ...) ,come from different parts of the world) ; moreover, different meals are prepared in a central place , then distributed around ... That explains the contamination of a lot of people from a unique preparation .

Those changes have led progressively to large outbreaks which have been known from the consumers : from 1992 , we have had to face different problems of Food safety . As an example , we can remember the problems of *Listeria monocytogenes* when the responsibility in different outbreaks have been discovered ; then, other foodborne diseases have taken place in the public debate .
The result of those food crisis , which were better and better investigated due to new techniques and new technologies have led , in Europe, to large changes in the apprehension of Food Safety : I want to speak of the creation of Food Safety agencies in quite all European countries and , in 2002, the creation of the European Food Safety Agency . All problems are now investigated according to a protocol of Risk analysis . In order to set up a good Risk assessment necessitates to have a lot of good and representative data – and that is very difficult in microbiology; in that context, the improvement of methods as well as the introduction of predictive microbiology can help but realistic quantitative risk analysis are only a few at the moment ... as microbes are alive and can grow and multiply in very different conditions ; a lot of analytical problems are not solved, in spite of many improvements ; virulence characterization is always very difficult to appreciate and may vary even for strains belonging to a same species; we have also to consider a lot of factors which may influence the growth or, simply, the survival of microorganisms (pH, temperature, water activity, oxygen, nature of the food and available substrates..) ; then, we must not forget the individual susceptibility ...A quantitative evaluation of microbial risk will always be very difficult to set up ...
We have to remember that we have a multiplicity of combinations food-microbe ; each time that looks like a new situation . The way in which we consider Food microbiology has completely changed from the past decades , changes we were not expecting ...and which lead to a lot of connections with different fields of Microbiology .

II- Connections of Food Microbiology with other fields of Microbiology

The final contamination of a product depends on the possibilities of contamination at different stages of production . From the concept " from farm to fork" well known from everybody nowadays, we can immediately undersee the connections with different fields of microbiology .

If you don't mind, I would like to take an example from my personal experience . as I think it is very significative .A long time ago , around 1970, as I was working in Ploufragan (Brittany) , in a laboratory devoted to poultry and eggs, the Ministry of Agriculture asked our Institute to participate in the setting up of standards for poultry further-processed products , as there was a tremendous development of turkey roasts and other products whose processing was quite unknown ; the authorities were very much concerned by the possible incidence on human health .
That is the reason why we began analyzing different products as turkey roasts and looked at the usual bacteria , including pathogens as *Salmonella* ..
From the first observations, we were somewhat anxious :10% of the turkey roasts were contaminated by *Salmonella* (most of the strains were *Salmonella saint-paul*) ; at that time , some official measures had to be taken against the industrials considered as responsible on the contamination of their production ; the difficulty was that we had been analyzing those products in a purpose of applied research ...that was the reason why we decided , in order to help industrials to improve their production, to look at the possible origin of those *Salmonella* .and that was the beginning of the story I shall summarize ...
We began by the processing plants and we found the same type of *Salmonella* on carcasses but also on some live birds entering the processing plant . So , we decided to look at the contamination of poultry farms , analyzing birds , but also the possible ways of those microorganisms to enter the poultry farm (chicks, feed, water, air , soil., farmers..) . If we want to summarize, we must say that different sources were important to consider ; the first one was chicks – and that is the reason why we decided to investigate hatcheries and breeders farms and we found the same serovar .. ; the second was the contamination of the environment and of what we called " resident" *Salmonella* ; the third one is feed which surely contributes to the contamination of breeders , of chicks and birds of the poultry farm , but the direct relationship is always difficult to establish

The reason why I give that example is that it shows immediately the connections of Food microbiology with different other fields of microbiology .
The first connection , which is the reason why we were interested in that problem is **Medical microbiology** ; presently , no controverse is observed , considering different outbreaks from poultry and eggs ..
The second connection is **Veterinary microbiology** and , if you allow me, I would like to share with you a short story : in March 1980 (a long time ago !) , I was giving a talk to a group of people (many of them were veterinarians)and I was explaining the problem of *Salmonella* on poultry may become a public health problem .. immediately , I received a lot of complains : I had no right to say such a thing as I could not be sure the type of *Salmonella saint-paul* found on breeders was identical to that found on turkey roasts .. that was partially true as molecular biology techniques were not used at that time ; there was only a high probability (but later , we knew that was true) . That shows , too , the connections with **Bacterial Taxonomy** which is also another branch of Microbiology ..
If we consider the importance of the environment in the final contamination of products , we see immediately the connections with the **Microbiology of** the **Environment** and with **Industrial Microbiology** ; moreover, we must not forget the important connections with **Biotechnologies** ...

I can add that, due to the new aspects of Food Safety , Food Microbiology is also closely connected with mathematics (**predictive microbiology**)

To improve the knowledge in all those fields, it is necessary to get quick results from a large number of samples ; for obvious reasons , rapid methods appeared at first in medical microbiology, then later in food microbiology which is the subject of the present symposium .Without giving too many details, I would like now to say a few general data about the techniques used in Food microbiology and their evolution .

III- Techniques used for detection and identification of microorganisms in foods

The first real developments in Food microbiology can be observed after the second World war only : as nowadays, the evaluation of Food safety always began by a visual inspection and microbiology was used as a help to the decision

The microbiological techniques used , which , in France, were taught from the early fifties by R.Buttiaux (who was a physician), in the Pasteur Institute in Lille (F) derived from the discoveries of Louis Pasteur . Those techniques are the conventional ones used at reference methods even if, of course , a lot of improvements have been afforded , new media have appeared of the market , a lot of specifications have been given in the purpose of accreditation, new technologies may be introduced in some cases (It has to be mentioned that, In France , the first regulation giving microbiological criteria , published 21st December 1979 , gave criteria and the related microbiological conventional methods). Quite at the same period , due to the necessity of examining a sufficient number of samples , representing as closer as possible the population , many scientists began to think to alternative methods which may be used in laboratories in order to control and maintain Food safety in most countries .

The first scientist to propose practical solutions is somebody who, providentially, is among us today . .and I like to tell you a story I repeat each year :I was participating in a symposium in Kiel(D) in 1974 . I can't forget it.. .I did not present anything on that day ; I was just learning and listening at the different papers when , suddenly , we had a very interesting and unusual presentation : the speaker , a young American Chinese was quite "dancing" , showing beautiful slides : the miniaturized methods used to detect or enumerate microorganisms from poultry was the subject of his presentation : he was using microplates instead of tubes and a micro-inoculator instead of platina loops ...He had a very simple principle" THINK SMALL" . As I was working on poultry meat microbiology and was very much interested by rapid methods in order to study a sufficient number of strains to get reliable results , I asked the speaker , at the end of his talk if he would be so kind as to send me more informations ... That was the beginning of a long story in Science and Friendship ! .....Dr Fung visited our Institute in 1976 and , from that time , miniaturized methods were introduced in our laboratory and used to identify a lot of strains to trace *Salmonella* , then different types of pathogenic microorganisms " from farm to fork"; we were so enthusiastic with those methods that we presented the technique as well as the results during different meetings in different laboratories : we wanted those methods be adopted by the scientific community ... and were somewhat successful .In fact , Dr Fung is really the "father" of all the commercial methods of identification which appeared on the market from the seventies and I must say I have been very lucky to stay in his laboratory during 2 months in 1980 .
Simultaneously , different scientists around the world began to think to new techniques derived from different branches of Science ; in fact , the microbiological controls taking place in industrial laboratories,they had to find a way in order to :

- Get a rapid answer in order to cancel any abnormality in processing food and, by that way, avoid to put on the market some products which would not fill regulatory standards or standards proper to the concerned industry
- Low cost in order to allow a high number of samples to be examined ,

All that being realized , keeping in mind the results have to be accepted by the scientific community .

In that context , many efforts have been proposed quite early , in order to set up methods based on the following principle : the microbial population is evaluated by detecting a signal related with the activity of microorganisms , most often an enzymatic or the concentration of a molecule (coenzyme, metabolite) or a change appearing in the medium (pH or Redox potential impedance, heat production variation) in connection with that activity .
Radiometric methods were also proposed .

Then, a lot of methods based on **immunology** have been developed but it seems that, during the last decade , the most spectacular developments have concerned the introduction an use of methods based on **molecular biology** , including now DNA chips , which means a fantastic technological evolution, for the characterization of strains ...During the week , you will be lucky to learn a lot about the evolutions but one of the difficulties, of course, has been to make the commercial techniques accepted for official controls . that is the reason why I would like to share with you an example of the difficulties encountered to introduce new technologies in regulations . Due to the evolution of techniques proposed, one important problem discussed during many ISO/CEN (I'll say a few words on those organizations in the last part) meetings has been the introduction of new technologies in Food microbiological methods and , to finalize the project, it took a very long time , many hours of discussions ...
Finally, an important resolution was taken during the joined meeting held in Parma (It) in April 2004.

. *" Each time a standard method is being revised , the possibility of using new technologies , including PCR, must be examined by comparing results with those obtained when using the official conventional method.*

- *For a given microorganism, in order to complete the existing method, the development of standardized methods based on new technologies can be proposed when the purpose to be obtained (for example pathogenicity level) makes it necessary .*
- *When new technologies , including PCR, are used as alternative methods , they must be validated against the reference method .*

Those sentences look probably as quite simple ,but I am sure you cannot imagine the number of hours of discussions which were necessary to obtain a consensus : that is " international cooperation " we shall discuss in the last part of my talk.

And that is a new facet of Food Microbiology , i.e **Standardisation**.and , consequently , relations with **Regulations** .

Concerning new facets in Food microbiological techniques, I think we can consider Food microbiology as a tree which has roots in different disciplines (**biochemistry , immunology, molecular biology** ...) and that, of course, necessitates a good Scientific cooperation I shall examine briefly in the last part of my presentation .

IV- Food Microbiology and Scientific cooperation

You know probably that Louis Pasteur said ": It is a characteristic of Science that it always open new horizons ". Personally, when I began to work , I was far to imagine all the new horizons appearing through new facets . Those facets appeared step by step .. I remember that , in 1981, Dr Fung kindly invited me to co-chair a session of the meeting on "Rapid methods and automation in microbiology" in Washington DC and the title of this session was something like " Rapid methods in Food microbiology : new horizons " .

New horizons appearing through new facets , that is so true in our fascinating field !

Let me have a look to different words I used previously :

- The first word is " **Food**" . I gave you the " official" definition and we saw that we must not forget that microbes may grow (or not) in the food environment ; you also know , of course , that Food Technology is a major field in the context of Food Safety .Most of you , I am sure , know that Josep and Marta have been involved , for a long time now , in the effects of high pressure on the microbiological quality of foods ; Food science and Food technology take an important place in many different other Universities : in KSU (KS), of course , but also in Wageningen (NL) and probably , the most important organization in that field is the Institute of Food Technologists (concerning IFT, I must say that Dr Fung who won a lot of awards, especially from IFT ,gave me the possibility to participate in one of the annual meetings (probably in 1986. in New Orleans (LA) and I am very grateful as it is possible , in that type of meeting, to meet a lot of scientists working in Food technology and connected fields ...I hope many of you will be as lucky as to participate in such meetings..

- The second word I used is " **microbe**" . You surely know there is a national society in quite each country , each of them being involved in the different branches we have examined, speaking of the connections with the different fields of Microbiology ;

  all the national societies for microbiology are in liaison through the International Union of Microbiological Societies (**IUMS**) which is one of the 29 Scientific Unions of the International Council of Science .

  " The objectives of the Union are to promote the study of microbiological sciences internationally , initiate, facilitate and coordinate research and other scientific activities which involve international cooperation , ensure the discussion and dissemination of the results of international conferences , symposia and meetings and assist the publication of their reports, represent microbiological sciences in ICSU and maintain contact with other international organizations"

  Then, after having given some comments concerning the connections of Food microbiology with other sectors of that discipline, I gave a short overview of the evolution of **techniques** and of difficulties encountered in some cases to make them officially accepted . Of course, the different societies or associations we have mentioned are concerned by techniques but , I would like to focus a few minutes in those which are especially involved in harmonization , **ISO** (International Standards Organization) and **CEN** (Comité Européen de Normalisation) :

ISO (International Standards Organization)

ISO was created in October 1946; its seat is located in Geneva (CH) ; the creation results from the fusion of two organizations :

ISA which was the International Federation of National Associations of Standardization, founded in New-York in 1926 and UNSC, i.e Committee for coordination of standardization of United Nations, created in 1944 .

The first national Assembly was held in 1949 in the great amphitheater in Sorbonne (Paris). ISO is composed of 247 committees . All standards are obtained by consensus ; however , they are not mandatory ..The technical committee in charge of Food microbiology is TC34 / SC9 ; the actual president is Bertrand Lombard (F); the meetings are held in a different country each year ; for example , in 2009, the meeting was held in Valencia (Spain) ; In June 2012 , the meeting was held in Brussels (B) ; as usual, it was a joined ISO/CEN meeting

CEN, Comité Européen de Normalisation *("European Committee for Standardization ")* has been created in 1961 in order to harmonize the standards elaborated in Europe ; that means that the standards are mandatory in all countries of EC (at the contrary, the standards which are elaborated by ISO are facultative , which means a great difference ... All members are members of ISO as well .

The seat of CEN is located in Brussels (B) .In the beginnings , it was created by the national organisms for standardization from France, Germany and Benelux countries .Nowadays, the full members are the 27 countries of EU and the three contries of AELE (Association Européenne de Libre Echange) which own such an organism (Switzerland, Norway and Iceland). CEN elaborates technical standards in favor of international trade .

CEN/TC275/WG6 was created in 1993 and I was nominated as the convenor ; nowadays , from July 2005, Alexandre Leclercq from Institut Pasteur in Paris , is in charge of the group .

From the beginnings, one main principle has been followed during the work of this group , i.e the **Vienna Agreement** ; this agreement requires that, as often as possible, ISO methods are taken In order to avoid any overlap, there is also an agreement between different groups of CEN that only one group is in charge of a particular method (the " Vienna agreement") . For example, TC302 in charge of milk and dairy products analysis may choose one specific technique . In this case, it requests TC 275/WG6 to refer to this specific technique in the standard method . The necessity of taking into account the experience of other groups around the world, for example AOAC and IDF (International Dairy Federation) , has also been emphasized from the beginning and , presently, the basis for a good cooperation has been set up .

In order to maintain a good international cooperation, one part of the meeting concerns the liaison with other organizations , ie : International Dairy Federation (**IDF**) Codex Committee on Food Hygiene , **AOAC** (Association of Official Analytical Chemists), **WHO** (World'Health Organisation), **IUMS** (International Union of Microbiological Societies) . The necessity of getting an international consensus , especially with **Codex alimentarius** , is always kept in mind ; I precise a short information about that organization : "The Codex Alimentarius Commission , established by FAO in 1963 develops harmonized international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair trade practices in the food trade . The Commission promotes coordination of all food standards work undertaken by international governmental and non- governmental organisations "

Finally , I would like to mention that Food microbiologists may find a lot of useful informations from different other organisations . I want to speak of **EFSA** (European Food Safety Agengy , whose seat is in Parma (It)) and the different national agencies (**FDA** and **USDA** in US), ...

Conclusion

It was , of course , impossible to give an exhaustive overview of all the horizons opened by Food microbiology and that was not my purpose... I only wanted to make you show that the field in which we are involved is really an "opened" world , a wonderful world opening anytime new horizons through new facets ..

And , finally, I wish you all the best for this week , and for the future ..

THANK YOU VERY MUCH FOR YOUR ATTENTION !