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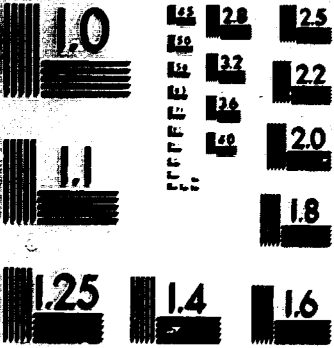

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Symposium on the Importance of Lactic Acid Fermentation in the Food Industry

Mexico City, Mexico, 27-29 November 1984

REPORT*. (Symposium on lactic acid fermentation).

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I. INTRODUCTION

1. Recent advances in the field of molecular biology are presently creating a revolution in agriculture as well as in the food industry. The food industry is, no doubt, the oldest user of biotechnology. For centuries humans have used milk from various sources to make fermented milk drinks and a large variety of cheeses. Vegetables are also fermented all over the world resulting in products such as sauerkraut, kimchi, pickles, gari, pozol, etc. Fermented meat, fish, and cereal products are also common in the diet of many cultures. Indigenous fermented foods, which have essentially remained unstudied, are also used by many populations in Third World countries to supplement their diet. Some beneficial characteristics of these many types of fermented foods include:

(a) Growth of spoilage and/or pathogenic micro-organisms is prevented and thus shelf-life is extended;

(b) In many cases the digestibility of the end-product is increased over the digestibility of the raw material;

(c) The nutritional value of the fermented food may be increased; and

(d) The fermentation may reduce the processing time before the food is consumed.

2. The applications of the new technologies of genetic engineering and biotechnology could vastly improve the characteristics of fermented foods and could consequently result in the development of local industries where new and traditional technologies could be merged without affecting the eating habits of the native population.

3. During a Seminar/Workshop on "Technology Transfer, Management and Development and the Implications of Newly-Emerging Advanced Technologies", Port-of-Spain, Trinidad and Tobago, 8-12 November 1983,

emphasis was placed on the impact that biotechnclogy could have on traditional techniques for the production of fermented food and on the need for assessing the state-of-the-art in fermented foods, particularly those involving lactic fermentation. The importance of lactic acid fermentation for developing countries was also discussed in a meeting sponsored by UNIDO for the creation of a Latin American Network of Biotechnology Centres, held at La Plata, Argentina, 19-22 March 1984. At this meeting it was agreed by the representatives of Argentina, Brazil, Costa Rica, Cuba, Chile, Mexico and Venezuela that lactic acid fermentation should receive priority consideration in the selection of topics for collaborative research and development.

4. The above-mentioned suggestions received special attention at this symposium, symposium on the Importance of Lactic Acid Fermentation in the Food Industry, sponsored by UNIDO and the Universidad Autonoma Metropolitana in Mexico and held at Mexico City during November 1984.

5. The objectives of the Symposium were: to strengthen technological capabilities of developing countries in fields of technological advances and their potential for accelerating the process of industrial development; to assess the state-of-the-art in the application of modern techniques of genetic engineering and biotechnology to fermented foods, identify the needs of developing countries, particularly with reference to upgrading present process techniques in increasing the nutrition value and manufacture of traditional food in some selected developing countries; and to improve and upgrade the capabilities of the network of national, regional and affiliated centres of the ICGEB in the application of the latest technology in the field of molecular biology, particularly in the area of lactic acid fermentation for the food industry.

6. The Symposium was opened by Dr. Reyes-Lujan, the Rector General of the Universidad Autonoma Metropolitana. The opening session was addressed by a member of the UNIDO secretariat, who reviewed the role it is playing in upgrading the technological capabilities of developing countries, particularly in the field of genetic engineering and biotechnology.

II. CONCLUSIONS AND RECOMMENDATIONS

7. The following conclusions and recommendations were made at the Symposium:

Directory of institutions

8. A list of all organizations involved in research and development of indigenous fermented foods in developing countries should be made available. Technical and scientific exchange programmes among groups having similar interests and objectives should be encouraged. A well-documented and coordinated survey of indigenous fermented foods is essential. Such a list exists in the book by K.H. Steinkraus entitled Indigenous Fermented Foods, and if possible this list should be kept up to date as new information becomes available. As stated by Steinkraus in his 1982 UNIDO report (UNIDO/IS.336):

> "Those involved in research on indigenous fermented foods recognize that we have only investigated the surface of a gold-mine of knowledge available on other indigenous fermented foods used daily in many relatively remote parts of the world. To complete our scientific knowledge requires that we bring all these fermentations to light, determine the essential microorganisms involved, study the biochemical changes that occur in the proteins, lipids, vitamins, and other components in the substrates, determine the flavours and textures produced and how they can be controlled, and finally give the world a broader view of how microorganisms can be grown on edible substrates and contribute more to the total proteins and nutrients available for man in the future."

9. After having isolated, identified, and studied the essential lactic acid bacteria in indigenous fermented foods and studied their products, growth conditions, and enzymology, it is possible to optimize the organism through genetic engineering techniques. This would result in the production of high quality fermented foods in regions where they are needed.

10. To accomplish the above-mentioned objective of improving these traditional fermentation processes, the following questions must be asked:

(a) What raw materials are involved in the fermentation process?

(b) What are the processing methods?

(c) What are the types and functions of the micro-organisms involved in the fermentation process?

(d) What is the role of fermented food in the diet and nutrition of the consuming population?

(e) What are the potential mechanisms for improving the fermentation and/or processes?

Process Development

11. Work should also proceed on development of improved processing methods for those fermented foods presently, or having potential of, being consumed by large numbers of people. Small-scale industries should be developed in those countries where the food will be consumed. An important prerequisite for meaningful small- or even large-scale industrial production of these selected fermented foods is the development of acceptable pure starter cultures. Meaningful projects associated with the development of acceptable starter cultures and processing must be implemented on a collaborative basis between developing countries and research centres of excellence. This should encourage effective and efficient characterization of results obtained where they are originally needed.

12. The rationale for the above-mentioned approach is that in many Third World countries fermented foods are consumed in large quantities. For example, in Thailand over 50 types of fermented products are consumed involving vegetables, fruit, fish, meat, cereals, or milk as the raw

material. About 200 fermentation type industries aid in producing many of these products. Similar industries could be established in other developing countries. Some of the technical problems that currently arise in production of indigenous fermented foods include:

(a) control of the lactic acid fermentation has not been adequately studied;

(b) no inoculum of lactic acid bacteria is used;

(c) tools and other implements are very simple;

(d) process of production is labour-intensive;

(e) sanitation conditions are usually inadequate; and

(f) no technique of genetics or genetic engineering have been used to improve upon the fermenting micro-organisms.

13. To solve these problems, the application of emerging concepts for the improvement of traditional fermented foods would be made easier by knowing the mechanism and function of lactic fermentations in traditional food systems which in turn would provide a common ground for cooperative work between different regions. These indigenous fermented foods within an area could then be studied and developed into industries to serve as a food source for the native population.

<u>Development of regional support centre</u>

14. There appears to be a need for the development of a centre in Latin America for the production of commercial dairy cultures. Lactic acid bacteria starters are currently being imported, but these imports may be reduced making it necessary to establish a centre in Latin America to mass produce commercial cultures. The Center of Reference for Lactic Acid Bacteria located in Tucuman, Argentina, could be one such centre. It has many years of experience in working with this group of organisms

and already has expertise in the area of taxonomy and dairy cultures. Other possible locations where such studies are being initiated would be the Instituto de Tecnologia de Alimentos in Campinas, Brazil and laboratories in Mexico. They would need to develop, possibly in collaboration with other laboratories, the expertise for mass production, preserving and shipping of active cultures. This expertise could also be used to develop cultures for inocula to be used for indigenous fermented foods. Latin American countries also appear in need of dairy cultures that function under local manufacturing conditions. New strains native to Latin America need to be isolated and made available to Latin American cheese-makers. Also the group at Tucuman has the opportunity of developing a starter culture for their native Argentinian cheeses. The latter could then be developed into a small business for local farmers.

15. The specific questions to be addressed in this area include:

(a) Can suitable strains of lactic acid bacteria be isolated from raw milk or green plant materials that will function in Latin American cheeses? These strains would need to be characterized with respect to stability, acid-producing ability, heat tolerance, salt tolerance, proteolytic activity, phage sensitivity and ability to produce agreeably flavoured cheese.

(b) Can phage resistant strains be developed?

(c) A critical question to be answered is how will the cultures be mass produced, preserved, and distributed? In tropical areas, will the cultures be made available in liquids, frozen, lyophilized, spray dried or other forms? How will stability and activity be maintained during distribution? Can "dry" cultures, such as those used in making bread be developed to overcome the distribution problems of liquid or frozen cultures?

Dairy industry

16. Dairy fermentation processes and the lactic acid bacteria involved were discussed extensively. These studies ranged from the isolation and characterization of strains involved in the production of a native Argentinian cheese, to biochemistry and genetics of phage infection, and to strategies for genetic engineering of dairy streptococci and lactobacilli. There is no doubt that these groups of bacteria will be the first to be genetically improved. This is mainly due to the fact that a critical mass of investigators exists in the field which provides the impetus for major achievements. Fundamental advances have been made in the genetics and recombinant DNA technology with dairy lactic acid bacteria. These studies should open new opportunities for both strain improvement and process development for a wide variety of lactic acid fermentation processes. The genetics and plasmid biology of lactic acid bacteria isolated from indigenous fermented foods could be facilitated by cooperation with those laboratories having expertise in these areas, such as those at the University of Minnesota, Massachusetts Institute of Technology, North Carolina State University, and many European universities.

Strategy for full utilization of potentials offered by lactic bacteria

17. A question that needs to be answered is whether the lactic acid bacteria which predominate in many of the traditional indigenous femented foods have unique biochemical and genetic properties. For example, it has recently been reported that there are strains of lactic acid bacteria which excrete proteins and produce amylases. These starch hydrolyzing strains could be essential in improving those fermentations where starch is the primary carbohydrate source (solid state fermentation processes). Studies on the genetics, plasmid biology, and mode of protein excretion by these strains is urgently needed and the results of such studies could lead to the genetic engineering of such strains for use in many indigenous fermentation processes. Collaboration is needed between laboratories in Mexico working with these unique strains of lactic acid bacteria and those laboratories having expertise in plasmid biology and genetics of lactic acid bacteria.

18. Specific questions in this area include:

(a) Can lactic acid bacteria with unusual ability to produce organic acids, essential amino acids, such as lysine or methionine, vitamins such as thiamine, riboflavin or B-12, or antagonistic compounds against spoilage and pathogenic organisms be isolated and then used in producing indigenous fermented foods?

(b) Can lactic acid bacteria producing enzymes such as proteinases, lipases, petinases or others be found and explored?

(c) Can the amylase producing strains of lactic acid bacteria be used to improve indigenous fermented foods based on starch as the carbohydrate source? Can these strains be used to produce SCP from starchy foods? Can the protein content of fermented foods, such as gari, be increased by using amylase producing lactic acid bacteria? Can genetic engineering principles be applied to these amylase producing strains to improve the organism? Such questions as regulation of amylase production, plasmid biology and mechanisms of protein excretion also need to be answered.

19. Lactic acid bacteria alone or in conjunction with other microorganisms are utilized for the manufacture of many different fermented foods. One unique association to be studied and exploited is that observed in pozol, a fermented maize product consumed in Mexico. The fermentation not only involves lactic acid bacteria but also a nitrogen fixing organism. The nitrogen content of the final product is greater than the original starting material. Basic studies need to be conducted on this microbial association.

20. Questions to be addressed in this area include:

(a) Has the nitrogen fixing micro-organism been fully characterized and what is the mechanism of nitrogen fixation?

(b) What is the role of lactic acid bacteria in the ability of this organism to fix nitrogen?

(c) Can the responsible micro-organisms be isolated in pure culture and then combined to form a nitrogen-fixing starter culture that could be used to increase the nitrogen content of those indigenous fermented foods naturally low in nitrogen?

(d) Could the genes responsible for nitrogen-fixation be transferred to a lactic acid bacteria species to develop a new culture that could be used in food fermentation processes?

(e) Could the laboratories in Mexico, which have the expertise on this nitrogen-fixing strain, work with other interested laboratories in order to rapidly develop the system?

21. In view of the potential exhibited by those systems, UNIDO was requested to do whatever possible in order to foster research in this area.

Non-traditional fermentation

22. The preservation of grass, i.e. silage, by the use of lactic acid bacteria would have a global impact if it could be consistently accomplished. A suitable starter needs to be developed that will function in tropical regions. Such a culture would aid in preserving

animal feed (silage) during those periods when fresh green grass is not available. Collaboration needs to be conducted among the various laboratories working in this area.

23. Lactic acid fermentations are also being extended to non-traditional raw materials for production of animal or human food. These efforts should continue as they may lead to ways of converting a waste material into an edible food source. Examples under study include production of fish silage combined with sugar cane wastes to produce animal food, the lactic acid fermentation of banana puree as a means of preserving bananas for human consumption, and the recycling of agricultural waste by lactic fermentations.

General recommendation

24. Biotechnology of lactic acid bacteria will occur. While it may occur first in those strains used in dairy fermentation processes, the true impact may come when this technology is applied to strains found in indigenous fermented foods. To accomplish this goal, the fundamental biology (metabolism, genetics, plasmids) of the strains involved must be understood. This could be accomplished by joint research ventures between the developing country where the properties of the indigenous fermented food could be studied and the organisms isolated and a laboratory which has the expertise in genetics and plasmid biology. Between the two efforts, strains to improve the fermentation process could be developed. For international cooperation however, a suitable source of financing would be required for initial investigative studies.

III. TECHNICAL PAPERS PRESENTED AT THE SYMPOSIUM

25. During the sessions devoted to technical presentations, twenty-one papers were presented. These papers and the discussions they generated formed the basis for the conclusions and recommendations of the Symposium.

26. The papers presented could be grouped into the following areas: basic biochemistry and taxonomy of lactic bacteria; molecular genetics of lactic bacteria in dairy industries; traditional fermented food; and new fermentation processes using lactic bacteria. A summary of each of the presentations made is as follows:

Advances on the application of lactic acid fermentation in the food industry

27. The food industry requires various lactic acid bacteria starter cultures for the preservation and processing of products of animal and plant origin. Starter cultures of higher activity and of better quality should promote the manufacture of food of improved quality. Modern food production can only be carried out on a large scale level if lactic acid fermentation is controllable.

28. The fermentation activity of microbes is extensively utilized in the dairy industry where different starter cultures are used in the production of cheese, butter, sour-milk products, cottage cheese, etc. It is used in the meat industry, where the decrease in pH inhibits the propagation of non-desirable bacteria and shortens the drying time. The technique is safer on a large scale and the quality of the final product could be standardized when applying starter cultures for the preservation of vegetables.

29. The bacterial conversion of malic acid to lactic acid and carbon dioxide in wine is a natural reduction of acidity and provides an improvement in sensory quality.

Behaviour of regional lactic starters

30. Microbial kinetics of the ripening of cheese "Tafi" was evaluated. The microorganisms involved are Streptococcus lactis, S. lactis subspecies diacetylactis, S. cremoris, S. faecalis, S. faecium, S. plantarum and

S. casei.

31. Their technological properties are:

(a) Optimal growth temperature does not necessarily coincide with optimal temperature for specific enzyme activities;

(b) The major proteolytic activity of mesophilic strains, grown at high temperatures (45-50C) could be attributed to heat enzyme activation;

(c) Optimal temperature of amino and imino-prolil-peptidase is around 37C;

(d) The acid production in mesophilic lactobacili and streptococci is higher at 15C but for thermophilic streptococcii the optimal temperature is 45C;

(e) Diacetyl production by lactic bacteria increase with decreasing temperatures;

(f) At the usual milk pH no proteolytic activity was observed nor diacetyl biosynthesis;

(g) Citrate addition to reconstituted non-fat milk (10 per cent) increases diacetyl production if pH is 6.2, whereas piruvate addition increases diacetyl production at all pH values to be considered;

(h) Lipolytic activity was shown in 60 per cent of all lactic starters;

(i) Ethanol treatment of lactic bacteria increases titrable acidity, decreases pH and reduces the clotting time of milk.

Ecology of lactic fermentation of starchy food

32. Lactic starch fermentations are important for the conservation and transformation of staple food such as cereal grains and tubers in may tropical countries and it is important to understand the ecology of this process for the future industrial development of the infant food and traditional food industries.

33. In this study, traditional microbial sources such as sauerkraut, "pozol" (fermented maize dough) and fresh cow dung was chosen. Using conventional microbial techniques, 76 lactic strains were isolated and classified according to genera (*Lactobacillus*, *Streptococcus*, *Leuconostoc* or *Pediococcus*) and type of reaction: homo or heterolactic. Their ability to use starch, glucose and $(NH4)_2SO_4$ or peptone as only carbon and nitrogen sources were analysed, using mesophilic or thermophilic conditions.

34. Statistical comparisons among those strains indicated the importance of the nature of the primary microbial source (inoculum) as a leading factor of such ecology. For example: thermophilic streptococcii were predominant in cow dung; more even distribution of strains according to genera were found in the other inocula.

35. It is possible to obtain thermophilic amilolytic homolactic bacteria with appropriate choice of samples and culture media. This type of result could be used for developing new industrial processes based on traditional or agricultural fermentations. As an example, lactic fermentation of enriched cassava meal (grown with Aspergillus-niger) was optimized as an inexpensive alternative procedure for the conservation of foodstuffs.

Evolutionary taxonomy of lactobacilli: enzyme homology

36. Bacterial macromolecules such as DNA, RNA and proteins, can be seen as archaic records useful for establishing taxonomic relations between micro-organisms. Having this in mind, primary structures of isofunctional proteins can be compared by means of immunological procedures, which in turn, make it possible to detect very small differences of amino acid sequences of such proteins. For this purpose homogeneous preparations of purified beta-galactosidase (E.C. 3.2.1.23) of three strains of lactobacilli: L. murinus ATCC 35020, L. Helveticus ATCC 15009 and L. bulgaricus ATCC 11842 were obtained. Purified enzyme preparations were used to immunize New Zealand rabbits in order to prepare the corresponding antisera. With these sera and the enzymes from 22 strains of lactobacilli, the following immunological techniques were developed: Wasserman and Levine complement microfixation and Ouchterlony immunodifussion. The first kind of technique was used to evaluate the differences between the enzymes, compared by means of dissimilarity and immunological distances, and those values were correlated to the number of amino acid substitutions in the primary structure of those proteins. The second type of immunological technique was used as a qualitative

comparison in order to supplement the former one. Those results were used in order to elaborate various phylogenetic trees of lactobacilli which were grouped as follows:

(a) A group of total homology: L. bulgaricus ATCC 11842 and wild L. bulgaricus;

(b) Close to the previous ones: L. lactis and L. leichmanni;

(c) Divergent groups: L. casei var. casei ATCC 393 and wild, L. acidophillus and L. helveticus;

(d) Some more divergent groups involving the rest of studied strains.

37. These results support the hypothesis of a common evolutionary origin of all members of this genus.

Manipulation of sugar lactic fermentation using heterogeneous populations of bacteria

38. Lactic acid fermentation of sugars is an ancient and common process for food transformation. Modern cheese and yoghurt industries use defined "starter" cultures in order to provide special flavours to final products and to also comply with sanitary regulations. Nevertheless, there are other lactic acid fermentations which are less demanding in flavour and sanitary standards. For example: silage, that is, lactic fermentations of fodder, or perhaps future industrial production of lactic acid as a chemical commodity. These types of processes could profit from non-aseptic cultures. Furthermore, recovery and utilization of traditional lactic acid fermentation could be improved if the basic principles of lactic fermentation were known.

39. The paper reviewed recent work on the biochemical manipulation of sugar lactic fermentation using pH, temperature, sugar concentration and the quality and quantity of the nitrogen source as environmental variables.

40. It is shown that the combined reactions of heterogeneous cultures of lactic bacteria, such as fermentations inoculated with cow dung, have a reproducable behaviour if the aforementioned variables are controlled within certain specified ranges. The kinetics of such systems could be modelled by simplified kinetic expressions reminiscent of those derived for the kinetics of pure cultures of lactic bacteria. In particular, the apparent yield of products and the requirement of nutritional factors seems to be appropriate for developing new lactic fermentation processes related to waste recycling for feed industries and the future mass production of lactic acid as a chemical feedstock. Results obtained in this work were compared with others using pure inocula.

Manipulation of sugar cane silage fermentation

41. The effect of sodium hydroxide addition when ensilaging sugar cane was reported when considering the nutritional value of this feed for ruminants. NaOH addition resulted in an increase in lactic acid and a decrease in ethanol production as well as an increase in the feed consumption by the ruminants with a corresponding weight gain at the same time. The fermentation's standards showed that silages treated with NaOH produced a greater amount of propionic acid which is the main gluconogenic agent in the ruminants. Sodic monensine reduced the feed consumption without modifying weight gain of the animals, therefore increasing the conversion yield.

Microbiology of cheese lactic fermentation

42. Lactic acid bacteria (L.A.B.) have played an important role from early times in the making of dairy products, especially cheese. The main starter cultures used in the manufacture of most cheeses are two mesophilic streptococci, S. lactis and S. cremoris with addition of S. diacetylactis of Leuconostoc, in some cases. In the other ones, S. themophilus and L. helveticus are used, but other L.A.B. may grow naturally in cheeses.

43. The major role of L.A.B. is the transforming of lactose to lactic acid thus lowering the pH of curd and contributing to syneresis. Secondly, proteolytic activity of L.A.B. is important both as a means of making N-compounds available for growth and as a part of the ripening process which gives cheeses their characteristic rheological and organoleptic properties. L.A.B. produce other aroma compounds which may contribute to flavour formation in some cheeses. At least CO2-producing species are useful in some cheeses where some "eye" formation is desirable.

44. In addition, the growth of spoilage bacteria and pathogens is controlled by L.A.B. inhibition resulting from competition for nutrients, decrease in pH and, in some cases, the formation of pH specific inhibitory compounds.

45. The rate of acid production by L.A.B. is affected by the manufacturing process, the composition of milk and eventually the presence of inhibitory substances, but the most important inhibitory factor however is phage attack, which constitute a real problem in cheese factories although there are measures to prevent it.

46. Different types of starter cultures are used according to the type of cheese and country, and frozen or lyophilized cell concentrates have been developed to inoculate bulk cultures and sometimes directly the vat.

Importance of local production of starter cultures for the dairy industry

47. Flavour is a crucial factor for the selection and acceptance of food products. It is for this reason that much value is given to flavour quality control. On the other hand, it is evident that the acceptance of a defined flavour is related to local food traditions and gastronomic preferences. Also, the characteristic aroma and flavour of fermented dairy products is mainly the result of the metabolism of component lactic strains.

48. In this study, it was postulated that R&D groups from developing countries interested in producing fermented dairy products should pay attention to local food traditions and study local conditions for the production and conservation of starter cultures in order to ensure the acceptance of these products.

49. In this work, some recent observations were reviewed related to the production of starters for the local dairy industry using appropriate raw materials for culture media and adequate methods for their preservation. Attention has been paid to evaluating the tasting threshold of Mexicans for various flavour components such as acidic, salty, bitter and sweet, in relation to the use of various starter cultures and the acceptance of standard type of cheeses.

Biochemical and genetic aspects in phage infection of lactic streptococci

50. Within the cultures used in the manufacture of cheese and other dairy products, lactic streptococci are preferentially attacked by bacteriophages. Phages specific for S. lactis were isolated from different samples of cheese whey and studied by electron microscopy. They had similar morphology although their dimensions were slightly different. On infection the phages were adsorbed on the bacterial surface by the free ends of the tail. Calcium ions were found essential for the effective adsorption and lysis. Phosphates and other complexing agents inhibit host phage interactions. Based on the acridine orange test, they were primarily identified as DNA phages. This information was confirmed by agarose gel electrophoresis after treatment with DNAse and RNAse. Previous alkaline treatment demonstrated that the DNA was double stranded. The calculated M.W. correspond to 11.25 Mdal. Restriction enzyme analysis using several specific endonucleases indicated differences in the cleavage patterns. Phage resistant mutants were isolated and used successfully in industrial trials. Morphological, biochemical and genetic characterization of phages, as well as the knowledge of those factors involved in infection analysis are important to develop strategies to prevent failure in lactic acid fermentations.

Presence of phages in starter cultures

51. The primary cause of slow acid production by lactic bacteria during industrial fermentations is lysis by bacteriophages. Many virulent and temperate phages have been isolated and characterized by electron microscopy but the genome structure was determined for only a few phages. A classification of phages has been proposed according to their host-range, their morphological type or their antigenicity, but DNA homology seems to be a more precise criterion to determine relatedness between phages. Lysogeny was shown to be widespread among lactic bacteria. It is not yet known to which extent temperate phages contribute to bacteriophage problem. Transduction with temperate bacteriophage has been demonstrated for the transfer of some genes of the lactic bacteria. Restriction-modification seems to be one of the main defenses of the lactic bacteria against their phages. Investigation into the mechanisms of phage resistance would provide information for construction of phage-resistant strains.

A restriction and modification system in Streptococus cremoris

52. A restriction/modification (r/m) system present in S. cremoris IL 839 is reported. In this strain, phage growth was restricted by a 10^5 factor. Strain 839 contained 8 plasmids designated pIL20 to pIL27 with respective sizes of c.a. 3, 5, 6, 9 , 13, 14, 17 and 46 kb. Using protoplast-induced curing method, derivatives cured in turn for each of the plasmids except for pIL21 retained in all derivatives were obtained. Phage sensitivity of these derivatives indicated that none of the plasmid cured encoded for the r/m system. To determine the role of pIL21, it was transferred into a plasmid-free, r-/m- strain of S. lactis using co-transformation with an indicator plasmid (pHV1301) conferring erythromycin resistance. Transformants containing pIL21 did not demonstrate r/m activity. It has been concluded that r/m genes were located on the chromosome. Work is currently in progress to clone chromosomal gene sequences which are implicated in the r/m system.

Plasmid biology, gene transfer systems, and strategies for genetic engineering of group N. streptococci

53. Plasmid biology has become an important area of investigation in dairy starter cultures. It is now established that N. streptococci used as starter cultures harbour plasmids of diverse sizes and that some of these plasmids code for properties vital for successful milk fermentation processes. Examples of plasmid mediated traits in this group of bacteria include lactose utilization, proteolytic activity, citrate utilization, production of antagonistic proteins, nisin resistance, and resistance to bacteriophage. Developments in the above area may lead to amplication of plasmid genes through the isolation of copy number mutants, thus increasing the efficiency of the fermentation process as well as the quality of the final product. Stabilization of these plasmid-mediated traits by integrating essential genes into the chromosome may also prove beneficial. Thus, the study of plasmid biology in dairy streptococci, as well as other lactic acid bacteria, has become a prerequisite for future strain improvement programmes. This knowledge, coupled with transduction, conjugation, protoplast fusion, and transformation systems applicable to dairy streptococci, is essential for gene cloning work within this group of bacteria. The worldwide interest in the genetics of lactic acid bacteria brings us closer to the time when genetically improved strains are actually used in commercial fermentation processes.

Genetic engineering of lactobacillus

54. Lactobacillus play a central role in various food fermentation processes as well as for the production of lactic acid. Despite their economic importance relatively little effort has been directed towards elucidating their genetics or biochemistry. Recent advances in the field of genetic engineering offer exciting prospects for strain improvement and manipulating catalytic activities if this technology were extended to lactobacillus. Currently several groups are developing recombinant DNA technology for Lactobacillus bulgaricus and Lactobacillus casei. These efforts have resulted in the elucidating of plasmid function,

construction of chimeric vectors and the establishment of a transformation system for introducing those chimeras into lactobacillus.

Fermented food industry in Egypt particularly related to milk products

55. Fermented dairy foods in Egypt are closely related to those produced in the Mediterranean and Middle East countries. Statistical data on production and per capita consumption of fermented milks in Egypt are given. The process of producing fermented dairy foods in Egypt varies greatly from primitive methods where fermentations occur spontaneously to the fairly advanced methods where dairy plants are established for their production under controlled conditions.

56. Results of recent work on properties of fermented dairy food in Egypt like sour milk (Laban Rakd), Zabadi (like yoghurt), sour buttermilk (Laban El-Salata) and Laban El-Zeer, Karish cheese, Mish paste and Mish cheese as well as Kishk were considered.

57. The technical problems and recommendations which could lead to the improvement of existing processes and products, trends and scope of future developments of fermented milks in Egypt, were discussed.

Fermented food industry in Nigeria

58. Fermented foods are consumed widely in Nigeria. These include alcoholic beverages, processed roots and tubers, cereal and milk products and condiments. The vast majority of these fermented foods are still being prepared entirely by "rule of thumb" methods.

59. Although information on the food industry in Nigeria is meagre the current status of the fermented food industry was reviewed, highlighting some technical and raw materials problems, success so far achieved, areas where more research and development are needed for increased production, possibly through small-scale industries.

Present status and development of lactic acid fermented foods in the Republic of Korea

60. The lactic acid fermented foods in the Republic of Korea can be classified into three groups: (a) the traditional non-milk fermented foods such as kimchi and simkchae, (b) fermented milk products, mainly yoghurt types and (c) pharmaceutical or unconventional food products. The lactic acid fermentation on different substrates is explained by taking kimchi as the typical lactic acid fermentation using vegetables, simkchae, using a mixture of fish and cereals, and yoghurt, using milk. Recent development in the study of lactic acid fermentation on vegetable milk products was also discussed. The production of beta-galactosidase and other enzymes from lactic acid forming micro-organisms will also be an important area attracting industrial interest.

Fermented food industry in Indonesia

61. The fermented food industry in Indonesia is mostly concerned with the production of traditional fermented foods. At present no less than 24 different kinds of products are known and widely consumed as beverages, staple foods as well as side dishes and snacks.

62. The fermentation of these products can be classified as follows: (1) fermentations producing a meat-like texture in a cereal grain/legume substrate by means of fungal mycelia that binds the particles together, e.g. in the production of different kinds of Tempe and Oncom, (2) fermentations involving proteolysis of vegetable proteins by microbial enzyme in the presence of salt and/or acid with the production of amino acid/peptide mixtures having a meat-like flavour, e.g. in the preparation of Kecap and Tauco, (3) fermentations involving the Koji principle whereby micro-organisms with desired enzymes are grown on a cereal grain or legume substrate to produce a crude enzyme concentrate that can be used to hydrolyze particular components in the desired fermentation, e.g. in the manufacture of different kinds of Tape, (4) fermentations in which organic acids are the major products, e.g. in the preparation of Sayur asin, (5) fermentation in which ethanol is a major product, e.g. in the production of Brem Bali and Cru.

63. The raw materials for most of these products, (e.g. Tempe, Oncom, Kecap, and Tauco) are soybeans or other legume seeds. Glutinous/sticky rice is used for the preparation of Tape Ketan and Brem Bali, whereas cassava is the raw mateiial for the preparation of Tape Ketela and Ciu. Sayur asin is made of green cabbage. With the exception of one or two products (e.g. Kecap), most of the traditional fermented foods in Indonesia are manufactured on a small scale as home industries using relatively simple tools with a mixed culture for the starter and under unsterile conditions. Hence the quality of the end-product often varies from batch to batch or from time to time. Thus appropriate microbial cultures as well as reliable and improved techniques of production should be developed if a product of uniform and better quality is desired.

Lactic fermentations in Thailand

64. Thailand, being a tropical agricultural country, has a wide variety of lactic fermentations, ranging from traditional, cottage-level production of fermented foods and feeds to large-scale industrial production for export.

65. Lactic fermentations in Thailand play a significant role in the country's economy and employment, and the nutrition of its people. With a few exceptions of imported technologies, most lactic fermentations are based on local, indigenous technologies. While many production processes remain traditional, some have evolved from traditional to modern, advanced processing.

65. Social and economic impacts of lactic fermentations in Thailand was described and the technological development illustrated.

67. Attempts were also made to identify potential research and development needs of Thailand in order to more effectively utilize the country's abundantly available agricultural raw materials for the industrial application of lactic acid fermentation technology.

68. Finally, potential collaboration and co-operation with other developing countries were discussed, particularly in relation to the exchange of microbial cultures with reference to the services of the Southeast Asian Microbiological Resource Center - the Bangkok MIRCEN.

Lactic acid fermentation of banana puree

69. Significant amounts of fresh banana not satisfying the requirements of the international market are discarded in Central America. A fermentation process to preserve banana puree using lactic acid producing bacteria, which could be applied to co-operatives in rural areas was developed. To prevent enzymatic browning, the optimum ripened and peeled fruit was immersed in boiling water for seven minutes; it was made into a puree and 1 per cent milk solids with 1 per cent inoculum (V/W) were added. From lactic bacteria studied, four were selected based on their ability to ferment the puree and reduce the pH below 4.5 in an adequate amount of time. L. plantarum ATCC E 8014, L. casei TISTR 390, L. fumentum TISTR 391 and L. cellobiosus TISTR 398 reduced the pH from 4.8 to 4.00, 3.95, 4.10 and 4.00 respectively in 24 hours at 37 C. At this time, the aroma flavour and colour of the resulting products were pleasant. After an additional 24 hours, the desirable organoleptic characteristics decrease. Fermentation must be stopped by refrigeration or pasteurization (boiling water) for 34 and cooling for 30 minutes. L. plantarum ATCC E 8014 was the final selected strain after a preferential response in the sensory evaluation. The fermented puree is suitable for use in many banana flour speciality food products like "bocadillo", which was chosen as the product with the greatest commercial possibilities in the Central American region.

The sugar canefish silage fermentation

70. The ensilaging of grass in temperate countries is mediated by a lactic process which preserves the material. Attempts to ensilage sugar cane produces ethanol-acetic acid, because it is mediated by yeasts selected by the high sucrose content. The incorporation of fish in the

fermentation (C:N ratio 15.1) with animal droppings as inoculum, redirects the fermentation to produce high levels of lactate to inhibit proteolysis and lead to a satisfactory silage which could be stored for up to twelve weeks.

Recycling agro-industrial waste by lactic fermentations: coffee pulp silage

71. A biotechnological process to make use of coffee pulp for animal feed has been developed. The process can be adapted to the existing coffee processing plants for drying the product once harvesting has finished. Unit operations involved are: pressing (optional), ensilaging, liming and drying.

72. Experimental work demonstrated that if the ensilaging operation is adequately handled, coffee pulp can be maintained over 100 days without signs of putrefaction. This length of time is usually equivalent to the actual Venezuelan coffee harvesting time. However, if molasses is added to the fresh pulp the silage quality can be strongly improved. Experimental results from 60 days coffee pulp silaged at the above conditions showed fatty acids and lactic acid formation of the following composition (g./100 g. of dry matter): acetic, 4.33; propionic, 0.46; isobutyric, 0.046; n-butyric, 1.52; isovaleric, 0.03; n-valeric, 0.12 and lactic acid, 1.12. Coffee pulp silage experimental production has been already suggested to local Venezuelan planning offices in order to get some more technical information for both nutritional and economical feasibility studies.

ANNEX I

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ANNEX II

PROGRAMME

27 November 1984

Opening Ceremony
S. Reyes Lujan, Genenal Rector of UAM

Discussion of technical papers:

"Advances on the Application of Lactic Acid Fermentation in the Food Industry"
A. Hoschke, P. Biacs, J. Beczner, Central Food Inst., Hungary

"Behaviour of Regional Lactic Starters"
G. Oliver, CERELA, Argentina

"Ecology of Lactic Fermentation of Starchy Food"
R. Velazquez, M. Raimbault, G. Viniegra, UAM-ORSTOM, Mexico-France

"Evolutionary Taxonomy of Lactobacillus: Enzyme Homology"
A.P. de Ruiz Holgado, CERELA, Argentina

"Biochemical Manipulation of Lactic Fermentation"
G. Saucedo, and J. Gomez, UAM, Mexico

"Manipulation of Fermentation in Sugar Silages"
A. Shimada, INIP, Mexico

General Discussion

28 November 1984

Discussion of technical papers:

"Microbiology of Cheese Lactic Fermentation"
J.L. Bergere, INRA, France

"The Importance of Local Production of Starter Cultures for Dairy Industry"
P. Perez-Gavilan, UNAM, Mexico

"Plasmid Biology, Gene Transfer Systems, and Strategies for Genetic Engineering of Group N Streptococcii"
L. McKay, University of Minnesota, USA

"A Restriction and Modification System in Streptococcus Cremoris"
A.L. Dos Santos, M. Gautier and Ms. Ch. Chopin, ITAL, Brazil

"Biochemical and Genetics Aspects in Phage Infection of Lactic streptococcii"
J.L. Parada, Univ. Buenos Aires, Argentina

"Genetic Engineering of Lactobacillus"
A.J. Sinskey and C.A. Batt, MIT, USA

"Presence of Phages in Starter Cultures"
M. Mata-Gilsenger, Univ. Lyon, France

General Discussion

29 November 1984

Discussion of technical papers:

"Traditional Lactic Fermentations of Egypt"
S.M. Khalafallah, Ain Shams Univ., Egypt

"Present Status and Development of Lactic Acid Fermented Foods in Korea"
C. Ho-Lee, Korea Univ., Korea

"Fermented Food Industry in Indonesia"
S. Saono, Nat. Biol. Inst., Indonesia

"Lactic Fermentations in Thailand"
M. Suwana-Adth and W. Daengsubha, Thailand

"Traditional Lactic Fermentation of Cereal Grains in Africa"
E.O. Iwo-Banigo, Fed. Univ. of Technology, Nigeria

"Lactic Acid Fermentation of Banana Puree"
Ma. C. Arriola, E. De Porres, R. Garcia and C. Rolz, ICAITI, Guatemala

"Fish Silage with Sugar Cane"
D. Ali, CARIRI, Trinidad and Tobago

"Recycling Agroindustrial Waste by Lactic Fermentation: Coffee Pulp Silage
V. Carrizales and J. Ferrer, CIEPE, Venezuela

General discussion, conclusions and recommendations

Closing remarks
G. Viniegra, UAMI, Mexico