Microbial Antagonism in Foods

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Introduction

General

The two components of the title of this review, microbial antagonism and foods, are so broad that it becomes necessary to impose some restrictions. Sterile foods do not exist in commerce but many foods exist in which microbial activity is very low, e.g. canned foods which had a 120D heat-treatment. Microbial antagonism (and its converse, microbial stimulation) presupposes actively developing microbes capable of affecting each other. As this does not exist in canned foods, they will not be considered. Dried foods, e.g. dried soups, numerous bakery products, also lack a growing microbial flora and will be excluded. However, one cannot exclude the possibility of even such safe products being subjects to manufacturing accidents and being abused. Canned foods can become contaminated after processing, or dried foods may be rehydrated with inadequate heating and then saved for use later. Under such circumstances microbial development can occur and antagonisms may be of practical importance. The same consideration also applies to frozen foods.

Microbial antagonism in the intestine has been known since the 19th century. Metchnikoff then suggested that harmful putrefying bacteria can be suppressed by acid producing lactobacilli, a factor said to contribute to longevity. The controversy which has arisen about this suggestion is not yet resolved. Modern studies with germ-free animals offer the best possibility of rational solutions. These studies have established that antagonisms do play an important part in the establishment of a microbial flora of the gut (for example Dubos and Ducluzeau, 1969a, b; Ducluzeau et al., 1971), and that the flora of the normal gut may play a significant role in host resistance to enteric pathogens (Luckey, 1963). Greenberg (1969) observed bacterial associations in the gut of blow-fly larvae which tended to eliminate Salmonella typhimurium. These examples are cited to illustrate the interest and complexity of the problem; in general the biochemical basis for dominance or for suppression is not yet known and germ-free animals provide a good tool for these studies. Also, there is an intimate connection between the intestinal flora of the animals and the food product which may be eventually derived from it.

Antibiotics have been tried as food-additives mainly to prolong shelf-life of products which may suffer organoleptic damage by adequate heat-processing. This subject was reviewed by Jarvis and Morisetti (1969).

Some Principles

Microbial antagonism in foods has been documented in raw meats, semi-preserved meats and thawing frozen foods, but in general food technologists have been content to observe and describe without interfering. Active interference in microbial development has taken place with fermented foods, e.g. pickles, dry sausages and some dairy products.

These will be discussed below because they offer the best examples of microbial antagonism being put to the service of preserving foods. In this context, we are prepared to accept high numbers of bacteria because they are known to be desirable. When foods contain high numbers of bacteria which develop by accident or negligence, the food is said to be spoilt. Therefore, our idea of spoilage is not necessarily the same thing as high numbers of microbes. Uncontrolled bacterial growth is frightening because conditions which enable spoilage organisms to develop are fre-
The initial infection of the substrate, (2) factors depending on the properties of the substrate (intrinsic factors), (3) factors depending on the conditions of storage (extrinsic factors), (4) properties of the dominant micro-organisms, which they term "implicit" factors.

Infection of foods can occur from ubiquitous depots such as water, air, dust, insects, human operators and these have been known to be highly dangerous (Mossel, 1971b). One of the most dangerous sources of infection is, however, the food itself as pointed out by Mol et al. (1971). Selection and enrichment of organisms can occur in the unprocessed food leading to contamination of the finished article.

Mossel and Ingram (1955) list the intrinsic factors affecting microbial development under three principal headings: chemical, physical and processing. The chemical factors are availability of nutrients, pH, redox potential and food preservatives. The physical factors are water vapour pressure, freezing and physical dispersion i.e. the colloidal state. The processing factors may affect the load of initial infection or any of the factors listed above. Probably the important extrinsic factors are the humidity during storage, temperature of storage and the composition of the gases during storage.

All the listed factors affect the development of microbes, but clearly the production of compounds inhibitory to other microbes is an intrinsic property of the microbe. Therefore, it is proper that further discussion should be under the heading of the name of the individual microbe. It is another question whether this intrinsic property can be expressed i.e. the compound in question synthesized, in the environment in which the microbe finds itself or even if it is made, whether the compound interacts effectively with the kind of microbes found in that environment. For example, strains of *Streptococcus lactis* make the antibiotic nisin which is known to be particularly effective against gram-positive organisms (Hurst, 1972). If cultures were to be applied to refrigerated carcass meat, these mesophilic bacteria would be unable to develop and produce the antibiotic. If the temperature was raised sufficiently for them to develop the antibiotic, it would be ineffective against the dominant gram-negative flora (Ayres, 1960; Pierson et al., 1970). Therefore, it is also proper that microbial interactions should be considered in relation to the different food products.

**Raw Meat and Fish**

The interior of carcass meat is generally much less contaminated than the surface. On the surface *Pseudomonas* and *Achromobacter* species predominate (Ingram 1962) their activity being controlled chiefly by temperature and the equilibrium relative humidity. Development of food pathogens is mainly controlled by temperature: at warm temperatures *Clostridium perfringens* develops first probably because it is the least strict anaerobe (Ingram and Dainty, 1971). Spoilage and development of fluorescent pseudomonads is rapid with aerobic packing. Under anaerobic conditions...
numbers of pseudomonads do not increase and the numbers of another potential spoilage organism, Microbacterium thermosphactum decrease (Pierson et al., 1970).

Ground beef at 7°C spoils more readily than whole meat probably because contaminating microbes become distributed. The gram-negative Pseudomonas-Achromobacter are again the main spoilage organisms but their development can be inhibited by adding lactic acid bacteria to the ground meat (Reddy et al., 1970). Untreated ground beef became spoilt after 2 days at 7°C but with a skim milk culture of Staphylococcus lactis and Leuconostoc citrovorum it was still acceptable after 7 days. Ascorbic acid (450 ppm) added at the time of grinding, greatly improved the color.

Gardner et al. (1967) found that the nature of the packaging material had a profound effect on the flora of pork. Aerobiologically pseudomonads and other gram-negative bacteria dominated at 2°C and 16°C storage. Packaging in a gas permeable film still permitted pseudomonads to dominate at 2°C but at 16°C pseudomonads only formed 22% of the flora; other gram-negative flora formed 62% of the flora. With a gas impermeable film at 2°C pseudomonads were 50%, M. thermosphactum 30%, and lactobacilli 20%. At 16°C the genus Kurthia dominated. Baran et al. (1970) found that CO2 inhibited bacterial growth on packaged hamburger and improved food color retention on storage. As might be expected, anaerobes were favoured by vacuum packaging in films which were gas impermeable.

Dominating spoilage organisms may not be a good indication of safety of meat. Bacterial spoilage does not manifest itself until high numbers of organisms are reached, about 10^8/cm^2 or per gram (Ingram and Dainty, 1971). However, sufficient toxin to cause food poisoning may be reached at 10^6 staphylococci/cm^2. Of the different enterotoxins produced by staphylococci, enterotoxin A is the most important one in foods. Markus and Silverman (1970) suggested that this was because growth and enterotoxin A synthesis are strictly correlated, whereas enterotoxin B is a secondary metabolite and it may or may not be produced during growth (also see further discussion below; Weinberg, 1971; Baird-Parker, 1971). These results are not in agreement with those of McCoy and Faber (1966) who used an enterotoxin A producing staphylococcus in 5 types of raw meat and were able to dissociate growth from enterotoxin A production. For example, beef and ham slurrries inoculated with Pseudomonas and Staphylococcus, allowed the staphylococci to grow to about 10^8/g but no enterotoxin A was detectable. The same result was obtained at 25 and 35°C. Many other gram-negative organisms tended to give similar, though perhaps less dramatic results.

The microbiology of fish and fish products has been reviewed by several people including Shewan (1971). The factors listed by Mossel and Ingram (1955) apply to fish also, namely: infection, intrinsic and extrinsic factors, and implicit factors. A complicating factor with fish appears to be that important microbiological qualities appear to depend on the environment from which the fish is caught. For example, North Sea fish caught near the British Isles does not appear to contain Clostridium botulinum type E but North Sea fish caught near the Norwegian coast does. Johansen (1965) favours the view that type E is of terrestrial origin. More recently Laycock and Loring (1972) observed a similar phenomenon in the Gulf of St. Lawrence. The distribution of type E could be correlated with terrigenous sedimentation. Sugiyama et al. (1971) found in the Green Bay area of Lake Michigan exceptionally high concentrations of type E. Fish acquire the type E and become carriers but the organism does not multiply in the living fish. Rather, type E propagates on vegetation and other bottom deposits; these results indicate that type E is an aquatic microbe and might be contrary to its terrestrial origin.

The synthesis of botulinum toxin E is complex and requires several steps. It has been studied by Sakaguchi and collaborators (1967). Progenitor toxin is first synthesized and becomes activated by partial proteolysis i.e. by trypsin acting at the suboptimal pH of 6.0 or by proteolytic enzymes made by other clostridia (Sakaguchi and Tohyama, 1955). Combined action of proteolytic enzyme and reduction of disulphide bonds leads to active toxin and the toxin has a diminished molecular weight. Since it is conceivable that these reactions can occur on fish during storage, this example may be regarded as stimulation — the opposite of antagonism. Boticins which are bacteriocins made by Clostridium botulinum may also affect production of type E toxin. Anastasio et al. (1971) studied the effect of a boticin made by a strain resembling type E. Boticin inhibited vegetative cells and was sporistatic to sensitive strains. Sensitive strains were always non-proteolytic so that resistance may be connected with production of proteolytic enzymes. Boticin itself was destroyed by trypsin but not heat (Anastasio et al., 1971).

The gram-negative flora of spoiling fish (Shewan, 1971) may be inhibited by peroxide producing Lactobacillus plantarum according to Price and Lee (1970). These workers isolated 81 microbial species from seafoods and the lactobacillus species from oysters. Their findings "may explain the abnormal shifts in microbial flora observed in foods where Lactobacillus species have overgrown the natural flora".

**Semi-Preserved Foods**

**British fresh sausage**

This product is made from raw pork or beef so that it may be regarded as raw meat. However, it contains SO2 at a legally permitted level of 450 ppm which gives it a shelf-life of several days at room-temperature so that it may be regarded a semi-preserved food. Dowdell and Board (1971) carried out a thorough ecological study of the microbial associations in British sausage. On storage at 4°C or at room temperature for 4 to 10 days the product spoilt by souring. Failure to isolate organisms capable of the anaerobic metabolism of lactate was taken to indicate that there was no ecological succession. When...
SO₂ was present, the dominant organisms developing were yeasts, lactic acid bacteria and microbacteria. Yeasts and *M. thermophila* grew in association so that they do not compete for a common growth limiting nutrient. Lactic acid bacteria tended to dominate at room-temperature especially if the initial numbers were high. When SO₂ was omitted, gram-negative bacteria predominated. Freshly prepared sausages thus had a heterogeneous population which could be ascribed to the contamination of the meat from which the sausages were prepared. Although this study has established the principal organisms of British fresh sausage, the factors underlying their selection remain poorly understood.

**Bacon**

The microbiology of vacuum packed sliced bacon was investigated by several workers (Hansen, 1969; Cavett, 1962; Tonge et al., 1964; Patterson, 1966; Miller, 1967; Baran et al., 1970). They found that the normal flora of spoiling bacon at room temperature was composed of micrococci and lactic acid bacteria. Pathogenic staphylococci did not develop at 20°C but coagulase positive staphylococci (and other bacteria) reached high numbers on storage at 30°C. Inoculation of bacon stored at 20°C and above with staphylococci caused the bacon to putrefy but at lower storage temperatures faecal streptococci tended to become dominant, spoiling the bacon by souring. Nisin is a permitted food additive in most countries (Jarvis and Morisetti, 1969) but its use did not delay spoilage of sliced vacuum packed bacon (Gibbs and Hurst, 1964). Packaging bacon in CO₂ resulted in reduction in total numbers of aerobes and lactobacilli and only a few *Cl. perfringens* were recovered (Baran et al., 1970).

**Ham**

Reerens (1955) discusses the factors leading to the development of clostridia in French hams. Giolitti et al. (1971) review the microbiology of Italian hams and the succession of microbial flora on ham rinds (Cantoni et al., 1971). Enterococci which are commonly found in hams may antagonize other microbes (Kafel and Ayres, 1969). These authors, observed that the processing temperatures used in the manufacture of canned hams does not always result in a sterile product. They noted that spore forming organisms were seldom present in pasteurized hams contaminated with enterococci in contrast to uncontaminated cans. They also remark that when bacilli, clostridia and enterococci were present immediately after processing, bacilli and clostridia could be recovered less frequently after storage. For example, bacilli were isolated in 13% of hams contaminated with enterococci and in 29% of hams that did not contain these microorganisms. These authors conclude that true antagonism by enterococci on other bacteria may take place in canned hams. However, this study does not reveal the nature of the antagonist, nor is there any critical comment about the acceptability or otherwise of large numbers of enterococci in canned hams.

**Other Cured Meats**

Recent observations on the microbiology of cured meats were made by Mitchell (1962), Miller (1964), Gardner (1968), Kempton and Bobier (1970), Reuter (1970a, b) and Mol et al. (1971). Kitchell (1962) reviewed the coagulase-negative staphylococci and micrococci and the antibiotic substances they produce (see below). Gardner (1968) and Kempton and Bobier (1970) observed that although initially lactic acid bacteria formed only a small part of the flora, they were the main spoilage agents of packaged cured meats. The spoilage was due to acid. After 15 weeks at 5°C bologna sausage accumulated 0.6 to 0.8% lactic acid and the pH dropped to below 5.0. Acid end products and the anaerobic packing would obviously favour the lactic acid bacteria. These results are similar to those reported by Reuter (1970). Authors of the last two papers just reviewed, remark on the poor correlation between total counts and organoleptic assessment of the products. Kempton and Bobier (1970) and Mol et al. (1971) observe that with adequate heating it should be possible to eliminate the lactic acid bacteria. However, the products become recontaminated during slicing and packaging.

**Food Poisoning and Cured Meats**

This was reviewed by Baird-Parker (1971). Cured meats, especially ham are frequently involved in *Staphylococcus aureus* food poisoning. Christiansen and Foster (1965) showed that *S. aureus* grows poorly in vacuum packed chopped ham. McCoy and Faber (1966) working with ham slurries found that food microbes could either inhibit the growth of *S. aureus* or if growth occurred, enterotoxin A toxigenesis could be prevented. On the other hand, Genigeorgis et al. (1969) found practically no conditions of manufacture or storage which could prevent the formation of enterotoxin B. For example, enterotoxin B was formed at any pH above 5.5, at any salt concentration up to 9.2% and at storage temperatures of 10°, 22° or 30°C. Baird-Parker (1971) found no enterotoxin B in vacuum packed bacon inoculated with *S. aureus* (10⁶/g) and stored for 14 days at 25°C. In aerobically inoculated bacon he detected enterotoxin B after 3 days at 37°C but not after 7 days at 28°, 30° or 32°C. This result is difficult to reconcile with that of Genigeorgis et al. (1969) and can be most easily explained by possible antagonistic microbes being present in the bacon examined by Baird-Parker (1971).

**Fermented Sausages**

Outside the dairy field, fermented sausages offer the best examples of the applications of microbial antagonism. Recent reviews of the microbiology of fermented sausages are by Giolitti (1967), Terplan (1969), Rozier (1969) and Kafel (1971). Coretti (1956) reviews work up to his time and concludes that cause and effect are difficult to distinguish. Is the flora selected by the conditions or does the flora cause the organoleptic changes? Niinivaara (1955) used a strain of *Micrococcus* (Mο) as a “starter” because this organism grew in meat and produced an antibiotic inhibitory to many of the miscellaneous and

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undesirable bacteria normally present in raw sausage mix. Examination of sausages from factories using the "starter" showed that there were many fewer defective products than were normally found. The most acceptable sausages contained mainly lactic acid bacteria and few micrococci. The lactic acid bacteria appeared to be resistant to the M₃₅ antibiotic and micrococci tend to disappear during smoking (Niinivaara and Pohja, 1957).

At about the same time as Niinivaara developed the Micrococcus starter, Niven et al. (1955, 1958) introduced Pediococcus cerevisiae as a starter culture. The same authors later describe the microbiology of curing (Deibel et al., 1961a, b) and the advantages of using P. cerevisiae. This is one of the lactic acid bacteria which becomes dominant towards the end of the fermentation and is a particularly strong acid producer. P. cerevisiae has been reclassified as P. acidilactici, and the culture originally available in the lyophilized form, is now available in the frozen form (Everston et al., 1970).

In a series of articles on the microbiology of salami type sausage in the Netherlands, Ten Cate (1960) found Group D (faecal) streptococci necessary for both the formation of desirable aroma, and the suppression of the undesirable gram-negative bacteria. A common additive to sausage meat is L-glutonolactone used for the production of acid in fermented sausages instead of starter bacteria. It hydrolyzes to gluconic acid, thus slightly acidifying the sausage meat with no effect on the meat microflora (Nurmi, 1956). In the U.S.S.R., Kuwahara et al. (1963) recommended the use of "multiple strain" starters, S. lactis being the main organism. Santa and Mugra (1969) and Reit (1970) recommended a 1:1 mixture of S. diacetylactis and L. plantarum to diminish the ripening period and improve aroma.

The influence of microbial populations on the quality of processed meats was studied by a new technique by Bothast et al. (1971). Bacteria on meat surfaces were first killed by a hot water dip and afterwards the meat was handled inside sterile plastic isolators. The meat was cured with and without P. cerevisiae, and the population of Bacillus cereus was reduced. Deibel et al. (1961b). In the meat with this diminished contaminating flora, lactobacilli dominated but P. cerevisiae dominated when added.

**Flora of Frozen Foods During Defrost**

Cavett et al. (1965) reported on the interaction of the dominant groups of bacteria which spoiled frozen thawed peas. Spoilage was delayed by the interaction.

Peterson et al., in a series of five papers (Peterson et al., 1962a, b; 1964a, b, c) investigated the development of staphylococci in mixed populations. Their interesting approach to potential food poisoning hazard can be best illustrated by quoting from one of their introductions (Peterson et al., 1962b):

"Reflection on the incidence of staphylococcal food poisoning indicated that a majority of cases occur in foods which have been treated to drastically reduce the bacterial population; staphylococci subsequently inoculated are without competition. Staphylococcal food poisoning also occurs in foods which favor selectively the growth of staphylococci while sharply inhibiting growth of other genera, or in foods which have a protective action on staphylococci through the action of substances such as eggs, starches, and lipids.

Kelly and Duck (1936) reported that meats of high salt concentration were selective for the growth of staphylococci. Staphylococci grew in concentrations of up to 10% salt. This concentration of salt prevented the growth of bacilli and allowed the staphylococci to overgrow other microorganisms present. Newman (1943) drew attention to the importance of high counts of mixed microbrial flora in milk in making it difficult for staphylococci to gain predominance before the milk spoiled. Takahashi and Johns (1959) reported that the number of non-staphylococci was one of the limiting factors influencing the growth of staphylococci in raw milk. Thatcher and Ross (1960), however, questioned the idea that milk with a high standard plate count was not conducive to multiplication of staphylococci.

Miller (1955), in a study of ground pork, reported that the natural saprophytic species of bacteria present outgrew added inocula of S. aureus by a tremendous margin at temperatures above 18°C. Sufficient numbers of S. aureus to cause food poisoning were never attained. Below 18°C, S. aureus failed to grow significantly. In a study of creamed chicken artificially and massively inoculated with S. aureus by Straka and Combs (1952), the food poisoning organisms were outgrown by the saprophytic organisms present.

Although these authors believe that antagonism and competition is a safe way of preventing staphylococcal development, their own results show that under conditions of abuse (incubation at 37°C) staphylococci were able to grow very well in some foods (Peterson et al., 1962a). The same general trend was observed in media: staphylococci could attain large numbers, in the presence of many competitive microbes, when they formed more than 15% of the initial population (Peterson et al., 1962b). A number of factors affecting development of staphylococci growing in competition were studied: pH, temperature and salt, sugar content of foods (Peterson et al., 1964a, b, c). In many of these tests, conditions were found which permitted staphylococci to develop.

Similar results were obtained by Silverman and Cohen (1971). Although neither enterotoxin A nor B was found in any food sample (even when the sole inoculum was S. aureus) these authors nevertheless showed that at temperatures above 20°C all food poisoning organisms were able to develop in the presence of competitive microbes. The only effective restraint to development was low temperature.

The general conclusions one may draw from this work is that (a) the natural flora of non-sterile foods exerts a strong inhibitory effect on staphylococci;
(b) that spoilage is likely to occur before staphylococci can reach dangerous numbers; (c) that this is not a suitable way of ensuring the complete safety of foods. In general, these conclusions are supported by the results of Dack and Lippitz (1962).

Other Foods

Sauerkraut: This subject is reviewed by Pederson (1960). This fermentation resembles that of sausages described above. The important organisms which develop in sequence are probably Leuconostoc mesenteroides, L. brevis and L. plantarum. Under certain conditions i.e. higher than normal temperatures or salt concentrations, two other species, S. faecalis, and P. cerevisiae, may play an important part in the fermentation. The gram-negative bacteria, so numerous on the leaves of fresh cabbage, play little or no part under normal conditions. The mechanisms, whereby lactic acid bacteria eliminate their competitors will be discussed further under the separate groups of bacteria.

Oriental Food Fermentations, Wang et al. (1970) described the antibacterial compounds produced by moulds commonly used in oriental food fermentation. They found that both Mucor and Rhizopus elaborated antibiotics when grown on rice, soyabeans or milk. Gram-positive but not gram-negative bacteria were affected.

Dairy Foods

Associative growth, inhibition and stimulation has been most extensively studied in dairy foods. These studies have been so successful that modern technology now enables almost any dairy food to be made in almost any part of the world. High standards of hygiene and intensive studies of starter microbiology have been two of the main causes for this success. Outbreaks of food poisoning associated with raw milk are rare but there are several reports concerning dried milks and cheese. (Baird-Parker, 1971; Minor and Marth, 1972b).

The number of papers dealing with microbial antagonism in dairy products are so numerous and can be dealt with from such diverse angles, that a separate review is required to cover this subject. Only a bare outline will be presented here.

Stimulation

Microbes may be stimulated by the chemical composition of the milk or by growth products resulting from the multiplication of other microbes. An example of the first is the stimulation of Streptococcus pyogenes by components of colostrum (Auclair and Hirsch, 1953) and examples of the second are formation of a peptide by S. faecalis which stimulates the growth of Roquefort cheese ripening bacteria (Devoyod and Desmazeaud, 1970a, 1970b, 1971), stimulatory peptides in milk cultures (Branen and Keenan, 1969), or the stimulation of lactic starter cultures by Pseudomonas (Claydon and Koburger, 1961).

Starter Dominance

The composition of starters composed of several strains of closely allied lactic acid bacteria, changes with subculturing, and one of the strains becomes dominant. Hoyle and Nichols (1948) were among the earlier workers investigating this phenomenon and their paper is still quoted as the standard reference. Collins (1961) showed that an organism became dominant in a day or two, it was probably due to antibiotics but if dominance was observed later (12 days) it was due to competitive growth. More recently, the problem was again studied by Reddy et al. (1971). Karlikanova et al. (1970) used multiple strain starters, all component strains being antibiotic producers.

Sour Milk and Tuberculosis

Milk soured with a nisin producing strain of S. lactis destroyed tubercle bacilli present in the milk. A culture producing lactic acid only did not have this effect (Mattick and Hirsch, 1946). However, Jacquet et al. (1961) obtained destruction of the tubercle bacilli with a non-antibiotic producing lactic acid bacterium.

Khristonova (1969) used antibiotic production to ferment koumiss and to destroy the tubercle bacillus. This fermented milk product is popular in the U.S.S.R.; yeasts play an important role in the fermentation. By using a selection method Khristonova found a yeast/Lactobacillus starter that gave a koumiss which could be diluted 1/320 and still inhibit Mycobacterium tuberculosis.

Cheese

Hirsch et al. (1951) were the first to use a nisin producing starter culture to preserve Swiss-type cheese from spoilage by clostridia. The same workers later used a similar method to preserve processed cheese (McClintock et al., 1952). However, cheese may contain nisin even when routinely made without a nisin producing starter. The nisin producing strains occur in nature and have been shown to be present in market cheese (Chevalier et al., 1957). This subject was recently reviewed (Hurst, 1972). Not only streptococci but also lactobacilli play an important role in the preservation of Swiss-type cheese (Hirsch et al., 1952). The preservative agent is not an antibiotic, but is likely to be hydrogen peroxide (Wheater et al., 1952).

Grecz et al. (1959) observed the inhibition of Clostridium type A in cheese heads made from aged, surface ripened cheese. The inhibition was attributed to antibiotic(s) made by Brevisbacterium linens (Grecz, 1964). The antibiotic has a remarkably broad spectrum and it kills the yeasts which are associated with it on the cheese. The yeasts can also make an antibiotic and it is not known which of these antibiotics is important in cheese (Grecz, 1964).

Propionic acid bacteria are important to the 'eye' formation of Swiss type cheese. In cheese, they have complex inhibitory/stimulatory relationships with other microbes; and this was recently reviewed by Hettiga & Reinbold (1972).

Principal Groups of Microbes Connected With Antibiotic Production in Foods

Introduction

In this section I shall be reviewing briefly anti-
biotic phenomena under four headings: Spore formers, lactic acid bacteria, staphylococci and micrococci, and gram-negative organisms. Antibiotic phenomena in these groups of microbes were recognized in the 19th century but this review will only cover more recent work. No attempt has been made to make this review comprehensive and citations are confined, as far as possible to those relevant to the microbiology of foods. The early history of antibiotics is reviewed comprehensively by Florey et al. (1945).

Stimulation

Pseudomonas antagonizes the growth of *S. aureus*, yet there are also reports of stimulation (Seminiano and Frazier, 1966; Graves and Frazier, 1963). De Repentigny et al. (1972) found that if the growth of *S. aureus* in mixed culture with *P. aeruginosa*, is antagonized by antimetabolites such as 5-methyl-trytophane, *S. aureus* is not inhibited. The deficiency can be made up by the pseudomonal.

Inhibitors Present in Foods

Some foods have "built-in" inhibitory properties which affect the development of undesirable microbes in these foods. For example the antimicrobial property of garlic oil is well known (see Baird-Parker, 1971; Al-Delainy and Ali, 1970). The lactocins of raw milk are also well known (Auclair and Hirsch, 1935; Patel, 1969). Staphylococcal enterotoxin A was less readily formed in raw milk than in heated milk (Tatini et al., 1971). Lysozyme in milk may also be antibacterial (Shahani, 1970), Busta and Speck (1968) reported on the antimicrobial properties of cocoa, and Dabbah et al. (1970) reported on the antibacterial action of some citrus fruit oils.

Antagonism by Gram-positive Bacteria

Spore Formers

The ecology of spore formers was recently reviewed by Slepecky (1972). They are seldom if ever dominant in foods which have not been heat treated. This is somewhat surprising because bacilli produce many antibiotics (reviewed by Hurst, 1969) although clostridia have not been reported to produce antibiotics.

Lactic Acid Bacteria

Almost all the lactic acid bacteria studied can inhibit other microbes with which they are associated. In consequence they frequently become the dominant microflora of foods (see above). The ecology of the lactic streptococci was recently reviewed by Sandine et al. (1972).

*Streptococcus lactis*. The antibiotic nisin produced by *S. lactis* has been extensively studied and the antibiotic itself has found wide application. It is one of the rare instances in this field of study where basic and applied research has gone hand-in-hand to the great benefit of better application. Nisin was reviewed recently (Hurst, 1972).

*Streptococcus cremoris*. This organism produces an antibiotic called diplococcin. It was partially characterized by Oxford (1944) and it appears that no work has been done on it since. Reddy et al. (1971) observed that in mixed cultures in milk with *S. lactis*, *S. cremoris* became dominant.

Aroma Producing Streptococci. Marsh and Hussong (1963) cultured skim milks with different strains of *L. citrovorum*. Skim milk filtrates inhibited *S. aureus* and gram-negative microbes but the inhibitor was not identified. Iandolo et al. (1965) observed the repression of *S. aureus* by *Streptococcus diacetylactis*. The inhibition was ascribed to competition for nutrients. Nicotinamide became unavailable to the staphylococci, especially at low pH. Pinheiro et al. (1968) isolated the inhibitory principle from cultures of *S. diacetylactis* and *S. citrovorum* effective against *Pseudomonas fragi*. They found the inhibitor to be acetic acid. Reddy et al. (1970) inhibited the development of *Pseudomonas-Achromobacter* in ground beef by inoculating the meat with mixed cultures of *S. lactis* and *S. citrovorum*. Practical applications, using *S. diacetylactis* to preserve foods were recently published by Daly et al. (1972).

Other Streptococci. Cultures of faecal streptococci inhibited *S. aureus* (Oberhoffer and Frazier, 1961). Spot tests on agar were used but results of such tests do not correlate with results in foods and moreover the inhibitor was not identified. Similar results were obtained later by Kao and Frazier (1966). Vanderzant (1968) also used tests on agar to investigate interactive phenomena among microbes originally isolated from dairy foods. Once again, it is not clear whether results obtained by an agar drop technique relate to situations existing in food products. Faecal streptococci were also reported to antagonize other microbes in canned hams. The mechanism of the inhibition was not investigated (Kafel and Ayres, 1969).

*Lactobacilli*

These organisms are capable of causing inhibition by virtue of strong acid production, H2O2, and antibiotics.

An antibiotic "lactolin" was reported by Kodama (1952). "Lactobacillin" reported about the same time was later shown to be probably hydrogen peroxide (Wheater et al., 1952). *Cl. botulinum* grew and formed toxin in pasteurized and sterilized milk but not in raw milk. Benjamin et al. (1956) ascribe this effect chiefly to lactobacilli. Vincent et al. (1959) concentrated an antibacterial factor from cultures of *L. acidophilus*. The properties of this concentrate would make it seem unlikely that it was H2O2 or acidity but there is no further work describing the nature of this substance. Sandine (1963) also reports on an antibiotic-like effect from *L. acidophilus* which Tramer (1966) believes was due to pH. Sandine found that the pH of the ager in which the zones of inhibition was measured, was uniform. Tramer believed that this was true only at the end of incubation. At first there was a drop to pH 3.5 when inhibition of the test-organism occurred followed by equilibration of pH. Vakil and Shahani (1968) also claim the isolation and partial purification of the antibacterial agent from *L. acidophilus* but this remains to be substantiated. Reuter (1971) working with lactobacilli of fermented sausages and well aware of the controversy described above, nevertheless con-
cludes that the inhibitory effect of lactococci cannot be accounted for in terms of acidity and peroxide, alone. Reddy and Shahani (1971) isolated a broad spectrum antibiotic from L. bulgaricus.

Dahya and Speck (1968) and Price and Lee (1970) confirm the strong inhibitory properties of peroxide produced by lactococci on a variety of gram-positive and gram-negative organisms. Inhibitor production paralleled H2O2 formation; the substance was inactivated by catalase.

Although there are many reports of antibiotics being synthesized by lactococci, one cannot conclude that this genus produces antibiotics. Some antibiotic claims have not been substantiated and in almost all cases, there is need for the reported antibiotic to be isolated and characterized. Work with this genus is particularly difficult because the organisms are fastidious. Growth hindrance may be due to non-antibiotic inhibitors, for example organic acids and peroxides. Antibiotics, if they are produced, occur in small yields.

Staphylococci and Micrococci

Jennings and Sharpe (1947) isolated antibiotic producing staphylococci from hospital environment. Soon after, Su (1948) described and characterized a peptide antibiotic, micrococcin, produced by a strain of Micrococcus. As already mentioned above, Niini­ vaara (1955) used a strain of antibiotic producing Micrococcus for starter in fermented sausage manufacture. Gardner (1949) reported and partially purified an antibiotic produced by S. aureus active against a wide range of other microbes. A similar antibiotic, probably a low molecular weight polypeptide, was isolated by Loeb et al. (1950). The antibiotic isolated from staphylococci from the udder and from milk by Jones and Edwards (1966) differs from the above in that “animal Staphylococcus antibiotic” had a narrow spectrum being effective only against other staphylococci and micrococci; it had virtually no effect on Corynebacteria. Yet a fourth kind of antibiotic produced by staphylococci was reported in a series of papers by Parker et al. (1953), Parker and Simmons (1958) and Barrow (1963a, b). These workers obtained their staphylococci from cases of impetigo contagiosa. Of large numbers of strains isolated, nearly one half produced antibiotic against Corynebac­ terium diphtheriae. Two types of cultures were observed: type 1 was numerous, produced sharp zones of inhibition on agar, killed C. diphtheriae but the ability to produce the antibiotic was readily lost on subculture. Type 2 was less numerous. They gave hazy zones of inhibition but this character persisted on culture. The antibiotic, as later reported by Barrow (1963b) appeared to be a polypeptide. An antibiotic made by the closely allied microbe Sarcina, was reported by Trust (1970) but it has not been characterized.

The peptide antibiotics so far described differ in many respects, but mostly they are thermostable and trypsin and pepsin resistant. Barrow’s (1963b) antibiotic was inactivated by trypsin. Luchowicz (1965), on the other hand describes an antibiotic which was thermolabile, trypsin and chymotrypsin sensitive and non-dialyzable.

A thorough study of some of these staphylococcal antibiotics was made by Hsu and Wiseman (1967, 1971). These substances, named epidermidins, were made by 5% of coagulase-positive and 8.5% of coagulase-negative isolates. Epidermidins are effective against a wide range of gram-positive bacteria, other staphylococci being especially sensitive. The antibiotics contain no lipipid, nucleic acid, carbohydrate, halogen or protein. They are dialyzable and resistant to heat and proteolytic enzymes.

It is hoped that this brief survey of some of the recent work on staphylococcal antibiotics will serve to interest applied microbiologists. It appears almost certain that a great variety of antibiotics are made offering challenging biochemical problems. The significance of these antibiotics in the ecology of these microbes is not known and merits further work. Antibiotics may play a part in securing the dominance of staphylococci and micrococci on the skin of man and in some foods.

Antagonism by Gram-negative bacteria

Pseudomonas and Achromobacter

Psychrophyllic members of these groups dominate the flora of refrigerated meat (Ayres, 1960) and they may also compete and inhibit each other (Vanderzant and Custer, 1968), and other gram-negative bacteria (Vanderzant, 1968).

There is considerable evidence that pseudomonads exist which inhibit S. aureus or inhibit toxicogenesis even when growth is not affected (McCoy and Faber, 1966). However, the converse may also be true, and stimulation may also occur. Seminiano and Frazier (1966) isolated a few strains which prevented growth of S. aureus. Earlier, Troller and Frazier (1966) showed that this might be due to competition for amino acids. Malda et al. (1970) reported results with a similar strain isolated from milk. McCoy and Faber (1966) observed that pseudomonads prevented staphylococcal enterotoxin A synthesis without affecting growth. Zyskind et al. (1965) isolated a wall lytic enzyme from a pseudomonad which was able to lyse S. aureus. In view of the many descriptive reports of the inhibition caused by pseudomonads, it is now desirable to isolate and characterize the inhibitor(s) in foods.

Coliforms and Proteus

Inhibition of S. aureus by coliform organisms is well documented (Neufeld and Kuhn, 1935; Wynne, 1947; Bowling and Wynne, 1957; Blackford and Paw, 1951; Wynne and Norman, 1953; Cook and Blackford, 1954; Higglibottom, 1960; Graves and Frazier, 1963). An “antibiotic” made by E. coli against S. aureus was probably glycine or serine (Troller and Frazier, 1963). Inhibition of staphylococci by Proteus, due to a combination of competition for nutrients and antibiotic substances was also reported (DiGiacinto and Frazier, 1966).


