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Culture

Antarctic micro-organisms: coming in from the cold

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Introduction

During the past two decades, there has been a large increase in research on cold-adapted micro-organisms, generally known as psychrophiles, driven by the realisation that they and their enzymes have a great potential for exploitation in biotechnology[1]. As a consequence, many more laboratories are culturing micro-organisms at low temperatures. However, many of the most commonly used growth media were designed originally for the culture of pathogenic micro-organisms that grow best at 37°C. Such media are also generally "rich", *ie,* in comparison with most natural environments they not only contain relatively high concentrations of carbon and nitrogen sources but also generous supplies of other necessary nutrients, usually in a readily assimilable form. Nutrient cornucopia is relatively rare on Earth and many habitats will be deficient in more than one important component of a micro-organism's nutrition. In addition, most of our earthly environment is (from a human perspective) relatively cold, *ie,* it is more or less permanently below 5°C.

Therefore, most micro-organisms are cold-adapted. They have special adaptations to their enzymes, membranes and other cellular components, enabling them to grow at low temperatures at rates comparable to those of mesophiles at moderate temperatures. These molecular changes are discussed in several reviews[2, 3]. This short review instead considers the growth of psychrophiles, and the requirements of isolating them from natural habitats and culturing them in the laboratory. Antarctica is used as the example of a cold habitat, which is not only one of the most extreme on Earth but also has been used both as a source of psychrophiles for biotechnological use and as a model in the search for extraterrestrial life.

Antarctica and cold-adapted micro-organisms

The major cold regions of Earth are the deep oceans (accounting for nearly 70% of the Earth's area), the Arctic and Antarctic polar regions, and high mountain ranges. The continent of Antarctica is a major cold habitat: it is larger than Australia or the sub-continent of Europe, and covers some 14 million square kilometres, of which all but about 1% is covered by ice and snow. Nonetheless, it is far from uniform and has environmental niches, including different soil types, sediments, rocks and meltwaters, as well as snow and ice (*Figure 1*), that vary in the constancy of their thermal regime, nutrient status, water activity and salinity. These factors are important when selecting the appropriate media for both initial isolation and subsequent laboratory culture of Antarctic psychrophiles. It is also reflected in the wide diversity of cold-adapted micro-organisms that include bacteria, archaea, yeast, fungi and microalgae[4]. Together they cover a wide range of nutritional types, including aerobes and anaerobes, heterotrophs and autotrophs, chemolithotrophs and chemoautotrophs, spore-formers and non-spore-formers.

Generally, the more extreme the conditions of an environmental niche the lower is the diversity of organisms that are capable of growing, and the most extreme environments are dominated by micro-organisms. In the sub-Antarctic islands and Antarctic peninsula one finds the grass, *Deschampsia* and abundant mosses growing; as you move to continental Antarctica mosses are to be found generally only in coastal areas and lichens are rarer, whilst in the Dry Valleys where the conditions are particularly harsh mosses are generally absent and it may even be difficult to find lichens. In all of these regions you can find bacteria, yeast, microalgae and fungi growing, with bacteria and yeast dominating the most extreme habitats and micro-niches.

Archaea, such as methanogens, have been isolated and cultured from Antarctic sediments and lakes, and these micro-organisms will require the use of media and anaerobic handling methods appropriate to their phenotype. In what follows, no distinction is made between bacteria and archaea: the terminology "bacteria" is used, since the general considerations of sampling and handling bacteria will apply also to archaea. Furthermore, the emphasis is on culturable microbes, and it should be borne in mind that, as for other environments, those of Antarctica contain many more that are unculturable and have been identified using molecular techniques[5].

What are psychrophiles?

Following nearly two decades of wrangling over the terminology of psychrophiles (named from the Greek for "cold-loving"), the definition given by R. Morita in 1975[6] became widely accepted. He based his definitions of cold-adapted bacteria on their cardinal growth temperatures, *viz.* lower limit, optimum and upper limit. Psychrophiles grow at or below zero (0°C) and have an optimum growth temperature

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Figure 1. The British Antarctic Survey experimental site at Mars Oasis, which illustrates the variety of Antarctic econiches, ranging from sea-ice in the foreground to sediment, soil, a summer melt-pond with seasonal ice cover, and rocks with (unseen) glacier ice and snow beyond. The orange-red, domed structure is a field laboratory.

≤15°C and an upper limit of ≤20°C. In contrast, psychrotolerants (also called psychrotrophs, particularly in the food industry), which can also grow close to zero, have optima and upper limits above these temperatures and may well grow at mesophilic temperatures with optima above 30°C; hence they could be considered as being cold-tolerant mesophiles.

Of course, any such classification is artificial, and individual cold-adapted micro-organisms may not fit the man-made definitions. For example, our experience is that Antarctic soil bacterial isolates may have both their optimum and upper limit between 15 and 20°C, or they may have an optimum that is <15°C but an upper limit of >20°C. The main difference between the two groups is the fact that psychrotolerants have a much broader growth temperature range (30-40 centigrade degrees) than do psychrophiles (~20 centigrade degrees). Psychrotolerants may grow as fast as psychrophiles at low temperatures, so there is an argument for substituting the terms psychrophile and psychrotolerant with stenopsychrophile and eurypsychrophile, respectively. This emphasises the fact that whilst both may grow at low temperatures, the former have a more restricted growth temperature range compared with the latter: the term psychrophiles would then be a more general one including both groups[7].

The values for growth temperature ranges cited above take into account only the positive temperatures over which cold-adapted micro-organisms grow, which ignores the fact that there are bacteria, fungi, yeast and microalgae that grow below 0°C[8]. The lowest recorded bacterial growth temperature that has been authenticated by growth curve data is –12°C for "Psychromonas ingrahamii", which has a doubling time of 4 hours at this temperature[9]. Viable bacteria can be found in some permafrost soils where the temperatures may be as low as –20°C[10], but it has not been established whether they are surviving via maintenance metabolism or actively growing and dividing at these low temperatures. The growth of yeast has been detected at –17°C. Determining sub-zero growth limits experimentally is difficult, and we have a poor understanding of how micro-organisms prevent the freezing of their intracellular water. As extracellular water freezes there will be osmotic loss of intracellular water, which concentrates intracellular salts and small molecules that will lower cellular freezing temperature to perhaps –10°C[8]. Compatible solutes are accumulated, but only some are effective cryoprotectants, and the evidence for microbial antifreeze proteins or other molecules is not well established. Given that freezing is used routinely for long-term storage of cultures, perhaps we should not be surprised if populations of micro-organisms are able to survive freeze-thaw cycles and regenerate their numbers in natural environments.

Isolation of micro-organisms from different cold habitats

The following sections summarise the isolation of psychrophilic micro-organisms from cold habitats, based on experience of working in Antarctica, as well as the cultivation and maintenance of cultures in the laboratory. A more detailed description of handling psychrophiles can be found in Ref 11.

Obviously, a prime requirement for isolating psychrophiles is that all sampling equipment, such as scoops, plastic bags, bottles, ice axes and small corers, etc. used for collecting and transfer, must be kept cold! Good planning is essential to ensure that all necessary field equipment and reagents are packed and sterilised where necessary. It is also a good idea to rehearse sampling protocols in order to minimise the number of transfers, which helps to reduce the chances of contamination.

Psychrotolerant isolates are likely to be more

Figure 2. A field camp site on Utopia Glacier (the tents can just be seen towards the top of the glacier) that provides ample cold storage of samples collected from the experimental site shown in *Figure 1*.

tolerant of thermal abuse (warming) than psychrophiles, but both are sensitive to warming at moderate temperatures, which damages the cellular membrane and causes the loss of essential nutrients and other constituents in a time- and temperature-dependent fashion[12]. In Antarctica it is usually possible to create natural "cryoholes" in ice or permafrost to act as temporary freezers/refrigerators (*Figure 2*). Keeping samples frozen during transportation to the national bases may pose a problem, but once on base there are facilities for refrigerating/freezing materials as well as transporting them to home laboratories by ship or air freight.

Permafrost soils and ice

The techniques for sampling frozen soil (permafrost) or water (ice) are essentially the same. Small samples can be obtained using sterilised ice hammers or picks, but the risk of contamination is less if larger samples are taken, which generally means that coring is done. Cores should be as large as possible (preferably >10 cm diameter), which usually requires the use of motorised augurs. Special drilling equipment and techniques have been developed, which, for example, avoid the use of drilling fluids that invariably introduce microbial contamination. The cores should be kept frozen throughout their sampling, transfer and storage to minimise the risk of contamination, since control experiments using genetically-modified marker bacteria, DNA or dyes have shown that penetration of cores is minimal as long as they remain frozen[13]. A number of protocols have been developed for collecting and handling permafrost soils and ice cores, and for their decontamination[14, 15]. They involve combinations of surface shaving and surface washing/sterilisation using detergents or other biocides, followed by removal of the outer layers before sampling the core centre, which is slowly thawed in isotonic media.

Unfrozen soils

The methodology for sampling unfrozen soils is essentially no different to that for temperate soils, the main concern (apart from keeping everything cool) being to ensure the statistical validity of the sampling regime. A particular problem with Antarctic soils is that they may be very gravelly and heterogeneous, and it may not be possible to take large samples because of transportation restrictions, as well as for reasons of environmental protection and to comply with the Antarctic Treaty[16].

Water and snow

Again, the methodology for sampling cold waters is essentially the same as that for temperate water bodies; it is well established for all types of habitat from small ponds and lakes to surface and deep oceanic collections, and is dealt with in a number of texts[17]. Generally, because of weight restrictions, water samples are filtered through membrane filters (0.45µm for vegetative cells or 0.22µm if spores are to be collected) using autoclavable or disposable plastic single-operation filtration units. Light, portable hand-driven pumps are useful in remote field locations, where generators are unavailable.

Freshly-fallen snow is best collected in sterile bags, in which it can be melted slowly at <5°C prior to filtration as for water samples. Compacted snow can be sampled via a snow pit, for example to collect historical information about microbial deposition.

If platings cannot be done in the field, then filtration should be done as soon as possible to avoid outgrowth of selected isolates and distortion of biodiversity profiles. It is preferable to prepare duplicate sets of filters, one set to be stored cool in 50% (v/v) sterile aqueous glycerol and the other frozen. Such a dual strategy is advisable since psychrophilic yeasts and fungi in particular will grow on the glycerol, but freeze/thawing will lyse some isolates and the glycerol acts as a cryoprotectant. Many Antarctic lakes and even seasonal meltwater ponds are highly saline, so salt should be included as appropriate in liquid storage media.

Recovery media

Strategies for the recovery of representative microbial populations from low-temperature environments have not been well developed, but it is advisable to change as little as possible the growth conditions of the micro-organism(s) compared with the natural growth habitat. The use of non-selective rich media such as nutrient broth for psychrophilic isolations makes it seem unlikely that the micro-organisms isolated are those most likely to be adapted to the often oligotrophic conditions of many Antarctic econiches – *eg,* the Dry Valley soils have some of the lowest organic carbon contents of any soils. The numbers of permafrost bacteria isolated on rich media are lower than those in dilute media, but the diversity is greater. The use of inappropriate media may fail to isolate those isolates actively growing in the psychrophilic niche, particularly if the salinity of the ecotype is ignored. Cold habitats often contain high concentrations of salts and other cryoprotective compounds. This is true of some Antarctic melt ponds, shoreline sediments and permafrost soils. For habitats such as ice and snow, an obvious strategy is to use sterilised *in situ* ice- or snow-melt medium, *ie,* without further

addition of a carbon/energy source.

As a general rule, it is better to use a range of different media (up to 10 is not unrealistic) when making primary isolations and always to include some diluted media, which may be supplemented with low concentrations of yeast extract (0.01-0.001%) to ensure that vitamin and cofactor requirements are met for fastidious organisms.

There are no media that are specific to psychrophilic representatives of the different groups of micro-organisms. Instead, the same specialised media for cyanobacteria, fungi, yeasts *etc.,* are used, with the general caveats given above. Psychrophilic microalgae grow particularly slowly, so special consideration may need to be given to the use of antibiotics as well as high pH media.

Recovery temperature and time

Generally, it is better to incubate plates and liquid cultures for primary isolations of bacteria below 5°C, which yields both psychrophiles and psychrotolerants. For yeasts, fungi and algae temperatures as low as 10°C are probably sufficient. Plates should be incubated for at least 2 weeks – the longer the better, because, although most psychrophilic bacteria form visible colonies within 1 month at 5°C, some grow more slowly. There is also the question of competition between psychrophiles and psychrotolerants, and it may be necessary to incubate plates at temperatures as low as –10°C in order to obtain slow-growing permafrost isolates, which can take up to a year to form visible colonies. Of course, for long-term incubations, the desiccation of solid media must be prevented by use of humid atmospheres.

Many psychrophilic micro-organisms can be maintained on solid media for up to a year at 0–4°C, with sub-culturing every few months, as long as care is taken to avoid freezing or drying out. Desiccation is best avoided by using slants in screw-cap bottles rather than plates for such medium-term storage.

For long-term storage it is necessary to use cryopreservation. There are no specific methods for psychrophiles and the usual cryoprotectants (*eg,* 20% glycerol or 20% dimethylsulphoxide) are suitable. The microbial cell suspensions should be frozen initially at –20°C and then stored at –80°C in multiple small aliquots so that repeated thawing of stored samples is avoided.

Biotechnological applications of psychrophiles

One of the driving forces for isolation of psychrophiles has been their potential for use in low temperature biotechnology[1], either as living organisms (eg, in environmental biosensors) or their isolated enzymes (eg, in washing powders). There are applications in a broad range of industries, including those concerned with food production, mining, waste processing, environmental bioremediations, speciality chemicals, agriculture, and medicine and molecular diagnostics. The food industry illustrates just how diverse are the potential applications. These include the use of cold-active β-galactosidase to lower the lactose content of refrigerated milk for lactose-intolerant persons; a wide variety of cold-tolerant microbes are used as starter cultures and in the cool ripening and flavour development of cheeses and other dairy products; some psychrophilic micro-organisms are an attractive alternative source of polyunsaturated fatty acids, such as docosahexaenoic acid (22:6n3) and arachidonic acid (20:4n6), which are essential for human health and widely used in the food, health and cosmetic industries; and a number of specialised enzymes are being explored for flavour modification or other specific biotransformations.

Antarctica and extraterrestrial microbial life

Antarctica has long been associated with attempts to push the boundaries of man's experience, and most readers will be familiar with the heroic attempts of Shackleton, Amundsen and Scott, successful or not, to reach the South Pole. Those early explorers knew little about microbial life and would not have imagined there to be active bacteria in the South Polar snow[18]. When much later man's attention turned to exploring the moon, the Dry Valleys of Antarctica were used as a lunar paradigm for testing not only unmanned rovers but also experiments aimed at detecting evidence of life in lunar soil.

Twenty-first century space exploration is now focussed on Mars and other even more distant planets, a key question being whether or not there was or still is life. Such life is expected to be microbial, quite possibly sub-surface, and once more experience in Antarctic microbiology is being used as the basis for designing experiments and equipment. For example, one of the harshest environmental niches in Antarctica is that inhabited by endoliths, communities of various combinations of bacteria, yeasts, fungi and microalgae that live within rocks a few millimetres below their surface[4]. Therefore, the focus of Antarctic microbial research includes the development of methods for isolating and culturing "difficult" organisms, the discovery of novel genera and species, and the use of new techniques to obtain a deeper understanding of microbial diversity. All of these avenues of research support attempts to discover extraterrestrial microbial life, which on the planets and their moons currently being explored is probably psychrophilic.

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Culture

Hurdle Technologies: Rational Procedures for Preservation

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Introduction

Microbial growth occurs in substrates that offer sufficient nutrients and accommodating environments. If micro-organisms have multiplied to high numbers they generally cause spoilage of these substrates, and some may be pathogenic or toxigenic. Therefore, substrates used by man (eg, foods, cosmetics, pharmaceuticals, and other perishable materials) should be preserved and free of micro-organisms or contain only harmless organisms, or organisms in low numbers. Many methods have been suggested for preservation, but preferable are those which are effective but not detrimental to the desired qualities of the substrate. Combined preservation methods, called hurdle technologies, are increasingly used for that purpose.

Preservation of Foods

For centuries foods have been preserved by heating, chilling, freezing, drying, salting, conserving, acidification, oxygen-removal, fermenting, adding various preservatives, etc., and often these methods were applied in combinations. Originally many of these procedures were derived empirically. More recently, the underlying principles of the traditional methods have been scientifically defined (ie, as values of F (heating), t (chilling, freezing), pH (acidifying), water activity (aw: drying, salting, conserving), Eh (oxygen-removal, addition of ascorbate, etc), competitive flora (fermenting), suitable additives, and effective limits of these factor for microbial growth, survival, and death were established. Food preservation as well as food quality depend in most cases on the empirical and, now more often, on the deliberate and appropriate application of combined preservative factors, ie, on hurdle technology. The simultaneous effect of different preservative factors may be additive or even synergistic. It also became obvious that potential new and emerging food preservation methods (eg, high hydrostatic pressure, high-intensity pulsed electric fields, high-intensity pulsed light, oscillating magnetic fields, as well as food irradiation) are most effective in combination with additional hurdles. With the current increase in consumers' desires for 'more natural' means for food preservation, herbs and spices have been evaluated because of their antimicrobial activities. Many herb and spice extracts have been shown to act synergistically, so that crude extracts are more efficacious than purified constituents[1]. Such combinations of 'natural hurdles' must have substantial potential, but still need further evaluation in practically meaningful situations. Thus, hurdle technology remains fundamental to the development of food preservation in the future[2].

In previous publications suitable hurdles for the preservation of foods have been discussed. Furthermore, topical applications of hurdle technologies in industrialized as well as developing countries were mentioned and the merits of hurdle technology for a sustainable food preservation were pointed out. Basic aspects of hurdle technology have been discussed, and a procedure for the design of hurdle technology foods was suggested. Details will not be repeated, but in brief the following could be said:

At present the most important hurdles for food preservation are high and low temperature, low water activity, low pH, reduced redox-potential, addition of various preservatives, addition of competitive flora. However, many additional hurdles could be taken into account. The number of potential hurdles for food preservation is great, especially with respect to the many only partially investigated "natural" preservatives which are effective in plants and animals as well as in biofilms. After these potential hurdles have been investigated thoroughly, at least 100 preservative hurdles are potentially available for food design[3,4]. Furthermore, when decontamination of food raw materials can be achieved (eg, by the application of hot water, steam, acids) or ultraclean packaging of foods undertaken (eg, in clean rooms), only a few micro-organisms will be present and thus only a few or lower hurdles will be sufficient to ensure stability of the products.

Foods preserved by hurdle technology are prevalent in industrialized as well as developing countries. In the past and often still today hurdle technology was applied empirically without knowing the governing principles in the preservation of a particular food. But with a better understanding of these principles and improving monitoring devices, the deliberate application of hurdle technology has advanced. Previously many examples of the application of hurdle technology in industrialized countries[3],

Figure 1. Examples of category Combi-SSP, preserved by equal hurdles, suggested for army provisions, as ready-to-eat and ambient-stable meats with fresh-like characteristics.

and developing countries[5] have been described. In this contribution four illustrative examples will be mentioned:

Provisions for the army. At the Federal Centre of Meat Research, Kulmbach (Germany), for the German army ready-to-eat and ambient-stable meat products, which have fresh-like characteristics, were developed in co-operation with the meat industry. German processors named 100 of their traditionally-produced shelf-stable products, and 75 indeed proved to be stable and safe without refrigeration. These meats were scrutinized in our laboratories for their physical, chemical, microbiological and technological characteristics and optimized. On this basis eight categories (product groups) were distinguishable, with a stability and safety based on different principles of hurdle technology. The processing of these products was improved and standardized using the HACCP concept. With permission of the German army our results have been published[6].

Fusion foods of China. A new category of meat products emerged and increased rapidly in China in the 1990s, which might be called fusion foods[7], because they have derived from German minimal-processed, ambient-stable, autoclaved sausages called F-SSP, but have been adopted to suit meat processing in China. The German F-SSP are hurdle products, since they are only mildly heated (to F 0.4 rather than to about 4 that is the heat treatment necessary for commercial sterilisation) in counter-pressure autoclaves, and their water activities are adjusted to a_w below 0.97 or 0.96[8]. In China counter-pressure autoclaves were not available and it was also not yet feasible to adjust the a_w of sausages. The Chinese fusion meats are called *retort sausages* or *ham sausages*, they are emulsion-type products to which 5-10% starch, 3-5% soya protein, 0.3-0.5% carrageen, and 0.3-0.5% polyphosphates are added, and they are heated at retort temperatures of 115-120°C for 20-30 minutes in order to achieve a shelf-life at ambient temperatures for at least 6 months. The quality of retort sausages could be improved if the microbial stability was achieved by more moderate heating combined with water activity reduction. Retort sausages are high volume and low cost foods which amount today to 30-50% of all processed meats consumed in China.

Novel fruit preservation. Fruits are important and abundant food commodities in Latin America. Therefore, researchers there have concentrated on improvements in the preservation of tropical and subtropical fruits, especially as high-moisture products by using combined preservative factors (hurdle technology). Objectives were to develop simple, inexpensive, energy-efficient processes for the local fruit industries, which could overcome seasonal production constraints and reduce post-harvest losses. The main goal was to preserve fruits in fresh-like condition even if stored for several months without refrigeration. Alzamora *et al.* have suggested five hurdles for the preservation of high-moisture-fruit-products (HMFP):

(1) mild heat treatment (to inactivate enzymes and lower the initial microbial load),
(2) slight reduction of a_w (by addition of sucrose or glucose),
(3) pH adjustment if necessary (by addition of citric or phosphoric acids),
(4) addition of preservative I (potassium sorbate or sodium benzoate), and
(5) addition of preservative II (sodium sulfite or bisulfite) in modest amounts.

This process results in stable and safe fresh-like products storable for at least 3-8 months (ie, from one harvest peak to the next) at ambient temperatures and with the use of modest packaging. If these hurdle-preserved fruits are stable they even autosterilize during storage; ie, any surviving micro-organisms, that are unable to multiply, decline in numbers due to 'metabolic exhaustion'. HMFPs are attractive alternatives to conventional fruit preservation[9].

Sustainable food preservation. It is gratifying to know that intelligent hurdle technology is equally applicable in industrialized and developing countries for the preservation of traditional and novel foods. Deliberate and intelligent application of hurdle technology allows a gentle but effective preservation of foods with respect to microbial stability and sensory quality and is advancing world-wide. *Hurdle technology* is a contribution to the global sustainable development. Therefore, a chapter on hurdle technology has been included in "The Encyclopedia of Life Support Systems (EOLSS)", that outlines all sustainable needs required for continuance of life on the globe. The application of hurdle technology for food preservation fits into the concept of sustainable world development, because

(1) it is not demanding on resources (reduced use of chilling and freezing saves energy;

Table 1. Example of rational multitarget hurdle technology preservation of a food

Hurdle	Effect
Reduce pH value	Force cells to expend energy to eject protons that leak into their cytoplasm
Add weak lipophilic organic acid (eg, sorbate, benzoate)	Accelerate leakage of protons into cytoplasm so that even more energy must be expended for their expulsion
Reduce water activity	Force cells to osmoregulate, accumulating 'compatible solutes' so as to avoid dehydration and loss of membrane turgor
Remove oxygen: vacuum or modified atmosphere pack; employ oxygen scavengers	Decrease energy (ATP) available for maintenance of cytoplasmic pH through the expulsion of protons, and for osmoregulation; inhibit strict aerobes
Reduce temperature	Limit flora capable of growth; enhance efficacy of preservatives
Consider other product-compatible adjuncts and treatments	
Raise level of carbon dioxide or other permitted preservatives	Gain additional inhibitory effects
Consider potential for use of naturally-occurring antimicobials	Gain additional inhibitory effects
Apply mild heat	Reduce microbial load; injure cells, further reducing ability to overcome stresses
Consider 'new and emerging' technologies	Inactivate micro-organisms using high hydrostatic pressure, pulsed high voltage electric fields
Consider modifying microstructure	Gain stability through emulsification and consequent compartmentalisation; modify structure to raise viscosity

simple equipment for food preservation limits energy consumption too; less packaging of foods reduces waste, etc),

(2) it could save precious food and thus would have social benefits (diminishing post-harvest spoilage; accommodating seasonal surpluses, eg, of tropical fruits),

(3) it could provide healthy foods (with balanced emphasis on microbial safety and stability on the one hand and sensory and nutritional quality of the food on the other; reduced use of chemical preservatives), and

(4) it helps to bridge the gap between the rich and the poor world (since hurdle technology is equally applicable in the industrialized and the developing countries it fosters North-South co-operation, and hopefully South-South co-operation as well)[10].

Basic aspects of Hurdle Technology

Food preservation implies exposing micro-organisms to a hostile environment in order to inhibit their growth, shorten their survival, or cause their death. The feasible response of the micro-organisms to such a hostile environment determine whether they grow or die. More basic research is needed in this area, because a better understanding of the physiological basis for growth, survival, and death of micro-organisms in hurdle foods could open new dimensions for food preservation[11]. Furthermore, such an understanding would be the scientific basis for an efficient application of hurdle technology in the preservation of foods. Advances have been made by considering the homeostasis, metabolic exhaustion, and stress reactions of micro-organisms, as well as by introducing the innovative concept of multitarget preservation for the gentle yet effective preservation of foods[11,12]. *Table 1* gives an example of such multitarget preservation as it might be developed for a food or other perishable material. Alongside the hurdles listed are indicated the rational bases for their efficacy. Together they act to apply stresses to microbial cells, then to overcome the cells' diverse reactions to those stresses[13].

Design of Hurdle Foods

Hurdle technology has proved useful in the development of novel products as well as in the optimization of traditional foods. In the development of hurdle foods, the concepts of hurdle technology, HACCP (Hazard Analysis and Critical Control Point) or quantitative GMP (Good Manufacturing Practice), as well as predictive microbiology (modeling of microbial growth) should be combined. A 10-step procedure for product design encompassing these concepts has been suggested and proved suitable when solving real product development tasks in the food industry[2].

Figure 2. Fusion meat products of China, based on German F-SSP and adopted to suit meat processing in China. These "retort sausages" are now high volume and low cost foods in China.

Preservation of Cosmetics and Pharmaceuticals

Until now the application of the concept of hurdle technology has been focused on the preservation of foods. However, it could be extended to the preservation of other perishable materials. Indeed, preservation of some aqueous cosmetics and pharmaceuticals already traditionally rely on hurdle technology. However, although there are many similarities between the preservation of foods, cosmetics, and pharmaceuticals there are also important differences which must be taken into account. These relate to the preservative systems in general use for the different substrates and the micro-organisms of significance[14]. For instance, for the preservation systems used in cosmetics, besides the hurdles pH, a_w, and antioxidants, which are important for foods too, also surfactants, fatty acids and esters, biomimetic phospholipids, aroma chemicals, and chelating agents are prominent. Whereas for foods, with a few exceptions (eg, Chinese *pi dan* eggs), only low pH is used for preservation, for some cosmetics (eg, shampoo) a high pH is employed for preservation. Multifunctional hurdles, which improve the quality as well as the preservation of cosmetics are desirable. For instance, the fatty acid ester monolaurin proved very suitable to improve the quality as well as the preservation of cosmetics[15], and this additive might also be used more frequently for foods. The packaging of cosmetics is even more critical than for foods, because cosmetics are often packaged not in single-use but in multiple-use containers in which the products are recontaminated with micro-organisms and possibly diluted with water by the consumer during use and this challenges the microbial stability of the formulas. For foods, besides the pathogens mainly sporeforming bacteria, are of most concern in preservation, whereas for cosmetics the pseudomonads, especially *Pseudomonas aeruginosa*, deserve particular attention.

A similarity between foods and cosmetics is the trend to "preservative-free" products. This often implies that these products are not sterile but harbour in small numbers living micro-organisms, however, these do not multiply because of the hurdles present. If additional micro-organisms are introduced to cosmetics, by the hands of consumers during repeated use, the microbial stability is at risk. However, in so-called "self-preserving systems" the number of micro-organisms decrease again to low levels. This is similar to some foods such as those mentioned above (eg, hurdle-preserved fruits of Latin America as well as German meats of the F-SSP or a_w-SSP types) which "autosterilize" during unrefrigerated storage.

Interactions of preservatives with each other and with other constituents of pharmaceuticals are particular problems because they may lead to losses of activity. Consequently, many attempts have been made to preserve or enhance activity, including the use of various combinations and potentiators[16]. Most simply, for example, various combinations of parabens (methyl, propyl, benzyl), each near to saturation, are employed with the intention of maintaining

greater total levels in the aqueous phases of multiphasic systems. In addition, incorporation of hydrophilic co-solvents (eg, ethanol, propylene glycol, glycerol) helps to reduce partition of active ingredients out of the aqueous phase[17]. Other practically useful synergistic combinations, summarised by Hiom[18], include parabens with imidazolidinyl ureas, with phenoxyethanol and with acrylic acid homopolymers and copolymers, and chlorochresol with phenoxyethanol. Chelating agents, particularly EDTA, enhance the efficacy of quaternary ammonium, parabens, phenolics, sorbate, and imidazolidinyl ureas.

Many of the effective combinations act by widening the antimicrobial spectrum. For example, use of hexachlorophane together with parabens does this, and allows lowering of hexachlorophane levels to usefully minimise toxicity. Addition of parabens with quaternary ammonium compounds also usefully widens the antimicrobial spectrum, while use of water soluble imidizolidinyl compounds together with lipophilic parabens acts differently, by ensuring satisfactory preservation throughout complex multiphasic products[19]. While the biochemical basis of many of the other such synergies remain obscure, EDTA is known to affect the Gram-negative outer membrane lipopolysaccharide, and so increase permeability to a wide range of active agents, even including such large molecules as lysozyme.

Conclusions

The concept of hurdle technology for mild and effective preservation of a variety of foods has attracted much attention in industrialized as well as in developing countries, and probably will be employed increasingly in the future for food preservation. In a book on *Antimicrobials in Food* (3rd edition), which was published in 2005 by CRC Press, the authors (P.M. Davidson, J.N. Sofos, and A.L. Branen) stated in their preface[20]: "More research is needed on the effectiveness of antimicrobial combinations and antimicrobials in combination with physical methods (eg, hurdle technology) that are effective against different groups of micro-organisms. Combinations could well be the ideal antimicrobials for which everyone is searching"

In the present contribution the application of hurdle technology is also discussed briefly for the preservation of cosmetics and pharmaceuticals because obviously there are similar challenges to achieve effective preservation, and ideally again with only modest amounts of chemical preservatives. If the scope of hurdle technology is broadened to include aqueous cosmetics and pharmaceuticals, also the preservation of foods could benefit, because many of the customary preservatives of these products may also be applicable to foods, especially those preservatives that display multifunctional and synergistic properties.

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