Chapter 10

Cultures and starter manufacture

Bacteria cultures, known as starters, are used in the manufacture of yoghurt, kefir and other cultured milk products as well as in buttermaking and cheesemaking. The starter is added to the product and allowed to grow there under controlled conditions. In the course of the resulting fermentation, the bacteria produce substances which give the cultured product its characteristic properties such as acidity (pH), flavour, aroma and consistency. The drop in pH, which takes place when the bacteria ferment lactose to lactic acid, has a preservative effect on the product, while at the same time the nutritional value and digestibility are improved.

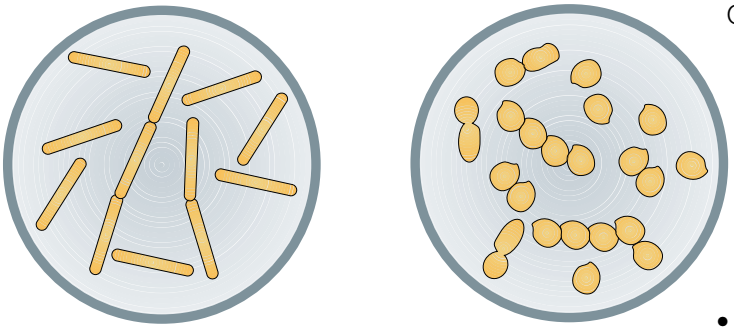

Fig. 10.1 *Bacteria in yoghurt: Lactobacillus bulgaricus, left, and Streptococcus thermophilus.*

Cultured dairy products have different characteristics, and different starter cultures are therefore used in their manufacture. Starter cultures can be classified according to their preferred growth temperatures:

- Mesophilic bacteria - optimal growth temperatures of 20 to 30°C
- Thermophilic bacteria - optimal growth temperatures of 40 to 45°C

The cultures may be of:

- Single-strain type (containing only one strain of bacteria);
- Multiple-strain type (a mixture of several strains, each with its own specific effect).

Mesophilic bacteria cultures can be further divided into O, L , D and LD cultures. Table 10.1, reproduced from the *Bulletin of the IDF* (263/1991), lists the new and old names of various cultures. The old names are used in this chapter.

Table 10.1

New and old names of various starters and their uses

Type	Old Name	New name	Product
Mesophillic			
O	*Streptococcus cremoris*	*Lactococcus lactis ssp. cremoris*	Cheddar cheese
	Streptococcus lactis	*Lactococcus lactis ssp. lactis*	Feta cheese Cottage cheese Quarg
L*	*Streptococcus cremoris*	*Lactococcus lactis ssp. cremoris*	Continental cheese (with eyes)
	Streptococcus lactis	*Lactococcus lactis ssp. lactis*	
	Leuconostoc citrovorum	*Leuconostoc mesenteroides ssp. cremoris*	Lactic butter
	Leuconostoc lactis	*Leuconostoc lactis*	Feta cheese
D**	*Streptococcus cremoris*	*Lactococcus lactis ssp. cremoris*	Lactic butter
	Streptococcus lactis	*Lactococcus lactis ssp. lactis*	
	Streptococcus diacetylactis	*Cit⁺ Lactococci****	
LD	*Streptococcus cremoris*	*Lactococcus lactis ssp. cremoris*	Continental cheese (with eyes)
	Streptococcus lactis	*Lactococcus lactis ssp. lactis*	Mould ripened cheese
	Streptococcus diacetylactis	*Cit⁺ Lactococci****	Cultured buttermilk
	Leuconostoc citrovorum	*Leuconostoc mesenteroides ssp. cremoris*	Lactic butter
	Leuconostoc lactis	*Leuconostoc lactis*	
Thermophillic			
	Streptococcus thermophilus	*Streptococcus salivarius ssp. thermophilus*	Yoghurt
	Lactobacillus bulgaricus	*Lactobacillus delbrueckii ssp. bulgaricus*	Mozzarella cheese
	Streptococcus thermophilus	*Streptococcus salivarius ssp. thermophilus*	Emmental cheese
	Lactobacillus helveticus	*Lactobacillus helveticus*	Grana cheese
	Lactobacillus lactis	*Lactobacillus delbrueckii ssp. lactis*	

* L = Leuconostoc ** D = diacetylactis

*** Cit⁺ = Abbreviation for citrate which is metabolised to flavour and aroma compounds

Some *Str. diacetylactis* bacteria are such powerful acidifiers that they can be used alone as acidifying cultures, but they are used primarily together with *Str. cremoris/lactis*. It is *not* however possible to use a pure *Leuc. citrovorum* culture, because growth of *Leuc. citrovorum* in milk is conditional upon the availability of nutrients produced by *Str. lactis* or *Str. cremoris*. *Leuc. citrovorum* grows very slowly in milk in the absence of acid-producing bacteria, and cannot produce aroma substances in such conditions.

Such bacterial characteristics as optimum growth temperature and salt tolerance are very important in the composition of a culture. The purpose of the component strains is to produce the desired result in *symbiosis*, not to compete with each other. Their characteristics must therefore be complementary in these respects. Table 10.2 lists essential data for some important culture bacteria.

Table 10.2

Characteristics of some important culture bacteria

Bacterium (old name)	Optimum growth temp, °C	Max salt tolerance for growth, %	Acid formation, ferment. %	Citric acid ferment.
I Streptococci				
Str. lactis	about 30	4 – 6.5	0.8 – 1.0	–
Str. cremoris	25 – 30	4	0.8 – 1.0	–
Str. diacetylactis	about 30	4 – 6.5	0.8 – 1.0	+
Str. thermophilus	40 – 45	2	0.8 – 1.0	–
Leuc. citrovorum	20 – 25	–	small	+
II Lactobacilli				
Lb. helveticus	40 – 45	2	2.5 – 3.0	–
Lb. lactis	40 – 45	2	1.5 – 2.0	–
Lb. bulgaricus	40 – 50	2	1.5 – 2.0	–
Lb. acidophilus	35 – 40	–	1.5 – 2.0	–

Dairies normally buy ready-mixed starters – commercial cultures – from special laboratories. These laboratories put much effort into research and development to compose special cultures for a given product, e.g. butter, cheese and a great number of cultured milk products. Thus the dairies can obtain cultures with selected properties for specific product characteristics such as texture, flavour and viscosity.

The dairies can buy the commercial cultures in various forms:

- Liquid, for propagation of mother culture (nowadays fairly rare).
- Deep-frozen, concentrated cultures for propagation of bulk starter.
- Freeze-dried, concentrated cultures in powder form, for propagation of bulk starter.
- Deep-frozen, superconcentrated cultures in readily soluble form, for direct inoculation of the product.

Stages of propagation

In recent years concentrated cultures have generally been used for direct manufacture of a bulk starter, see figure 10.2, as well as for direct use in production. Future handling of cultures will most probably be based on specially designed, concentrated cultures that can be used direct in production without any further propagation at the dairy.

There are however many dairies that still propagate their own bulk starters in successive stages via a mother culture, as shown in figure 10.3; the technique will therefore be described here.

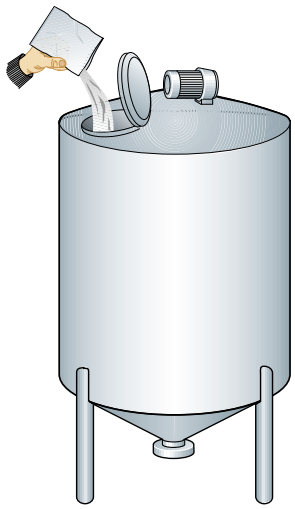

Fig. 10.2 *Bulk starter manufactured from freeze-dried or frozen commercial cultures.*

The process may involve two or more stages. Cultures in various stages of propagation are known by the following names:

- **Commercial culture,** master culture – the original culture that the dairy buys from the laboratory.
- **Mother culture** – the culture prepared from master culture at the dairy. The mother culture is prepared daily and is, as the name indicates, the origin of all cultures made at the dairy.
- **Intermediate culture** – an intermediate step in the manufacture of large volumes of bulk starter.
- **Bulk starter** – the starter used in production.

Process technology

Starter manufacture is one of the most important and also one of the most difficult processes in the dairy. Production failures can result in heavy financial loss, as modern dairies process large quantities of milk.

Very careful attention must therefore be paid to the manufacturing technology and choice of process equipment. Starter production demands the very highest standard of hygiene. The risk of airborne infection by yeasts, mould fungi and bacteriophages must be reduced to an absolute minimum. Mother cultures should be prepared in a separate room supplied with filtered air at a pressure slightly above normal atmospheric pressure. The cleaning system for the equipment must also be carefully designed to prevent detergent and sterilant residues from coming into contact with the cultures and spoiling them.

Manufacture of intermediate culture and bulk starter can take place close to the point of production, or in the same room where the mother culture is prepared. Each transfer of culture to the next stage of manufacture should preferably take place under aseptic conditions.

Stages in the process

The process, presented in figure 10.4, is essentially the same for production of mother culture, intermediate culture and bulk starter. It comprises the following stages:

- heat treatment of the medium
- cooling to inoculation temperature
- inoculation
- incubation
- cooling of the finished culture
- storage of the culture

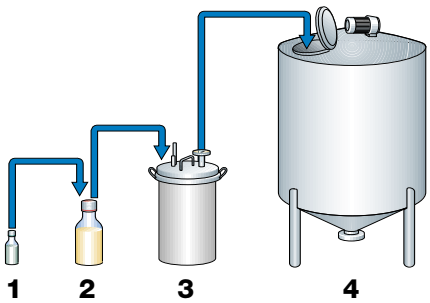

Fig. 10.3 *Steps in the manufacture of starters.*
1 Commercial culture
2 Mother culture
3 Intermediate culture
4 Bulk starter

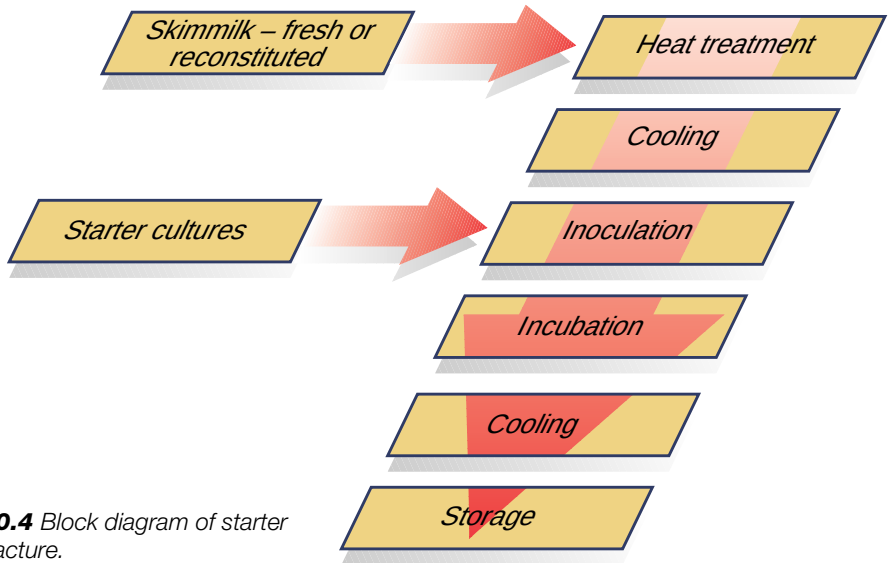

Fig. 10.4 *Block diagram of starter manufacture.*

Skimmilk is the medium most frequently used for starter production, but reconstituted skimmilk with 9 – 12% dry matter (DM), made from top-grade skimmilk powder, is another alternative.

The basic reason for using fresh or reconstituted skimmilk is that anomalies in the flavour of the culture are much more readily apparent. Fresh milk from selected farmers is also used in some dairies.

A medium with constant composition, such as reconstituted antibiotic-free skimmilk, is more reliable than ordinary skimmilk.

The medium can also be modified by addition of growth factors such as Mn^{2+} (Manganese), example: 0.2 mg $MnSO_4$ per l culture, which is supposed to promote growth of *Leuc. citrovorum*. Phage-inhibiting media (PIM) offer an alternative for production of single-strain or multi-strain starters. These media contain phosphates, citrates or other chelating agents which make Ca^{2+} (Calcium) insoluble. The reason for doing this is that most phages require Ca^{2+} for proliferation. Removing Ca^{2+} from the medium protects the lactic acid bacteria from being infected and thus avoids failure of starter activity. Skimmilk powders with PIM are available on certain markets.

Heat treatment of the medium

The first step in starter manufacture is heat treatment of the medium. It is heated to 90 – 95°C and held at that temperature for 30 to 45 minutes. This heat treatment improves the properties of the medium through

- destruction of bacteriophages
- elimination of inhibitory substances
- some decomposition of protein
- expulsion of dissolved oxygen
- destruction of original living micro-organisms

Cooling to inoculation temperature

After heat treatment the medium is cooled to inoculation temperature, which differs according to the type of bacteria culture used. It is important that the temperatures recommended by the producer of the commercial culture, or empirically determined optimum temperatures, are maintained.

In propagation of multi-strain cultures, even small deviations from the proper incubation temperature may favour growth of one strain at the expense of the other(s), resulting in failure to obtain the desired typical characteristics of the end product. Figure 10.6 demonstrates what happens when typical yoghurt bacteria are incubated at a progressive temperature range.

Typical inoculation temperature ranges are 20 – 30°C for mesophilic types of bacteria and 42 – 45°C for thermophilic types.

Inoculation

For inoculation, a determined quantity of bacteria culture is transferred to the heat-treated medium after the temperature has been adjusted to the correct level. To prevent any deviations in the culture it is most important that the starter dosage, the propagation temperature and the time are kept constant throughout all stages — mother culture, intermediate culture and bulk starter.

The amount of starter used can also affect the relative proportions of different bacteria which produce lactic acid and aroma substances. Variations in the amount of starter can consequently cause variations in the product. Each manufacturer must therefore determine which practical conditions suit his particular production process best. Figure 10.5 illustrates how the amount of starter used for inoculation affects the acidifying process in a culture. The curves represent dosages of 0.5% and 2.5% respectively. The inoculation temperature is 21°C in both cases.

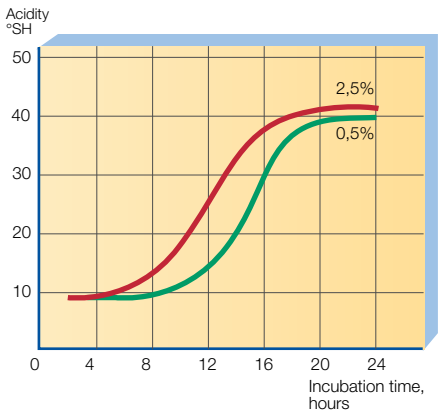

Fig. 10.5 *Acid development curves for inoculation with 0.5% and 2.5% of a mesophilic culture. Incubation temperature 21 °C.*

Incubation

As soon as inoculation has taken place and the starter has been mixed into the medium, the bacteria begin to multiply – incubation begins. The incubation time is determined by the types of bacteria in the culture, the inoculation dosage, etc., and can vary from 3 to 20 hours. It is most important that

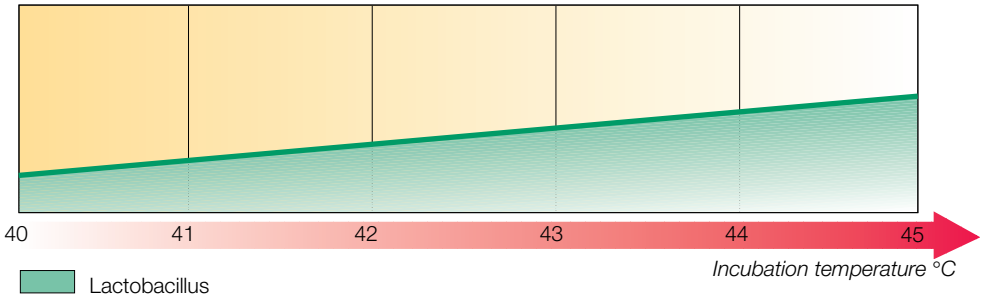

Fig. 10.6 *Effect of incubation temperature on relative counts of cocci and bacilli at constant dosage and incubation time.*

the temperature is carefully controlled and that no contaminants are allowed to come into contact with the culture.

During incubation the bacteria multiply rapidly and ferment lactose to lactic acid. A culture containing aroma-producing bacteria will also produce aroma substances such as diacetyl, acetic and propionic acids, ketones and aldehydes of various kinds, alcohols, esters and fatty acids as well as carbon dioxide.

The importance of a correct incubation temperature is illustrated in the graph in figure 10.6, which refers to a yoghurt culture. The culture contains two strains of bacteria, *Str. thermophilus* and *Lb. bulgaricus*, which coexist in symbiosis and together produce the desired characteristics of the yoghurt, such as pH, flavour, aroma and consistency. Most yoghurt has a ratio of cocci to bacilli between 1:1 and 2:1. The bacilli must never be allowed to gain the upper hand, as the flavour will then be too acid.

An example of growth of *Str. thermophilus* and *Lb.bulgarius* with resulting aroma formation is demonstrated in figure 10.7.

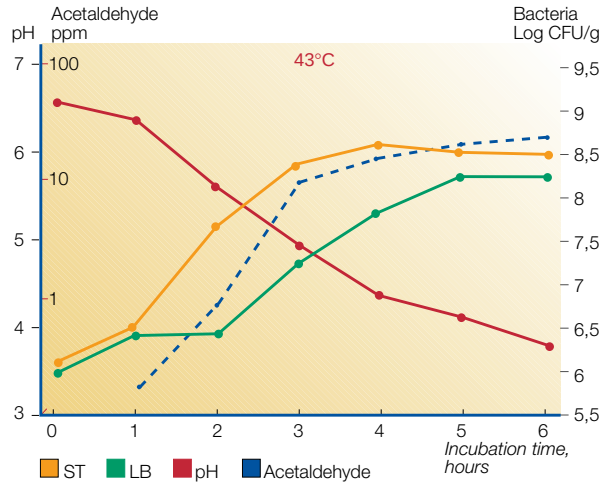

Fig. 10.7 *Growth of* Str. thermophilus *and* Lb. bulgaricus *with resulting aroma development, at 2.5% inoculation.*
The curves are derived from information received from Chr Hansen A/S.

In this context it may be mentioned that acetaldehyde is recognised (Pette and Lolkema, 1950 c; Schultz and Hingst, 1954) as the principal flavour component in the flavour of yoghurt. A principal role in acetaldehyde production is attributed to *Lb. bulgaricus*, although various strains of this species show considerable differences. In the associated growth of *Str. thermophilus* and *Lb. bulgaricus*, the rate of acetaldehyde production is considerably increased compared to the single *Lb. bulgaricus* species (Bottazzi *& al*., 1973). Thus the symbiotic relationships between these species favourably influence production of acetaldehyde in the manufacture of yoghurt. During production of yoghurt, formation of acetaldehyde does not become evident until a certain level of acidification, pH 5.0, has been reached. It attains a maximum at pH 4.2 and stabilises at pH 4.0 (A.Y. Tamime & R.K. Robinson, Yoghurt - science and technology).

The optimum aroma and flavour of yoghurt are usually obtained with an acetaldehyde content ranging between 23.0 and 41 ppm and a pH value of between 4.40 and 4.00.

One of the factors affecting the ratio of cocci to bacilli is the incubation temperature. At 40°C the ratio is about 4:1, while at 45°C it is about 1:2 (see fig. 10.6). The optimum temperature for inoculation (and incubation) in yoghurt manufacture is thus 43°C to achieve a cocci-to-bacilli ratio of 1:1, with a rate of inoculum of 2.5 – 3% and an incubation time of 2.5 – 3 hours.

During the incubation period it is essential that the person responsible for production regularly checks acidity development and otherwise follows the routines found to give optimal results.

Careful handling of all starter cultures is a very important aspect of the processing of cultured milk products; this task should therefore always be given to skilled personnel.

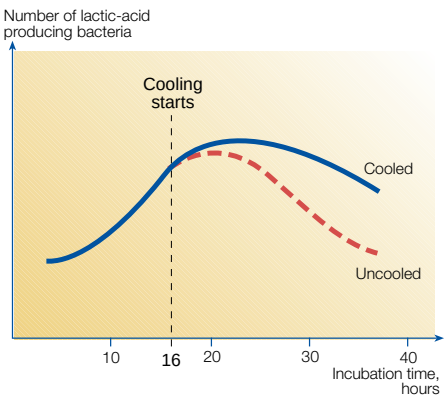

Fig. 10.8 *Growth of lactic-acid producing bacteria with and without cooling at the end of incubation.*

Cooling the culture

Cooling is started at an empirically determined acidity to stop bacterial growth and thus to preserve the activity of the culture at a high level. Figure 10.8 demonstrates the course of events for an ordinary lactic-acid-forming culture inoculated with 1% mother culture at 20°C.

Cooling to 10 – 12°C is often practised when the culture is going to be used within the next 6 hours. If the culture needs to be stored for an extended period, more than 6 hours, it is advisable to cool it to about 5°C.

In large-scale production, or production during more than one shift, it is more convenient to prepare starters at regular intervals of, say, 4 hours. This means that active cultures are available at all times, making it easier to follow the prescribed processing schedule and to assure consistently high quality in the end products.

Preservation of starters

A great deal of research work has been done to find the best way to treat starters in order to preserve their activity during storage. One method is freezing. The lower the temperature, the better the cultures keep. Freezing with liquid nitrogen to –160°C and storage at that temperature preserve cultures very well.

Modern forms of starter cultures – concentrated, deep-frozen or freeze-dried (lyophilised) – can be stored for a considerable time provided that the manufacturers' recommendations are followed.

Table 10.3 shows the recommendations issued by Chr Hansen A/S of Copenhagen, Denmark.

It should be noted that deep-frozen cultures require lower storage temperature than lyophilised cultures. The former, moreover, are supplied in insulated polystyrene boxes packed with dry ice; time in transit should not exceed 12 hours. The latter, on the other hand, can be transported at temperatures up to some 20°C for up to 10 days without shortening the stated shelf life, provided that they are stored at the recommended temperature after arrival at the buyer's premises.

Table 10.3

Storage conditions and shelf lives of some concentrated cultures. (Chr Hansen A/S, Denmark)

Type of culture	Storage	Shelf life, months
1. Freeze-dried DVS	Freezer below –18°C	minimum 12
2. Deep-frozen DVS	Freezer below –45°C	minimum 12
3. Freeze-dried REDI-SET	Freezer below –18°C	minimum 12
4. Deep-frozen REDI-SET	Freezer below –45°C	minimum 12
5. DRI-VAC	Refrigator below +5°C	minimum 12

1. Freeze-dried, superconcentrated culture (for direct inoculation of product)
2. Deep-frozen
3. Freeze-dried, concentrated culture (for preparation of bulk starter)
4. Deep-fozen, concentrated culture (for preparation of bulk starter)
5. Freeze-dried culture in powder form (for preparation of mother culture)

Manufacture of cultures under aseptic conditions

Since the new generation of concentrated, frozen and lyophilised cultures was introduced, there is no longer so much demand for the specific equipment for aseptic production of cultures in dairies. However, this does not mean that the need for hygiene in starter propagation can be neglected. The recommendations given by the suppliers of the new types of starter should be carefully followed to obtain optimal results.

Where traditional starter preparation is still required, the procedure can be accomplished as outlined below.

A typical system for production under aseptic conditions of a bulk starter via an intermediate culture, also produced under aseptic conditions, is illus-

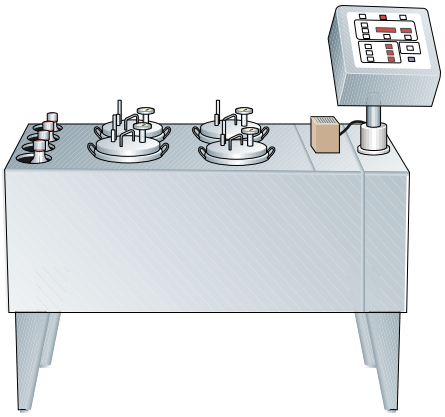

Fig. 10.9 *Incubator with four intermediate containers and four compartments for mother culture flasks. The temperature of the heating water bath is accurately controlled from the panel. (Laboratorium Wiesby, Germany.)*

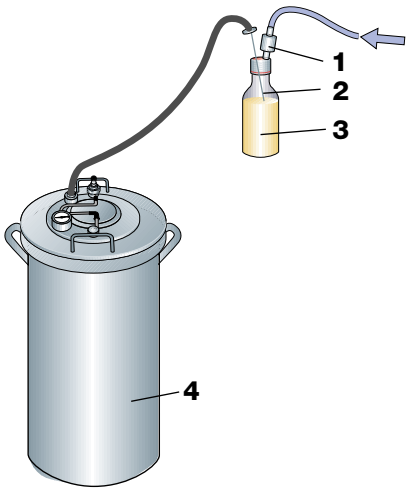

Fig 10.10 *Aseptic transfer of mother culture to intermediate culture container.*
1 Sterile filter
2 Aseptic needle
3 Mother culture flask
4 Intermediate culture container

Fig. 10.11 *Aseptic transfer of intermediate culture to bulk starter tank.*
1. Incubator
2. Intermediate culture container
3. Bulk starter tank
4. HEPA filter
5. Air valve
6. Steam filter
7. pH measurement unit

trated in figures 10.10 and 10.11. The following conditions apply:
1 The mother culture is traditionally prepared in 100 ml bottles provided with a cap containing a membrane.
2 The bottle is filled with skimmilk, autoclaved, and then cooled to the appropriate inoculation temperature.
3 The master culture is injected into the bottle for mother culture with a sterilised syringe inserted through the membrane.
4 Following a suitable period of incubation and subsequent cooling, the mother culture is inoculated into the milk, usually skimmilk, used for the intermediate culture; the milk is first heated to a minimum of 95°C for 30 – 45 minutes and then cooled to adequate incubation temperature. Heating and cooling are done in a specially designed incubator.
5 After an appropriate incubation period and cooling to about 10 – 12°C, the intermediate culture is transferred by displacement with filtered air through a tube to the bulk starter tank.
6 Before being inoculated the milk, often skimmilk, is heated by circulation of heating medium and coolant through the tank jacket. Air supplied to and evacuated from the the tank passes through a sterile HEPA (High Effiency Particle Air) filter.

Bulk starter tanks

The normal practice is to use two tanks in rotation for manufacture of bulk starter. One contains ready-made starter for use in the day's production, while the starter for the following day is being prepared in the other one.

The tanks should be of aseptic design, i.e. hermetically sealed and triple jacketed. They should also be capable of withstanding negative pressures up to 30 kPa (0.3 bar) and positive pressures up to 100 kPa (1 bar). The agitators should be double-sealed and powered by two-speed motors. In addition, they should be fitted with HEPA filters (4) to exclude airborne infection from the air that is drawn in when the tank is cooled after cleaning and when the heated medium is cooled to incubation temperature.

The bulk starter tank can be equipped with a stationary integrated pH meter (7) designed to withstand the great temperature differences that occur during cleaning and heat treatment of the medium.