A QUANTITATIVE STUDY OF BACTERIOPHAGES FOR "CHEESE STARTER BACTERIA": THEIR OCCURRENCE AND DISTRIBUTION IN THE ATMOSPHERE OF CHEESEMAKING ROOMS

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ABSTRACT

The object of this study was to examine quantitatively the phage distribution in the atmosphere of a cheesemaking room so as to obtain an overall picture of: phage build-up in the air during the cheesemaking process, the areas where phage was most abundant, and the rate at which phage disappeared. A detailed knowledge of the typical fluctuations of phage in the atmosphere of a cheesemaking room throughout the day should provide a basis for intelligent mitigation of the problem of phage infection in cheesemaking.

Several methods for estimating phage in the air were employed. As a rough guide, the well-known sedimentation method of exposure to the air of agar plates spread with the host organism was used throughout the work. Experiments showed that regardless of whether the plates were spread with the host organism shortly before exposure, or held overnight in the refrigerator after being spread, little difference was made to the results. Using another sedimentation method, Petri dishes of peptone-salt solution were exposed to the air. The phage particles in the suspensions were estimated by a plating method. The slit sampler method, where air was drawn over the surface of agar plates spread with the host organism, was used only on some occasions. The most reliable method used was that of filtration of air through calcium alginate wool. In this method a measured volume of air was drawn, by means of a vacuum cleaner motor, through several layers of calcium alginate wool held in a glass tube. The phage which collected in the fibres of the calcium alginate wool was estimated by a plating method. It was at this stage, when developing a suitable method, that grave difficulties were encountered, as the hexametaphosphate solution used for dissolving the calcium alginate apparently sequestered some of the calcium needed for phage proliferation, with the result that plaque counts were adversely affected. Calcium was, therefore, supplied by the addition of $CaCl_2$ but, when calcium alginate and sodium hexametaphosphate were present during estimations, some calcium combined with phosphate, and this resulted in the production of plates which were cloudy and difficult or impossible to read. After much experimental work with different quantities of agar media and of $CaCl_2$, and trials with and without phosphate in certain layers, a four-layer method was eventually devised in

which calcium was supplied by diffusion to the phage-organism layer, and this gave satisfactorily high counts and plates which could be easily read. Experiments were also carried out to determine a suitable age for the culture used as host in the estimations.

During the course of the work it was necessary to prepare the phage aerosols needed for experiments in which the efficiency of the method could be checked. Forcing air through a rubber tube into which phage suspension was injected by means of a hypodermic needle proved to be satisfactory for producing the required aerosol. Investigations of the survival of aerosols were also carried out.

Surveys were made at three commercial factories (two mechanized, one non-mechanized) by carrying out sedimentation tests at various places in the cheesemaking room at regular intervals throughout the day. Air filtration counts were also carried out at regular intervals, and the phage content of the whey released during cheesemaking was determined on occasions.

Results showed that the phage content of the cheese factory atmosphere depended on the amount of phage development in the milk and whey and the extent to which the whey droplets were dispersed into the air during the cheesemaking operations. The times for maximum distribution of phage were after cheddaring (milling, salting, etc.). The whey separator dispersed large quantities of phage into the air. The majority of aerosolized phage particles normally sedimented fairly rapidly. The unwrapping of cheese bandages on the morning following cheesemaking resulted in extra phage being distributed into the air.

The agar plate sedimentation method gave an approximate idea of comparative amounts of phage in the atmosphere.

As a result of the various experiments carried out, an overall picture of phage distribution in the air of the cheesemaking room during the cheesemaking process was secured.

PREFACE

The work described in this thesis was undertaken with the object of obtaining a comprehensive view of the quantitative distribution of airborne phage in the making rooms of cheese factories during the manufacturing process. It was not intended to define the level of airborne phage which would be critical to the making process but rather to investigate the variations in levels of airborne phage at different times, in various areas near the cheese vats, and under varied conditions of manufacture.

As the methods for evaluating airborne phage available hitherto were qualitative at best, the development of a more precise method formed an important part of the work.

There already exists some knowledge regarding the quantities of phage in the curd, whey and atmosphere during cheese manufacture. Amplification of this knowledge should be of value in dealing with phage-contamination problems which are ever likely to occur in the cheesemaking industry.

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