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Studies on Continuous Sampling of *Serratia marcescens* Using a Slit Sampler

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Although much information is available on the comparative efficiencies of various samplers for sampling air-borne vegetative cells of microorganisms, a search of the literature failed to reveal many instances where sampling had been carried out for periods of more than 20 min. In certain research organizations, hospitals, and industries, it is advantageous to monitor air systems for much longer periods of time with a minimum of personnel and equipment being employed.

MATERIALS AND METHODS

Utilizing the slit sampler developed by Decker and Wilson (1954), studies have been conducted on several critical factors affecting the efficiency of air sampling when vegetative cells of microorganisms are collected for extended periods of time. In these studies, *Serratia marcescens* was used as the test organism and collection times up to 12 hr were investigated. This sampler is shown in figure 1. The principle of operation is based on the passage of air by means of a vacuum source through the slit opening in the top of the sampler at the rate of 1 cu ft per min. The organisms in the air are impinged on the agar plate, which is rotated by means of a timer mechanism located in the base of the sampler. In these samplers, 1-, 2-, 5.5-, and 12-hr timers were used. For example, the plate in a 2-hr sampler makes one complete revolution in 2 hr, and, likewise, the plate in a 12-hr sampler makes one complete revolution in 12 hr.

The samplers were operated side by side so that direct comparisons of results utilizing the various timers could be made. The organisms were collected on Wilson's peptone agar in the 150 by 20 mm culture plates

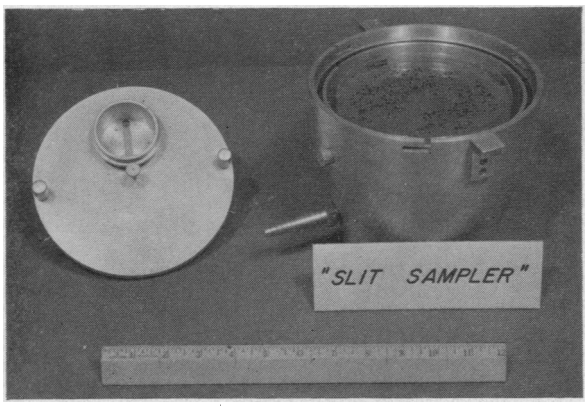

FIG. 1. Slit sampler showing agar plate

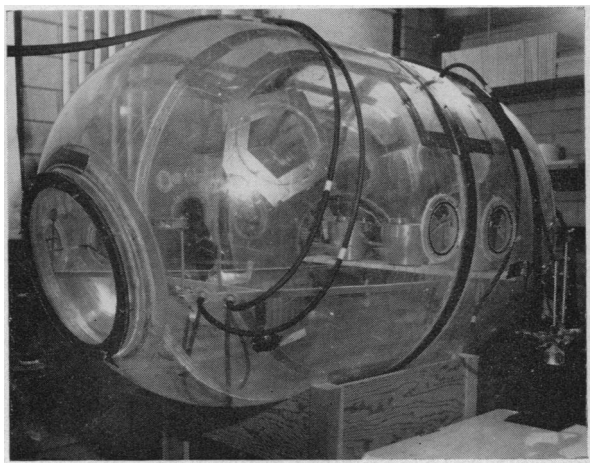

FIG. 2. Aerosol chamber

incorporated in the samplers, and were incubated at 37 C for 24 hr before counting.

The organisms were aerosolized in a 1,500-L plexiglas chamber at a temperature of 20 to 25 C and relative humidity of 40 to 50 per cent. A dynamic aerosol was maintained by atomizing a suspension of *S. marcescens* with a Darby[1] nebulizer. Eighty per cent of the particles produced were less than 2 μ in size. The test chamber is shown in figure 2.

The factors involved in prolonged sampling periods which were investigated in this study were (1) the effect of evaporation of moisture from the agar medium, (2) the effect of varying the concentration of agar in the nutrient medium (3, 1.5, 1.0, 0.8 and 0.5 per cent agar was used), (3) the effect of addition of certain chemical compounds to the medium in an attempt to inhibit spreading of the organisms on the surface, and (4) the actual viability of the organisms on the 1-, 2-, 5.5-, and 12-hr sampler plates.

Results

Preliminary tests in which Wilson's peptone medium containing 3 per cent agar was used (which is the concentration used in Wilson's peptone at Fort Detrick) showed that cracking of the agar sometimes occurs after prolonged sampling, and also that a dry, tough surface results. Consequently, studies were performed to investigate the effects produced when lower concentrations of agar are used in the same medium. When a concentration of 1.5 per cent is used, the medium remains in better physical condition and gives higher recoveries (figure 3). However, a concentration of 1 per cent or less agar results in spreading of the test organism.

Attempts to prevent spreading, by the addition of one one-hundredth per cent and one-tenth per cent of the silicone compounds Dow-Corning Antifoam A[2] and GE 60 antifoam,[3] and 10 per cent glycerol added separately to 1 per cent agar were employed. None of these compounds decrease spreading of the colonies.

[1] Vaponefrin Co., Upper Darby, Pennsylvania.

[2] Dow-Corning Corp., Midland, Michigan.

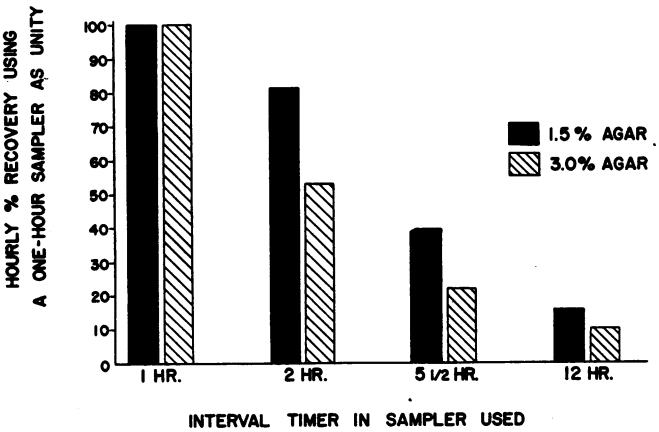

FIG. 3. Recovery of *Serratia marcescens* at end of 1 hr using 1.5 per cent and 3.0 per cent agar for collection with various timers in slit samplers.

The results of 1-hr tests on 1.5 and 3 per cent agar using 1-, 2-, 5.5- and 12-hr clock samplers are shown in figure 3. These are hourly per cent recoveries of the various samplers, using a 1-hr sampler as unity. In these tests, the samplers collected *S. marcescens* for 1 hr. The 2-, 5.5-, and 12-hr samplers then sampled sterile 50 per cent-humidified air for the remainder of one complete revolution. For example, the 12-hr sampler sampled *S. marcescens* for 1 hr and then was aerated for 11 more hr. It can be seen that when a 1-hr sampler is used there is no difference in recovery between 1.5 and 3.0 per cent agar. However, with all other samplers, 1.5 per cent agar permits greater recovery. With a 2-hr sampler, the hourly per cent recovery is 81.4 per cent for 1.5 per cent agar, and 53.0 per cent for 3 per cent agar, based on a 1-hr sampler as unity. Using a 5.5-hr sampler, the hourly recovery is 39.3 per cent for 1.5 per cent agar, and 21.5 per cent for 3 per cent agar. When a 12-hr sampler is used, the hourly recovery is 15.5 per cent for 1.5 per cent agar, and 9.8 per cent for 3 per cent agar. These data show that the hourly per cent recovery decreases as the revolutions per hr decrease. This is probably due to the increase in the total volume of air passing over equal areas of agar.

Experiments further indicate that once organisms are collected on the agar surface continued sampling on the remainder of the plate has little effect on those already collected. This was shown by allowing two 5.5-hr samplers to sample *S. marcescens* for 1 hr, after which one plate was removed and incubated. The second sampler sampled sterile 50 per cent-humidified air for 4 more hr. The resulting 1-hr counts are not appreciably different. This is illustrated in table 1. Another series of tests were made in which the plate in a 5.5-hr sampler sampled sterile air for 4 hr prior to sampling *S. marcescens* for 1 hr. The same per cent recovery is obtained as

[3] General Electric Corp., Silicone Products Dept., Waterford, N. Y.

TABLE 1. *The effect of aeration on sampling of Serratia marcescens in a 5.5-hr slit sampler*

Sampling Time of *Serratia marcescens* Aerosol	Per Cent Agar Used	Avg Per Cent Recovery Compared with a 1-Hr Slit Sampler (1.5% agar)*
1 hr, with no subsequent aeration	1.5	47.9
1 hr, followed by 4 hr aeration	1.5	39.3
1 hr, preceded by 4 hr aeration	1.5	41.7
1 hr, followed by 4 hr aeration	1.5 plus 10% glycerol	38.1

* Each figure represents an average of 8 to 22 tests.

when sampling is followed by 4 hr aeration (table 1). That is, the same recovery is obtained on the fifth hr of sampling as is obtained on the first hr of sampling. These results show that the slit sampler collection efficiency is constant over the entire sampling period.

Incorporation of glycerol to prevent rapid dehydration was also tested. Ten per cent glycerol added to 1.5 per cent agar shows no increase in recovery at 5.5 hr over that using 1.5 per cent agar alone. This is also shown in table 1.

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SUMMARY

The data presented indicate that increasing the volume of air per given area of agar is detrimental to the viability of *Serratia marcescens*. It also shows that these samplers can collect an aerosol of *Serratia marcescens* continuously for periods up to 12 hours on 1.5 per cent agar and still recover a significant number of the organisms at 40 to 50 per cent relative humidity. Once the organisms are impinged on the surface of the agar, continued sampling over the remainder of the plate has little effect on the organisms already collected. Use of the slit sampler affords a time-concentration relationship, permits air sampling for longer periods of time at a good collection efficiency, and requires a minimum of labor, personnel, and equipment to take the samples. The standard bubblers and impingers do not fulfill all of these requirements.

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A Continuous Freeze Drier for Laboratory Studies

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Laboratory and production scale batch-type freeze driers are described in several reviews on the use of freeze-drying for preserving biological materials (Flosdorf, 1949, 1954; Greaves, 1946), but relatively few descriptions of continuous-type freeze driers have been published. A pilot plant continuous freeze drier developed for drying orange juice (Campbell *et al.*, 1945; Sluder *et al.*, 1947) and similar driers developed for production of dried food materials (Anon., 1955a, b) have been described. Naturally, these pilot plant and production driers have been large and not readily adaptable for preparing biological materials in amounts of one ounce to several pounds. A continuous freeze drier was needed for studies to relate quantitatively measured properties of dried preparations with variations in freeze-drying processes and such a drier developed for laboratory studies is described in this paper.

A continuous drier, small enough so that many trials may be performed, and large enough so that sufficient materials may be prepared for adequate measurement of critical properties of the dried preparations, was designed and constructed at Fort Detrick in 1952 for drying biological materials. Studies made since then at Northern Utilization Research and Development Division of Agricultural Research Service and Fort Detrick have shown that the basic design of the apparatus is satisfactory, and the drier may be adapted to many investigations of variables in freeze-drying processes. The construction and typical use of this Fort Detrick Continuous Drier for preparing dried materials containing viable organisms is described in this report. The characteristics of some of the suspensions dried and the dry materials produced will be described elsewhere (Maister *et al.*, 1958a, b).

[1] One of the divisions of the Agricultural Research Service, United States Department of Agriculture.

GENERAL CONSTRUCTION AND OPERATION OF THE DRIER

The Fort Detrick Continuous Drier was designed so that material frozen in the form of pellets[2] (*ca* 1/8-in. diameter) can be fed into the drying chamber and the dry material be removed without interrupting the drying process. The drier was designed to be easily constructed and to incorporate as component units readily

[2] The pellets used with the continuous drier were made by freezing drops of suspension in a bath of liquid Freon cooled by dry ice. A mixture of equal parts Freon 11 and Freon 113 maintained at −50 C was satisfactory. The suspension was fed into the bottom of the Freon bath through a 13-gauge hypodermic needle with a square cut end. The size of the pellets (drops) was controlled by the feed rate.