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Development of a New Sampling Medium for Bioaerosols

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Objective To develop a new sampling medium for detecting of bioaerosols. **Methods** The sampling media were tested by using *Escherichia coli*, *Staphylococcus aureus* and *Serratia marcescens* under static and active conditions, preliminary applications were performed using AGI-10 and high volume sampler. **Results** The average recovery rates were raised to 24.7%, 58.2%, 40.5%, 44.1%, 20.5%, and 15.4%, respectively in six consecutive experiments under static condition for 60 min at room temperature. Four kinds of sampling media were singled out after static experiments, which were referred to as "samplutions" PD1, PX2, TD1, and TX2, respectively. Under the active condition, the protective efficacy of PD1, PX2, TD1, and TX2 was 226% (153/47), 553% (111/17), 150% (120/48), and 268% (419/114), respectively. **Conclusion** The samplutions have some effects on the subsequent nucleic acid detection, which could be avoided by employing standard nucleic acid extraction procedure. The newly developed samplution can be applied to the detection of bioaerosols.

Key words: Sampling medium; Bioaerosols; Airborne microorganisms

INTRODUCTION

The term bioaerosols is used to describe aerosols composed of particles of biological origin[1]. These particles may comprise microorganisms, which are the most important parts of bioaerosols. Airborne microorganism is a potential source of a wide variety of public and industrial health hazards. Bioaerosols are associated with wastewater treatment processes, nosocomial infections, fermentation facilities, biological warfare, and the release of genetically engineered microorganisms to enhance agricultural productivity. Also, the airborne transmission of pathogenic microorganisms and newly recognized pathogens are of growing concern[2].

Effective monitoring of bioaerosols requires the efficient collection of microorganisms from the air[2]. However, it is very important to maintain the integrity of the sample all the way from collection, handling to final analysis. Since microorganisms are sensitive to stress induced by the sampler and the collecting medium, each stage will decrease the viability of microorganisms[3]. By whatever means, a necessary step for analysis involves collecting microorganisms onto certain kinds of media such as agar and collection media, or filters[2,4-6]. Collecting media or sampling media are essential for maintenance and recovery of airborne microorganisms[7]. However, this feature is often neglected by researchers and scientific workers. Most researches have largely focused on the samplers[3,6] and analysis techniques[5,8-9], few studies on sampling media are available so far.

To date, even a standard term is not available when describing the collecting media. The following descriptions of sampling media have been historically used alternatively, for example, adsorbing fluid[10], collecting fluid[3,11-14], collection buffer[2], collection fluid[4,15-21], collection liquid[22], collection media[3,23-27], liquid medium[28], sampler fluid[17,29], sampling liquid[22], sampling media[28,30-31], and scrubbing liquid[32], etc. In the following paragraphs, if not particularly specified, we will use the term "sampling media" for consistency.

The sampling medium, if not a nutrient medium for direct incubation, should ideally be formulated so that the sample is preserved intact with no occurrence of growth and death[3]. In fact, this is perhaps the only impartial and correct description of collecting medium and should be a gold rule when formulating the sampling medium.

From this point of view, we have carried out a series of experiments to develop a new sampling medium named as "samplution" for airborne microorganisms. Orthogonal table was introduced in the

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experiment design, the sampling media were tested by using *Escherichia coli*, *Staphylococcus aureus* and *Serratia marcescens* strains under static and active conditions, preliminary applications were performed using AGI-10 and high volume sampler. The results showed that the newly developed samplution can be applied to the detection of bioaerosols, it has good protective effects on the microbes in the samples, but does not stimulate the growth of microorganisms.

MATERIALS AND METHODS

Materials

Strains of *Escherichia coli*, *Staphylococcus aureus* and *Serratia marcescens* were from Laboratory of Analytical Microbiology, National Center of Biomedical Analysis, China. Most of the chemicals and reagents were purchased from Sigma Company (USA), all the other chemicals were of analytical grade. Bioaerosol generator TK-2, AGI-10 impinger, high volume sampler (500 L/min) and 500 L aerosol chamber were products from Institute of Microbiology and Epidemiology, Beijing.

Protective Effects of Samplution Under Static Condition

Five reagents, A (PBS, pH 7.4 or Tris-HCl, pH 7.4), B (inositol), C (trehalose), D (glycerol) and E (betaine) were chosen as the main ingredients of samplution, each ingredient had 3 levels of concentration (Table 1). An orthogonal design was introduced in evaluation of the protection effects of samplution under static condition. The orthogonal design was based on the orthogonal table $L_{27}(3^{13})$, three concentrations of each ingredient were referred to three levels, five different reagents were defined as five factors[33-34]. Interactions between A and B, A and C, A and E were covered in the design based on the research experiences and reagent properties (Table 2). Orthogonal experiments of *Escherichia coli*, *Staphylococcus aureus* and *Serratia marcescens* were carried out simultaneously. To perform the experiment, 0.5 mL of bacterial suspension was added to a test tube containing each aliquot of samplution solution, the test tubes were then put at room temperature for 60 min. One milliliter of suspension was taken from each test tube and spread onto two agar plates for viable counting. The control sample was 10 mL of autoclaved distilled and deionized water. The results were analyzed by SAS software package.

TABLE 1

Factors and Levels in Orthogonal Matrix

Factors	A	B	C	E	D
Level 1	0.01 mol/L	0.5%	0.5%	5%	0.1 mol/L
Level 2	0.02 mol/L	1%	1%	10%	0.2 mol/L
Level 3	0.05 mol/L	2%	2%	30%	0.5 mol/L

TABLE 2

$L_{27}(3^{13})$ Orthogonal Matrix Used in the Present Study

Test No.	1(A)	2(B)	5(C)	8(E)	11(D)
1	1	1	1	1	1
2	1	1	2	2	2
3	1	1	3	3	3
4	1	2	1	2	3
5	1	2	2	3	1
6	1	2	3	1	2
7	1	3	1	3	2
8	1	3	2	1	3
9	1	3	3	2	1
10	2	1	1	1	1
11	2	1	2	2	2
12	2	1	3	3	3
13	2	2	1	2	3
14	2	2	2	3	1
15	2	2	3	1	2
16	2	3	1	3	2
17	2	3	2	1	3
18	2	3	3	2	1
19	3	1	1	1	1
20	3	1	2	2	2
21	3	1	3	3	3
22	3	2	1	2	3
23	3	2	2	3	1
24	3	2	3	1	2
25	3	3	1	3	2
26	3	3	2	1	3
27	3	3	3	2	1

Note. Each level denotes a value of the parameter, 1 indicating low level and 3 indicating high level.

Test of Samplution Under Active Condition

The preliminary active test of samplution was performed in a 500 L aerosol chamber, suspension of *E. coli* was aerosolized with TK-2 bioaerosol generator.Five minutes after aerosolization, samples were taken using an AGI-10 impinger at a flow rate of 7 L/min for 5 min. Aerosolization was continued throughout the whole process, two samples were taken simultaneously for parallel results, one impinger contained 10 mL of candidate samplution, the other served as a control, containing 10 mL PBS (pH 7.4, 0.03 mol/L). Similarly, 1 mL of suspension was taken from each test tube and spread onto two agar plates for viable counting. Recovery rate was calculated after plate counting[8]. The remaining liquid of each impinger was subjected to nucleic acid amplification[9].

RESULTS

Protection Effects of Samplution Under Static Condition

The recovery rate and protection effect were calculated by the following equations:

$$\text{Recovery rate} = \frac{\text{Colonies of samplution}}{\text{Original colonies}} \times 100\%$$

$$\text{Protection effect} = \frac{\text{Colonies of samplution} - \text{colonies of control}}{\text{Original colonies}} \times 100\%$$

where “colonies of samplution” stands for the viable count of samplution samples 60 min after incubation at room temperature, “colonies of control” stands for the viable count of control samples 60 min after incubation at room temperature, “original colonies” indicates the viable count of control samples without incubation.

The results showed that the average recovery rate was raised to 24.7%, 58.2%, 40.5%, 44.1%, 20.5% and 15.4% respectively in each of the six consecutive experiments under static condition for 60 min at room temperature (Table 3).

TABLE 3

Results of 6 Orthogonal Tests

No.	(P1/P2/T1/T2/Sm/Sa)* (CFU/mL)	Recovery Rate (%)	Protection Effects (%)
1	335/143/157/242/756/243	89.8/77.3/53.2/71.8/99.5/105	68.9/48.1/44.1/17.5/33.2/31.0
2	350/138/175/229/748/205	93.8/74.6/77.6/68.0/98.4/89.1	72.9/45.4/50.2/13.6/32.1/14.5
3	264/108/141/238/648/184	70.8/58.4/47.8/70.6/85.3/79.2	49.9/29.2/38.6/16.3/18.9/4.51
4	266/76 /146/238/666/169	71.3/41.1/49.5/70.6/87.6/73.5	50.4/11.9/40.3/16.3/21.3/-1.1
5	265/167/160/261/771/223	71.0/90.3/54.2/77.4/100 /97.0	50.1/61.1/45.1/23.1/35.1/22.3
6	307/172/163/265/690/233	82.3/93.0/55.3/78.6/90.8/101	61.4/63.8/46.1/24.3/24.5/26.7
7	364/161/162/247/739/207	97.6/86.5/54.9/73.3/97.2/90.0	76.7/57.8/45.8/19.0/30.9/15.4
8	252/108/118/269/693/223	67.6/58.4/40.0/79.8/91.2/97.0	46.6/29.2/30.8/25.5/24.9/22.3
9	336/149/152/277/672/171	90.1/80.5/51.5/82.2/88.4/74.3	69.2/51.4/42.4/27.9/22.1/0.0
10	314/141/127/252/752/277	84.2/76.2/43.1/74.8/98.9/120	63.3/47.0/38.6/20.5/32.6/45.8
11	327/150/165/262/672/218	87.7/81.0/55.9/77.7/88.4/94.8	66.8/51.9/41.7/23.4/22.1/20.1
12	240/98 /97 /236/618/199	64.3/53.0/32.9/70.0/81.3/86.5	43.4/23.8/24.1/15.7/15.0/11.9
13	258/107/117/235/686/199	69.2/57.8/39.7/69.7/90.3/86.5	48.3/28.6/27.1/15.4/23.9/11.9
14	331/146/185/211/683/273	88.7/78.9/62.7/62.6/89.9/119	67.8/49.7/40.3/8.30/23.6/47.1
15	341/147/179/170/645/195	91.4/79.5/60.7/50.4/84.9/84.8	70.5/50.2/40.7/-3.9/18.6/10.2
16	319/157/141/195/739/227	85.5/84.9/47.8/57.9/97.2/98.7	64.6/55.7/44.1/3.60/30.9/24.1
17	204/87 /104/235/676/202	54.7/47.0/35.3/69.7/88.9/87.8	33.8/17.8/20.3/15.4/22.6/13.0
18	342/129/139/340/756/186	91.7/69.7/47.1/100 /99.5/80.9	70.8/40.5/34.6/46.6/33.2/6.25
19	295/133/125/308/745/214	79.1/71.9/42.4/91.4/98.0/93.0	58.2/42.7/35.9/37.1/31.7/18.4
20	346/135/151/289/718/203	92.8/73.0/51.2/85.8/94.5/88.3	71.8/43.8/36.6/31.5/28.2/13.6
21	216/99 / 91/240/569/192	57.9/53.5/30.8/71.2/74.9/83.5	37.0/24.3/24.4/16.9/0.09/8.86
22	223/80 /123/190/670/209	59.8/43.2/41.7/56.4/88.2/90.9	38.9/14.1/18.0/2.00/21.8/16.3
23	292/139/153/285/745/200	78.3/75.1/51.8/84.6/98.0/87.0	57.4/45.9/38.0/30.3/31.7/12.3
24	352/135/143/326/741/184	94.4/73.0/48.5/96.7/97.5/80.0	73.5/43.7/36.6/42.4/31.2/5.38
25	324/151/144/279/679/196	86.9/81.6/48.8/82.8/89.3/85.2	66.0/52.4/42.0/28.5/23.0/10.6
26	194/99 /93 /220/558/196	52.0/53.5/31.5/65.3/73.4/85.2	31.1/24.3/24.4/11.0/0.07/10.6
27	324/141/130/248/662/162	86.9/76.2/44.1/73.6/87.1/70.4	66.0/47.0/38.6/19.3/20.8/-4.1

*Note.**: P1, P2, T1, and T2 represent 4 trials of test results of *E. coli*; Sm represents test result of *S. marcescens*; Sa represents result of *S. aureus*.

Computer analysis of results by SAS software package showed that ingredient D was of statistical significance, there were no interactions between A and B, A and C, or A and E, suggesting that the five ingredients could be pooled together at any level (SAS statistical results were omitted).

Four kinds of samplution, namely PD1 (A1B3-C3D1E3), PX2 (A1B3C3D1E1), TD1 (A1B3C3D2-E3), and TX2 (A1B3C3D2E1), were chosen as candidates based on the static condition test.

Results of Samplution Under Active Condition

The increased protective effect was determined by the formula: [(A-B)/B×100%], where A refers to colonies of samplution, B stands for colonies of control. The protective effects of 4 kinds of samplution were raised to 226% for PD1 [(153-47)/47], 553% for PX2 [(111-17:)/17], 150% for TD1 [(120-48)/48], and 268% for TX2 [(419-114)/114]. The samplutions did have some effects on the subsequent nucleic acid amplification. When the samplution volume was 10% of the total amplification system, there was no significant variation of the PCR results. If the samplution volume increased to 40% of the total amplification volume, the PCR was strongly affected due to the high salt concentrations. Because of the perfect sensitivity of PCR, the impact of samplutions could then be neglected due to the restricted volume of samplution.

DISCUSSION

Although sampling medium is a necessary element in bioaerosol sampling, there is not a special research report about it until now. Sampling media used in previous studies were either distilled water, PBS, gelatin, milk, BSA, or other culture media. Since no standard for sampling media and protocol has been reported, the comparison of data between researches is not available.

For best recovery, one may think that the suitable culture medium is the best choice of sampling media. However, this rule does not work under certain circumstances. The sample should be analyzed immediately after aerosol sampling, but under most circumstances, there is a delay in sample transportation and handling. If a nutrient broth is employed, microorganisms grow rapidly under ideal conditions, such as high temperature, suitable relative humidity, O_2 and CO_2 concentrations, leading to an overestimation of bacterial concentration.

The best way to solve the problem is to develop a new sampling medium, which has a good recovery ability and does not stimulate the growth of microorganisms[3]. Based on this principle, we developed the new sampling mediurn, which has little effect on the growth of microorganisms. In conclusion, samplution has good protection effects and does not increase the growth of microorganisms. Further standardization of it may lead to a sampling medium for bioaerosol sampling, environmental surveillance, indoor air quality control, etc.

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