The Development and Optimization of a DNA Extraction Method for Aerosol Samples Collected using Polyvinylchloride Filter Media

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Background

Inhalation exposure to poultry dust is associated with adverse respiratory outcomes.

Poultry dust contains a diverse microbial community.

The microbial community in poultry dust has yet to be characterized and may present an occupational hazard.

Molecular analysis of DNA is used to characterize microbial species in poultry dust.

Commercial kits are primarily used for DNA extraction of filter based air sampling media.

However, limited data are available on the optimization of DNA extraction methods for filter based air sampling media.

Objectives

Develop and optimize a method for DNA extraction from aerosol samples collected using polyvinylchloride (PVC) sampling media.

Methods

Three DNA extraction methods [*i.e.*, MoBio PowerSoil DNA Isolation kit, Cetyltrimethyl ammonium bromide (CTAB), and sucrose/tris-HCl/EDTA (STE)] were selected to evaluate collected aerosol samples.

Extraction of DNA was completed with samples having a minimum dust mass of 0.1 mg.

Extraction methods were evaluated using a spectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, DE) to observe ultraviolet light absorbance at A260 nm (*i.e.*, DNA) and A280 nm (*i.e.*, protein).

The ratio of absorbance (*i.e.*, A260/280) was used to assess the purity of DNA.

Absorbance at 230 nm is also an indicator of contamination (*e.g.*, phenol).

Ratios between 1.7-2.0 were considered acceptable for downstream analysis.

Both ratios (*i.e.*, A260/280 and A260/230) were reported for samples extracted using all three DNA extraction protocols.

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Results

MoBio PowerSoil DNA Isolation Kit:

Table 1. Dust mass on the filter, absorbance ratios for A260/280 and A260/230, and DNA concentration for selected samples for the MoBio PowerSoil DNA Isolation Kit

Sample No.	Dust (mg)	A260/280	A260/230	DNA conc. (ng/µL)
16	0.530	1.74	0.91	0.39
17	0.330	1.78	0.65	2.30
36	1.072	1.85	0.80	2.34
38	0.479	1.74	0.52	3.70
52	0.532	1.99	1.67	0.49
53	0.516	1.75	1.11	0.82
55	0.270	1.77	1.72	2.00
65	0.363	1.87	1.02	3.20
72	0.337	1.71	0.31	1.70
83	0.194	1.80	0.76	4.20
90	0.249	2.03	1.20	2.30
91	0.402	1.76	0.60	3.60
93	0.146	1.81	0.64	3.10
95	0.179	1.78	0.69	2.60
102	0.078	1.95	0.82	3.90

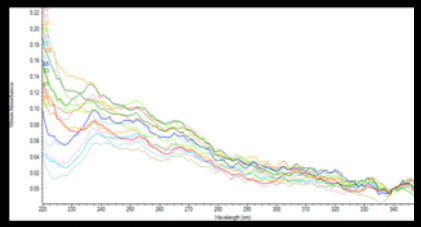

Figure 1. MoBio PowerSoil DNA Isolation Kit absorbance spectrum. Graph represents all samples from Table 1, in reference to absorbance vs. wavelength.

CTAB Extraction Method:

Table 2. Dust mass on the filter, absorbance ratios for A260/280 and A260/230, and DNA concentration for all samples for the CTAB extraction method.

Sample No.	Dust (mg)	A260/280	A260/230	DNA conc. (ng/µL)
108	0.183	1.39	0.68	55.40
110	0.153	1.41	0.64	54.20
183	0.051	1.43	0.49	5.30
184	0.265	1.42	0.66	23.60
256	0.617	1.29	1.20	17.40
258	0.354	1.38	0.76	108.60
263	0.454	1.48	0.86	42.10
272	0.297	1.66	1.52	18.60
278	0.206	1.44	0.90	101.20
281	0.388	1.47	0.77	141.60
318	0.787	1.27	4.43	1.30
319	0.595	1.66	1.47	21.30

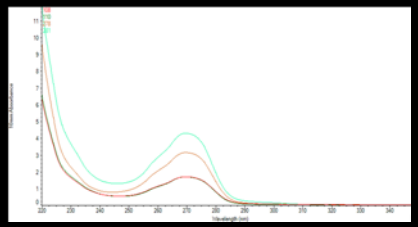

Figure 2. CTAB extraction method absorbance spectrum. Graph represents samples 108, 110, 278, and 281 from Table 2, in reference to absorbance vs. wavelength.

STE Extraction Method:

Table 3. Dust mass on the filter, absorbance ratios for A260/280 and A260/230, and DNA concentration for all samples for the STE extraction method.

Sample No.	Dust (mg)	A260/280	A260/230	DNA conc. (ng/µL)
301	1.248	1.79	1.57	51.00
386	1.048	1.90	2.09	65.30
387	1.177	1.87	2.12	59.20
388	1.025	1.89	2.29	57.80
389	0.773	1.79	1.81	58.70
390	0.746	-2.42	1.76	0.40
392	0.526	1.75	1.94	6.40
393	0.514	1.95	2.91	1.90
395	0.608	1.84	1.63	57.10
396	0.598	1.92	2.06	50.40
400	0.523	1.88	1.88	42.00
401	0.715	1.90	2.03	61.20
402	0.986	1.85	2.31	60.00
403	0.788	1.87	2.08	56.60
404	1.283	1.86	1.99	56.00
405	1.340	1.95	2.09	91.30

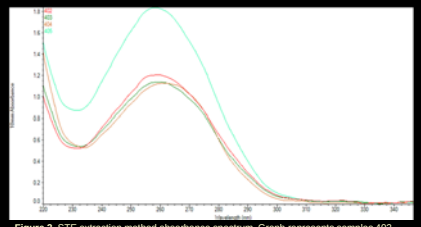

Figure 3. STE extraction method absorbance spectrum. Graph represents samples 402, 403, 404, and 405 from Table 3, in reference to absorbance vs. wavelength.

Results

The MoBio kit (n=64) yielded 24% and 0.02% of the absorbance values in the desired range for A260/280 nm and A260/230 nm, respectively (Table 1).

MoBio Kit spectrum shows no distinct peaks (Figure 1).

The CTAB extraction method (n=12) yielded 0% of the absorbance values in the desired range for both ratios (Table 2).

CTAB method spectrum shows distinct peaks at 270 nm, which suggests the presence of contaminants (Figure 2).

The STE extraction method (n=16) yielded 93% and 62% of the absorbance values for A260/280 nm and A260/230 nm, respectively (Table 3).

STE method spectrum shows distinct peaks at A260 nm, which suggests the presence of nucleic acids (Figure 3).

Discussion

Using the STE extraction method, contamination was decreased and DNA purity was increased among poultry dust samples compared to a commercially available kit and detergent based DNA extraction method.

This observation is important as commercial kits are primarily used to extract DNA in samples for bioaerosol analyses.

Extraction of DNA should not be performed with samples that have a minimum poultry dust concentration less than 0.5 mg.

We recommend using the STE method for DNA extraction from PVC filter media.

Conclusion

A method for bacterial DNA extraction has been developed and optimized for aerosol samples collected using PVC filter media.

Future Research

After extraction, the bacterial DNA solutions will be analyzed using Real-Time Quantitative Polymerase Chain Reaction (qPCR).

Evaluation of DNA extraction method using varying filter media should be performed.

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