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Short communication

Enumerating actinomycetes in compost bioaerosols at source – use of soil compost agar to address plate 'masking'

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Abstract

Actinomycetes are the dominant bacteria isolated from bioaerosols sampled at composting facilities. Here, a novel method for the isolation of actinomycetes is reported, overcoming masking of conventional agar plates, as well as reducing analysis time and costs. Repeatable and reliable actinomycetes growth was best achieved using a soil compost media at an incubation temperature of 44ºC and 7 days' incubation. The results are of particular value to waste management operators and their advisors undertaking regulatory risk assessments that support environmental approvals for compost facilities.

Keywords: actinomycetes, bioaerosols, compost, enumeration, risk assessment

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1 Introduction

Actinomycetes are a heterogeneous group of filamentous bacteria resembling fungi. They are a major component of bioaerosols emitted from composting facilities (Strom, 1985; Lacey, 1997; Niemi *et al.*, 1982; Swan *et al.*, 2003). Mesophilic and thermophilic actinomycetes have growth profiles of 20ºC-50ºC and 30ºC -60ºC, respectively. They grow as branching microorganisms with short chains of spherical spores, 0.7–1.3 µm in diameter. Prolonged inhalation of actinomycetes is linked to adverse human health effects (Douwes *et al*., 2003), including allergic alveolitis and other respiratory responses (Millner, 1982; Lacey and Crook, 1988, Lacey and Dutkiewicz, 1994; Reboux *et al.*, 2001; Herr *et al.*, 2003), in which inflammation of the lung is believed to be caused by glycopeptides and protein allergens released from spores, as is the case for 'farmer's lung disease' (Edwards, 1972).

Actinomycetes concentrations, reported in colony forming units per cubic metre of air (cfu/m^3), are among the micro-organisms that comprise the atmospheric bioaerosol burden emitted from composting facilities (Composting Association, 1999; Taha *et al.*, 2005; 2006). In the UK, a quantitative estimation of bioaerosol load is expected as part of risk assessments for composting facilities within 250m of sensitive receptors, prior to the development or extension of such facilities and to inform permitting of Waste Licences (Environment Agency, 2001). Meaningful risk assessments rely on quality source term data (Pollard *et al*., 2006); particularly at source, so that downwind concentrations may be reliably approximated.

In bioaerosol sampling methods based on the capture of culturable micro-organisms, problems associated with the identification and quantification of micro-organisms include colony 'masking', where colonies overlap (Chang *et al.*, 1994; Chang *et al*., 1995; Chen *et al.*, 1998; Kalogerakis *et al.*, 2005). Masking impedes the visual inspection of the media plate and enumeration of individual species, which may lead to colony counting errors. Lacey (1997) suggests that the method used to collect samples may influence the growth of other bacteria, and that this problem is particularly associated with isolating actinomycetes

from compost samples. Researchers have also commented that selecting the appropriate media for the environment in which the sample is collected may also improve the enumeration results (Balestra and Misaghi, 1997).

Here, we introduce an alternative media, soil compost agar, for the improved enumeration of actinomycetes from bioaerosols sampled close to source at two green waste composting facilities. Source term samples, we have found, are especially prone to masking and yet are most relevant to estimating the bioaerosol flux from compost facilities and so a reliable enumeration method must be established. We believe that the method proposed here is of timely importance due to the current regulatory interest in occupational and public exposures to bioaerosol emissions (Swan *et al*., 2003; Herr *et al*., 2003).

2 Materials and methods

2.1 Bioaerosol and compost sampling

Bioaerosol sampling was performed at two green waste windrow composting facilities, with capacities of 2000 m^3 and 1000 m^3 per annum respectively. Air and compost samples were taken on nine separate occasions between October 2003 and March 2005, covering the winter, spring and autumn seasons. The samples were taken (i) >15 m upwind of the facility boundary; (ii) within the site; and (iii) at 15, 100 and 200 m downwind of the composting facility site boundary. Further details and the background context for our bioaerosols studies at these composting facilities have previously been reported (Taha *et al.*, 2005; 2006; 2007).

Sampling employed medium flow, personal aerosol filter samplers (SKC Universal dust and vapour sampling pump; models SKC Aircheck™ 224-PCXR8 and 224-PCTX8). Pumps operated at 2.0 ± 0.1 l/min and were fitted with SKC dust sampling IOM (Institute of Occupational Medicine) heads connected using 10 mm i.d. Tygon™ tube. IOM heads were loaded with 25 mm diameter, 0.8 µm MCE (mixed cellulose ester, SKC) filters. The IOM samplers were attached to a stand, 1.8m above ground, similar to the method used by

Wheeler *et al*. (2001) and Environment Agency (2007). The sampling time was 30 minutes, reduced to 10 minutes where the expected bioaerosol concentration was high. Two air samples were taken simultaneously in each case using two samplers, with one pump kept as a standby, as recommended by the Composting Association (1999). Both samples were treated identically in further analyses and the collection of simultaneous samples was used to increase the statistical significance of the results. All pumps were calibrated before use.

Compost grab samples (*ca.* 30 g) were also taken from the windrows associated with bioaerosol release. The grab samples were taken from as close to the area where the air samples were taken as possible, usually at a depth of 0-10cm into the compost pile, which had already been shredded and turned so that there was homogenisation of the material. This procedure was adopted so as to sample the area most closely representing the area where the air samples were collected.

2.2 Isolation

Micro-organisms were quantified using the plate count analysis steps of the CAMNEA-method (Collection of airborne microorganisms on nuclepore filters, estimation and analysis; Palmgren *et al*., 1986) with subsequent visual enumeration. Filters and compost grab samples were placed, whilst at the site, into 30 ml vials containing 10 ml 0.05% ^{v}/v Tween-80™ mixed with 0.1% w/v NaCl to prevent osmosis. Samples were transported at < 4°C and, on return to the laboratory, re-suspended by separating the filter from the head and shaking in a vial for 2 min. The solution was diluted to a common logarithm order (10^{-1}, 10^{-2} and 10^{-3}) and inoculated onto both media plates. All equipment was sterilized before use.

2.3 Enumeration

Media preparation, inoculation, dilution and sterilization were performed in accordance with BS 5763: Part 0:1996 (British Standards Institution, 1996). Two media were used: (a) half strength nutrient agar (NA) (Oxoid CM3 nutrient agar); 14 g/l, in ionised water; and (b)

soil compost agar (SCA); a supernatant of 100g loam compost (John Innes™ N^{o}.1) with 1000ml deionised water was vigorously shaken for 2 min. and centrifuged at 500 rpm for 5 min. The SCA agar was prepared using 7g of agar-agar powder with 500ml deionised water, with no need for pH adjustment. After preparation, both media were autoclaved (121ºC for 15 minutes), left to cool to below 47°C and treated with 1% $^{w}/_{v}$ antifungal cycloheximide, dissolved in 2ml of HPLC grade ethanol. In each case, the agar medium (12ml) was poured into petri dishes to a depth of *ca.* 2mm and allowed to cool on a horizontal surface before being inoculated with the sample solution as described above. Colonies grown at an incubation temperature of 44°C on both media were enumerated visually after 7 days, employing the BS 5763 standard (British Standards Institution, 1996).

3 Results and discussion

Actinomycetes were enumerated by estimated count from 76 air samples inoculated on NA plates. However, 60 of these samples showed either full or partial overgrowth of other bacteria ('masking') that obscured actinomycetes growth and it was therefore difficult to count the colonies of actinomycetes (Figure 1). This resulted in these plates being rejected. From the 24 compost samples inoculated on NA plates, all showed 'masking' by overgrowth of other bacteria (Table 1). Serial dilution of the samples was not able to address this masking effect that we attribute to a very high bacterial burden in samples taken very close to source.

In contrast, the SCA plates, incubated at a temperature of 44°C, showed no masking by eubacteria or fungi (Table 1), after allowing 7 days for the actinomycetes to grow and sporulate. In each case, the enumeration of actinomycetes colonies was proportionate to the dilution factor applied in constructing the plates, improving the confidence interval limit of the study. The actinomycetes grown on SCA were easily identified by the white powdery colour of their spores and spider web-like formation appearing on the colonies (Figure 2). Of the 13 samples where no actinomycetes were found on the SCA plates, 1 of the NA plates

showed bacteria growth with no actinomycetes, 7 of the NA plates showed no actinomycetes and 5 NA plates showed masking by other bacteria.

Several media have previously been used for the identification and enumeration of actinomycetes (Table 2). The Composting Association (1999) suggests the use of half-strength nutrient agar. Most significantly, Crawford *et al*. (1993), during their experiments on plant roots in British soils, secured optimum enumeration using a low nutrient organic media and compost extract agar and using this, the overgrowth of media by eubacteria and fungi was dramatically reduced. Curtis and Beuchat (1998) argue that a common problem related to quality control of culture media is the non-inhibition and growth of unwanted micro-organisms on selective media. High colony counts of unwanted micro-organisms may lead to significant colony overlap on the growth media plate, 'masking' the growth of the species of interest.

The enumeration methodology described here offers substantive methodological improvements over existing approaches (Composting Association, 1999) and allows a reliable estimation of the actinomycetes load, especially where concentrations are expected to be high, such as at source. This method has proved useful in achieving consistent results. In addition, it has proven to be cost effective and simple, as well as reducing analysis time. This method is also inline with the findings of Griffiths and Stewart (1999), who suggest the tailoring of micro-organism analysis for the application of interest, as it focuses on composting. This is because no one single assay is suitable for the study of micro-organisms.

The results presented here are encouraging for the further development of this method. Future work will include the analysis of the consistency of the extracted compost supernatant, as well as testing batch to batch variation and the use of different types of compost.

4 Conclusions

A novel method for the enumeration of compost-derived actinomycetes close to source and in bioaerosol samples has been supported by evidence from over 70 air and compost

samples taken over 9 separate sampling days. The method is suited to the enumeration of actinomycetes sampled from green waste compost facilities and significantly reduces the masking of actinomycetes by other bacteria. It provides substantive improvements over the nutrient agar media (*e.g.* Nielsen *et al*., 1997; Würtz and Breum, 1997; and Breum *et al.*, 1997) for source term samples. The method is cost effective, reduces analysis time and produces replicable results. Its application is particularly significant for practitioners enumerating bioaerosols in support of regulatory risk assessments for the waste management sector.

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5 References

Andreeva, I.S., Belan, B.D., Borodulin, A.I., Buryak, G.A., Zhukov, V.A., Panchenko, M.V., Penenko, V.V., Petrishchenko, V.A. and Safatov, S.A., 2001. Variability of the content of live microorganisms in the atmospheric aerosol in southern regions of western Siberia. Doklady Biological Sciences, 318, 1-6, 530-534.

Asan, A., Ilhan, S., Sen, B., Erkara, I.P., Filik, C., Cabuk, A., Demirel, R., Ture, M., Okten, S.S. and Tokur, S., 2004. Airborne fungi and actinomycetes concentrations in the air of Eskisehir city (Turkey). Indoor and Built Environment, 13, 63-74.

Balestra, G.M., and Misaghi, I.J., 1997. Increasing the efficiency of the plate counting method for estimating bacterial diversity. Journal of Microbiological Methods, 30, 111-117.

Breum, N.O., Nielsen, B.H., Nielsen, E.M., Midtgaard, U. and Poulsen, O.M., 1997. Dustiness of compostable waste: a methodological approach to quantify the potential of waste to generate airborne micro-organisms and endotoxin. Waste Management & Research, 15, 169-187.

British Standards Institution, 1996. Methods for microbiological examination of food and animal feeding stuffs — Part 0: General laboratory practices, British Standard BS 5763-0:1996, British Standards Instiution, London, 41 pp.

Chang, C.W., Hwang, Y.H., Grinshpun, S.A., Macher, J.A. and Willeke, K., 1994. Evaluation of counting error due to colony masking in bioaerosol sampling. Applied and Environmental. Microbiology, 60, 10, 3732 – 3738.

Chang, C.W., Grinshpun, S.A., Willeke, K., Macher, J.A., Donnelly, J., Clark, S. and Juozaitis, A., 1995. Factors affecting microbiological colony count accuracy for bioaerosol sampling and analysis. American Industrial Hygiene Association Journal, 56, 10, 979-986.

Chen, C.C., Yu, T.S., Chang, J.Y., Chang, C.W., Shih, T.S., Hwang, J.S., 1998. A computer simulation study on bioaerosol colony counting error due to masking effect. Annals of Occupational Hygiene, 41, 7, 501-510.

Composting Association, 1999. Standardised protocol for the sampling and enumeration of airborne micro-organisms at composting facilities. ISBN 0 9532546 2 3, 30 pages.

Crawford, D.L., Lynch, J.M., Whipps, J.M. and Ousley, M.A., 1993. Isolation and characterization of fungal actinomycete antagonists of a fungal root pathogen. Applied and Environmental Microbiology, 59, 11, 3899-3905

Curtis, G.D.W., Beuchat, L.R., 1998. Quality control of culture media – perspectives and problems. International Journal of Food Microbiology, 45, 3-6.

Douwes, J., Thorne, P., Pearce, N. and Heederik, D., 2003. Bioaerosol health effects and exposure assessment: progress and prospects. Annals of Occupational Hygiene 47, 3, 187-200.

Dutkiewicz, J., Olenchock, S.A., Krysińska-Traczyk, E., Skórska, C., Sitkowska, J. and Prażmo, Z., 2001. Exposure to airborne microorganisms in fibreboard and chipboard factories. Annals of Agricultural and Environmental Medicine, 8, 191-199.

Edwards, J.H., 1972, The isolation of antigens associated with farmer's lung. Clinical and Experimental Immunology, 11, 341-355

Environment Agency, 2001. Agency position on composting and health effects, Bristol. Available online at http://www.environment-agency.gov.uk

Environment Agency, 2007. Bioaerosols in waste composting: deriving source terms and characterising profiles. Science Report, Environment Agency, Bristol, in press.

Folmsbee, M.and Strevett, K.A., 1999. Bioaerosol concentration at an outdoor composting center. Journal of Air and Waste Management Association, 49, 554-561.

Griffiths, W.D. and Stewart, I.W., 1999. Performance of bioaerosol samplers used by the UK biotechnology industry. Journal of Aerosol Science, 30, 8, 1029-1040.

Herr, C.E.W., zur Nieden, A., Jankofsky, M., Stilianakis, N.I., Boedeker, R.-H. and Eikmann T.F., 2003. Effects of bioaerosol polluted outdoor air on airways of residents: a cross sectional study. Occup Environ Med, 60, 336-342.

Kalogerakis, N., Paschali, D., Lekaditis, V., Pantidou, A., Eleftheriadis, K. And Lazaridis, M., 2005. Indoor air quality – bioaerosol measurements in domestic and office premises. Aerosol Science, 36, 751-761.

Lacey, J. and Dutkiewicz, J., 1976. Methods for examining the microflora of mouldy hay. Journal of Applied Bacteriology, 41, 13-27.

Lacey, J. and Crook, B., 1988. Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. Annals of Occupational Hygiene, 32, 515-533.

Lacey, J. and Dutkiewicz, J., 1994. Bioaerosols and occupational lung disease. Journal of Aerosol Science, 25, 1371-1404.

Lacey, J., 1997. Actinomycetes in Compost. Annals of Agricultural and Environmental Medicine, 4, 113-121.

Lavoie, J. and Alie, R., 1997. Determining the characteristics to be considered from a worker health and safety standpoint in household waste sorting and composting plants. Annals of Agricultural and Environmental Medicine, 4, 123-128.

Millner, P.D., 1982. Thermophilic and thermotolerance actinomycetes in sewage-sludge compost. Developments in Industrial Microbiology, 23, 61-80.

Nielsen, B.H., Wuertz, H. Breum, N.O. and Poulsen, O.M., 1997. Microorganisms and endotoxin in experimentally generated bioaerosols from composting household waste, Annals of Agricultural and Environmental Medicine, 4, 1, 159-168.

Niemi, R.M., Knuth, S. and Lundström, K., 1982. Actinomycetes and Fungi in Surface Waters and in Potable Water. Applied and Environmental Microbiology, 43, 2, 378-388.

Palmgren, U., Strom, G., Blomquist, G. and Malmberg, P., 1986. Collection of airborne micro-organism on nuclepore filters estimation and analysis – CAMNEA method. Journal of Applied Bacteriology, 61, 401-406.

Pollard, S.J.T., Smith, R., Longhurst, P.J., Eduljee, G. and Hall, D., 2006. Recent developments in the application of risk analysis to waste technologies. Environment International, in press.

Reboux, G., Piarroux, R., Mauny, F., Madroszyk, A., Millon, L., Bardonnet, K. and Dalphin, J.C., 2001. Role of molds in farmer's lung disease in Eastern France. American Journal of Respiratory and Critical Care Medicine, 163, 17, 1534-1539.

Recer, G.M., Browne, M.L., Horn, E.G., Hill, K.M. and Boehler, W.F., 2001.Ambient air levels of aspergillus fumigatus and thermophilic actinomycetes in a residential neighborhood near a yard-waste composting facility. Aerobiologia, 17, 99-108.

Strom, P.F., 1985. Identification of thermophilic bacteria in solid-waste composting. Applied and Environmental Microbiology, 50, 4, 906-913.

Swan, J.R.M., Kelsey, A., Crook, B. and Gilbert, E.J., 2003. Bioaerosol components and hazard to human health, *in* Occupational and environmental exposure to bioaerosols from composts and potential health effects-a critical review of published data, research report 130, HSE Books, pp. 15-20.

Taha, M.P.M., Pollard, S.J.T., Sarkar, U. and Longhurst, P.J, 2005. Estimating fugitive bioaerosol releases from static compost windrows: feasibility of a portable wind tunnel approach. Waste Management, 25, 445-450.

Taha, M.P.M, Drew, G.H., Longhurst, P.J., Smith, R. and Pollard, S.J.T., 2006. Bioaerosol releases from compost facilities: Evaluating passive and active source terms at a green waste facility for improved risk assessments. Atmospheric Environment, 40, 1159-1169.

Taha, M.P.M, Drew, G.H., Tamer, A., Hewings, G., Jordinson, G., Longhurst, P.J., and Pollard, S.J.T., 2007. Improving bioaerosol exposure assessments of composting facilities – comparative modelling of emissions from different compost ages and processing activities. *Atmospheric Environment,* in press.

Wheeler, P.A., Stewart, I., Dumitrean, P. and Donovan, B., 2001. Health effects of composting: a study of three compost sites and review of past data. R & D Technical Report P1-315/TR, Environmental Agency, Bristol, UK (2001).

Würtz, H. and Breum, N.O., 1997. Exposure to microorganisms during manual sorting of recyclable paper of different quality. Annals of Agricultural and Environmental Medicine, 4, 129-135.

Figure captions

Fig 1. Actinomycetes growth using half strength nutrient media (NA). Plates show clear evidence of masking effect, as individual colonies of actinomycetes are difficult to distinguish.

Fig 2. Actinomycetes growth using soil compost agar (SCA). Plates show clear evidence of improved visual inspection using soil compost agar, as individual colonies of actinomycetes are clearly visible.

Table captions

Table 1: Actinomycetes growth from compost and air samples for the soil compost agar (SCA) and the half strength nutrient agar (NA) (Samples were taken on 9 separate occasions between 26/10/2003 and 10/03/2005).

Table 2: Media used for the identification and enumeration of actinomycetes.

Table 1: Enumeration outcome for air and compost samples with actinomycetes growth using soil compost agar (SCA) and half strength nutrient agar (NA) (samples taken on 9 occasions between 26/10/2003 and 10/03/2005).

enumeration outcome	air samples				compost samples			
	SCA		NA		SCA		NA	
	Samples[a]	%[b]	Samples[a]	%[b]	Samples[a]	%[b]	Samples[a]	%[b]
actinomycetes enumeration achieved	28	37	0	0	23	96	0	0
actinomycetes enumeration achieved by estimated count	33	44	6	8	1	4	0	0
full masking	0	0	48	63	0	0	22	92
partial masking	0	0	12	16	0	0	2	8
no actinomycetes	13	17	8	11	0	0	0	0
bacteria, no actinomycetes	1	1	2	3	0	0	0	0
total samples	75	100	76	100	24	100	24	100

[a]The number of samples that achieved each enumeration outcome

[b]The percentage of samples for each media that achieved each enumeration outcome

Table 2: Media used for the identification and enumeration of actinomycetes

medium	reference
nutrient Oxoid agar	Nielsen *et al*. (1997), Würtz and Breum (1997) and Breum *et al.* (1997)
half strength nutrient agar	Composting Association (1999)
10% ^{w}/v nutrient agar	Nielsen *et al*. (1997), Würtz and Breum (1997) and Breum *et al.* (1997)
starch-casein (CS) agar	Folmsbee and Strevett (1999)
Czapek's solution agar (CZ)	Asan *et al*. (2004)
starch-ammonia medium	Andreeva *et al.* (2001)
half-strength (50%^{w}/v) nutrient	Lacey and Dutkiewicz (1976); Recer *et al*. (2001)
tryptone soya with casein hydrolysate agar	Lacey and Dutkiewicz (1976); Recer *et al*. (2001)
half-strength tryptic soya agar	Dutkiewicz *et al.*, (2001)
trypticase soya agar	Lavoie and Alie (1997)
low nutrient organic media and compost extract agar	Crawford *et al*. (1993)

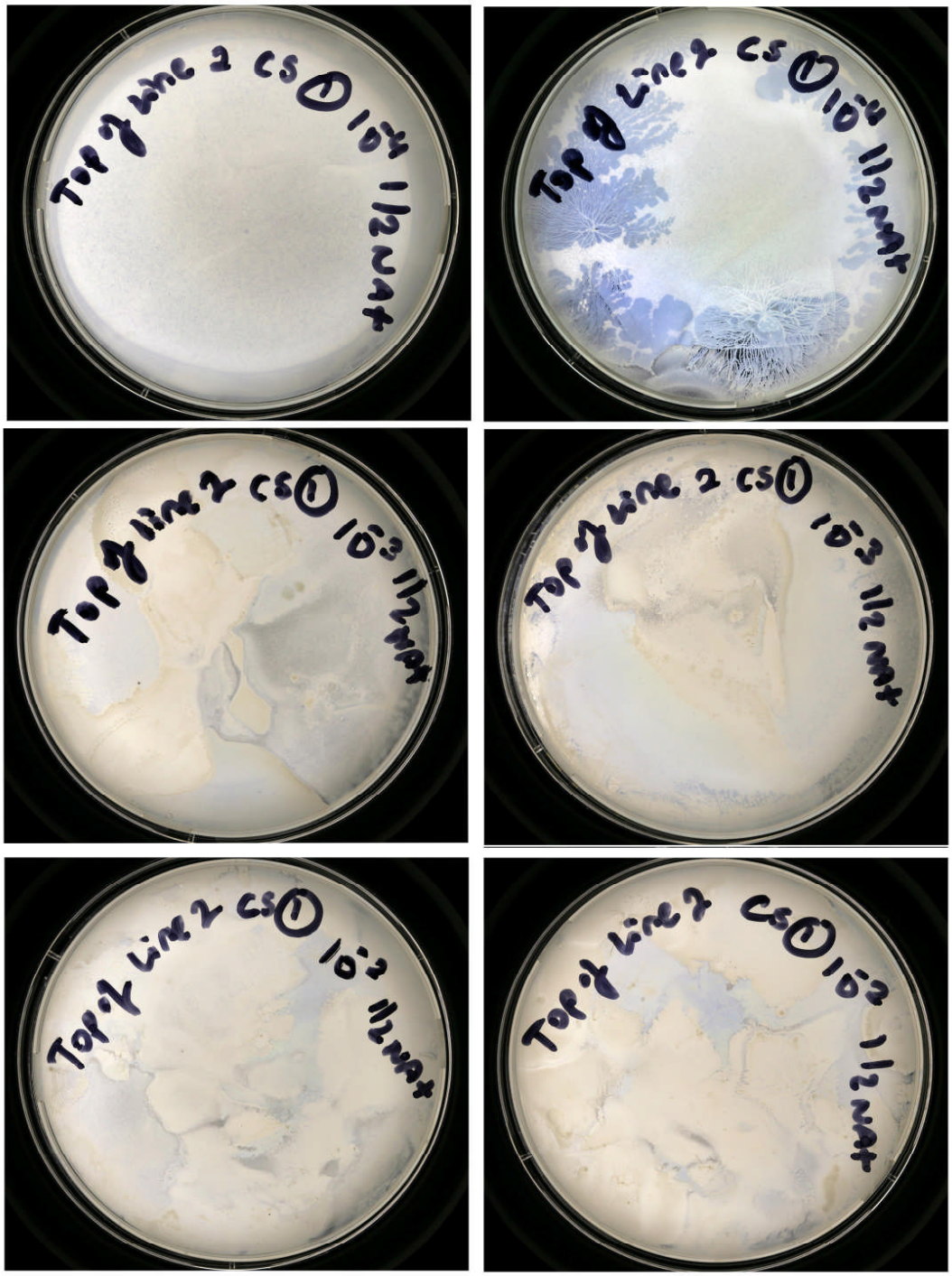

Fig 1. Actinomycetes growth using half strength nutrient media (NA). Plates show clear evidence of masking effect, as individual colonies of actinomycetes are difficult to distinguish.

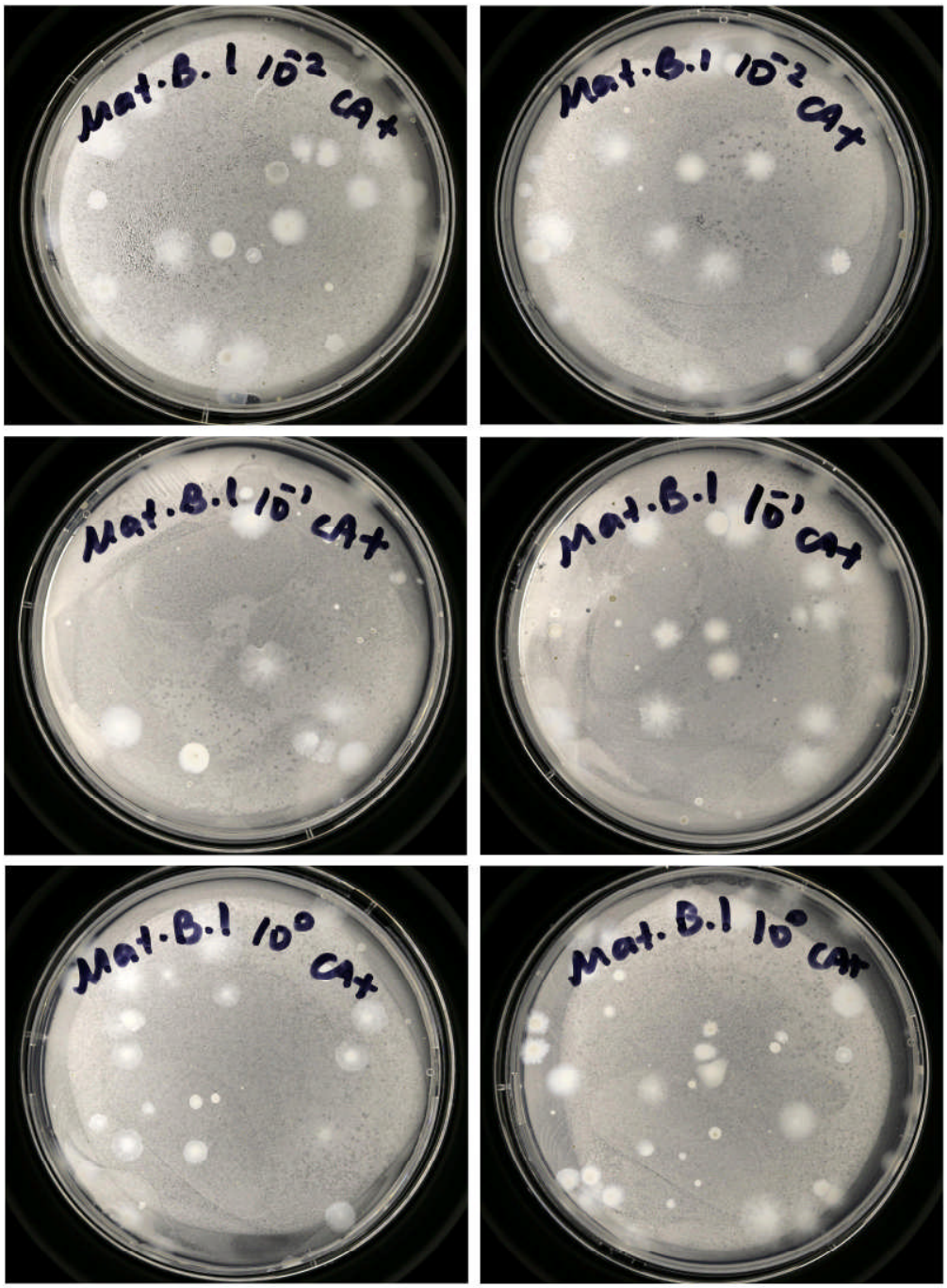

Fig 2. Actinomycetes growth using soil compost agar (SCA). Plates show clear evidence of improved visual inspection using soil compost agar, as individual colonies of actinomycetes are clearly visible.

Enumerating actinomycetes in compost bioaerosols at source—Use of soil compost agar to address plate ‘mask

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