CSIRO PUBLISHING

LAB REPORT
https://doi.org/10.1071/MA22011

Microbiology Australia

Detection and control of off-flavour compound-producing streptomycetes on locally produced nuts using streptophages

Laura G. Dionysius[A], Peter R. Brooks[A] and D. İpek Kurtböke[A,*]

For full list of author affiliations and declarations see end of paper

*Correspondence to:
D. İpek Kurtböke
Centre for Bioinnovation and the School of Science, Technology and Engineering, University of the Sunshine Coast, Maroochydore DC, Qld 4558, Australia
Email: IKurtbok@usc.edu.au

Received: 17 January 2022
Accepted: 17 February 2022
Published: 23 April 2022

Cite this:
Dionysius LG *et al.* (2022)
Microbiology Australia
43(1), 36–39. doi:10.1071/MA22011

ABSTRACT

Members of the phylum Actinomycetota are the most prominent part of the soil microbiota, more specifically the species within the genus *Streptomyces* of this phylum. Key functions of *Streptomyces* species (or streptomycetes in general terms) include nutrient cycling and plant growth promotion and disease protection. However, these species can also produce volatile organic compounds, predominantly geosmin, which is responsible for musty and mildew scents that are unpleasant to humans and can negatively impact the nut crop industry as odorous nuts generally lose their market value. Bacterial viruses, called bacteriophages have been previously used successfully in agriculture and aquaculture to remove such odorous species and they may therefore be applied to the nut industry. To eliminate these compounds, the producer streptomycetes may be selectively removed from nut surfaces using streptophages. The removal of *Streptomyces* species from nut surfaces can then be expected to minimise geosmin production, therefore removing the unpleasant off-flavours and benefiting the nut industry.

Keywords: actinomycetes, bacteriophages, food taints, geosmin, nuts, Streptomyces, streptophage, volatile organic compounds.

Introduction

Streptomycetes as the producers of volatile organic compounds

Over a thousand microbial volatile organic compounds (VOCs)[1] have been identified from streptomycetes, many of which are acids, alcohols, aldehydes, alkenes, benzenoids, esters, ketones, pyrazines, and terpenes.[2] VOCs produced by *Streptomyces* species can benefit agriculture via the production of bioactive compounds, which can assist in bacterial and fungal growth inhibition, plant growth promotion or inhibition, and invoke resistance mechanisms.[2]

Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), 2-methylisoborneol (2-MIB; 1,2,7,7-tetramethyl-exo-bicyclo-heptan-2-ol), and dimethyl disulfide are three of the major VOCs produced by *Streptomyces* species.[3–5] Geosmin and 2-MIB are semi-volatile and terpenoid secondary metabolites.[6] Streptomycetes produce these compounds via the 1-deoxy-D-xylulose 5-phosphate/2-*C*-methyl-D-erythritol 4-phosphate (DOXP/MEP) and melavonic (MVA) pathways.[7] Likewise, dimethyl disulfide is a volatile sulfur compound produced via methionine degradation followed by methanethiol oxidation.[8,9]

Taste and odour compounds in environment

VOCs are also known as taste and odour compounds (T&Os) due to their detectability by humans. Humans can detect these T&Os-VOCs at concentrations of 4 ng/L, due to their olfactory sense.[4,10] Furthermore, geosmin synthase genes, which enables geosmin production, are broadly distributed within the members of the genus *Streptomyces*.[4,11] These compounds arise in food products, such as nuts and fish, via bioaccumulation from plant debris, soil, and water use.[6,12,13] Further accumulation of geosmin can occur in storage silos if left unmaintained due to continued growth of streptomycetes, not only resulting in strong odours but also giving rise to organic dust toxic syndrome.[14] Geosmin has an earthy flavour,[15,16] yet there has been no successful technique to remove these VOCs due to the ineffectiveness and high cost of current methods.[17] A recent study conducted at the University of the Sunshine Coast (USC) aimed to remove VOCs on locally-produced and openly sold nut samples using streptophages.

Bacteriophage safety in agricultural products and humans

Bacteriophages have seen a rise in use as biocontrol agents in agriculture, bioprocessing, and healthcare.[18,19] Bacteriophages are regarded as safe for animals, humans, and plants, further promoting their use in the previously mentioned industries.[20–22] Chibani-Chennoufi *et al.*[23] reported that mice exposed to an oral four-phage cocktail did not experience a decline of their commensal *E. coli* biota. Bruttin and Brüssow[24] also reported that human volunteers orally exposed to phage T4 maintained their commensal *E. coli* population.

Findings of an example study from the Sunshine Coast region

Streptomycetes

Eight streptomycetes were isolated from seven different locally produced and openly sold nut samples using two different isolation methods (air compaction using an air sampler[25] and conventional serial dilution[26]) and incubation temperatures (28°C and 37°C) to maximise the chances of detection of these odorous species. Details of these isolates are given in Table 1.

Streptophages

Like bacteria, bacteriophages are present in agricultural environments where the host bacteria reside.[27] Usually bacteriophages are host specific, however, they also display polyvalency within the host's taxonomic rank.[22] The three major families of actinophages are Myoviridae, Siphoviridae, Podoviridae. Siphoviridae morphology is the most abundant one, particularly in soil among the actinophages.[28,29] This group of phages is mostly polyvalent within the Streptomycetaceae family to which the genus *Streptomyces* belongs. They are commonly known as streptophages[30] and morphologically consist of a long and flexible noncontractile tail. The siphoviridae heads contain portal protein at the vertices, which connects the head and tail segments, while the other vertices contain capsid proteins[31] and the tails usually are 100–400 nm in length depending on the species.[31]

Bacteriophages target host populations via phenotype modification, predation, and lysogeny.[27] Soil is the major reservoir for actinophages, and they most commonly target actinomycete genera *Streptomyces*, *Actinoplanes* and *Mycobacterium*.[27]

Application of streptophages onto nut samples and testing for the presence of the VOCs

Nine different polyvalent streptophages from USC's Microbial Library[32] were selected and used to create a composite phage suspension (Fig. 1) at a concentration of 10^8 pfu/mL. A composite streptomycete suspension was also created by mixing all eight streptomycete isolates at a concentration of 10^4 cfu/mL. This concentration was selected as it represents unacceptable contamination value determined by the NSW Food Authority in their guidelines[33]

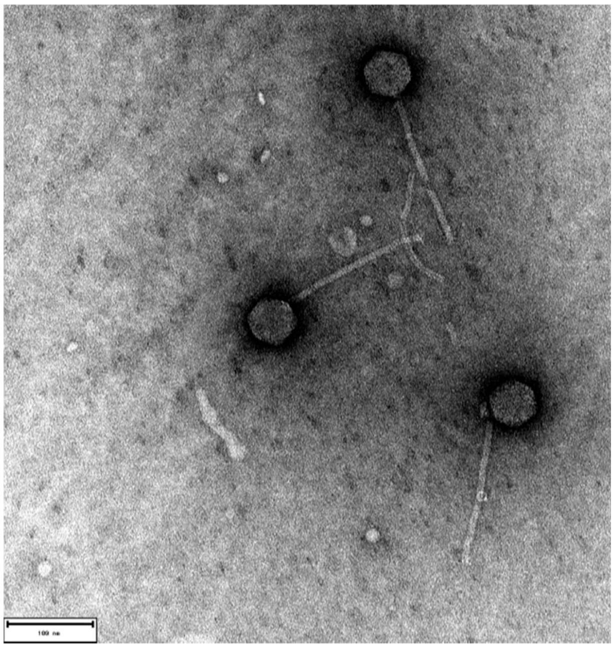

Fig. 1. TEM micrograph of the streptophages in the composite phage suspension displaying typical siphoviridae morphologies.

Table 1. Key characteristics of the Streptomyces isolates from nut samples.

Isolate code	Nut type and isolation method used	Closest relative identified using *16S* ribosomal RNA gene, partial sequence Blast analysis https://blast.ncbi.nlm.nih.gov/Blast.cgi
USC-7000	Corn kernels, air sampler,[A] 28°C	*Streptomyces* sp. strain 219202
USC-7001	Roasted peanuts, air sampler,[A] 28°C	*Streptomyces* sp. strain GS10
USC-7002	Raw peanuts, air sampler,[A] 28°C	*Streptomyces* sp. strain HBUM206355
USC-7003	Raw peanuts, air sampler,[A] 28°C	*Streptomyces* sp. strain HBUM206419
USC-7004	Raw almonds, air sampler,[A] 37°C	*Streptomyces werraensis* strain IIPR:KR05:01
USC-7005	Raw peanuts, air sampler,[A] 28°C	*Streptomyces werraensis* strain IIPR:KR05:01
USC-7006	Raw peanuts, serial dilution, 37°C	*Streptomyces werraensis* strain IIPR:KR05:01
USC-7007	Roasted peanuts, air sampler,[A] 37°C	*Streptomyces werraensis* strain IIPR:KR05:01

[A]Sampl'air Lite (https://www.biomerieux-usa.com/industry/samplair).

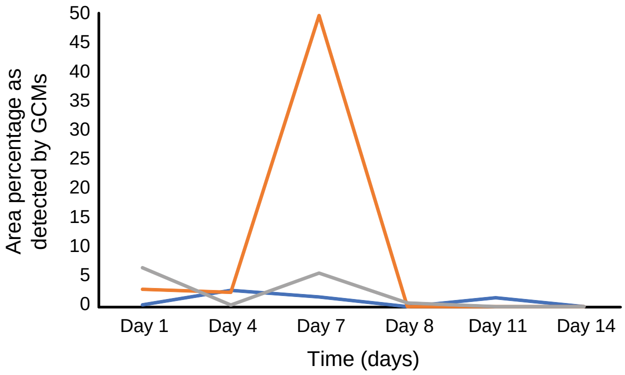

Fig. 2. Decreased values of geosmin after phage application. Key: blue – geosmin production (control); orange – geosmin production with streptomycetes application; grey – geosmin production with streptomycetes application then streptophage application after day 7.

for microbiological quality of ready to eat foods in Australia. Surface sterilised and UV irradiated nut samples using the methods by El-Tarabily[34] and Thomas and Puthur[35] were deliberately infected with this composite sample of streptomycetes. After 3 days of incubation streptophage composite sample was applied onto the streptomycete inoculated nuts with a host/phage ratio of 1:2. This ratio was selected due to past successful applications of phages onto hosts.[36] VOC production was examined using Headspace-Gas chromatography mass spectrometry (HS-GC/MS) throughout the 14 days of incubation in tightly capped bottles. A mixed standard of Geosmin and 2-MIB (Sigma-Aldrich) was used to detect Geosmin, which is known to be detected at 8.83 min.

Findings indicated a sharp decrease immediately in geosmin production after the composite phage suspension application onto streptomycete infected nut samples (Fig. 2). After day 8, the geosmin levels were near zero and streptomycete cfu/mL came down to the acceptable levels by the NSW Food Authority ($<10^2$).

2-MIB was not detected on the streptomycete or streptomycete plus phage treated nut samples at any stage of this study. Yanxia *et al.*[17] reported a strong correlation between geosmin and 2-MIB concentration, indicating that 2-MIB may be dependent on geosmin production so the sharp decrease in geosmin might be the reason of its absence.

Conclusions

The continual rise in demand of agricultural products has resulted in an increase in preferences in the use of environmentally and human health friendly methods replacing other synthetic agents. Therefore, bacteriophage treatments gained attention to minimise product losses and nutritional properties from disease causing bacteria. Like the previous successful treatments of potatoes[37–39] and strawberries,[40] the observed success of streptophage treatment on nuts in this study may indicate similar positive outcomes might be possible. Therefore, information generated via the studies like the one presented here can contribute toward development of effective phage biocontrol methods targeting different problems in agriculture. Such methods might subsequently reduce the economic losses of the growers due to unmarketable product including the ones possessing earthy-musty smells.

References

1. Whitfield FB (1998) Microbiology of food taints. *Int J Food Sci Technol* **33**, 31–51. doi:10.1046/j.1365-2621.1998.00156.x
2. Cordovez V *et al.* (2015) Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Front Microbiol* **6**, 1081. doi:10.3389/fmicb.2015.01081
3. Schrader KK, Blevins WT (2001) Effects of carbon source, phosphorus concentration, and several micronutrients on biomass and geosmin production by *Streptomyces halstedii*. *J Ind Microbiol Biotechnol* **26**, 241–247. doi:10.1038/sj.jim.7000121
4. Becher PG *et al.* (2020) Developmentally regulated volatiles geosmin and 2-methylisoborneol attract a soil arthropod to Streptomyces bacteria promoting spore dispersal. *Nat Microbiol* **5**, 821–829. doi:10.1038/s41564-020-0697-x
5. Wilkins K (1996) Volatile metabolites from actinomycetes. *Chemosphere* **32**, 1427–1434. doi:10.1016/0045-6535(96)00051-3
6. Jonns JA *et al.* (2017) Streptophage-mediated control of off-flavour taint producing streptomycetes isolated from barramundi ponds. *Synth Syst Biotechnol* **2**, 105–112. doi:10.1016/j.synbio.2017.04.002
7. Zaitlin B, Watson SB (2006) Actinomycetes in relation to taste and odour in drinking water: myths, tenets and truths. *Water Res* **40**, 1741–1753. doi:10.1016/j.watres.2006.02.024
8. Han D *et al.* (2016) Dimethyl disulphide residue analysis and degradation kinetics determination in soil using gas chromatography–mass spectrometry. *Int J Environ Anal Chem* **96**, 694–704. doi:10.1080/03067319.2016.1180383
9. Schöller CEG *et al.* (2002) Volatile metabolites from actinomycetes. *J Agric Food Chem* **50**, 2615–2621. doi:10.1021/jf0116754
10. Asquith EA *et al.* (2013) The role of actinobacteria in taste and odour episodes involving geosmin and 2-methylisoborneol in aquatic environments. *J Water Supply: Res Technol—AQUA (Print)* **62**, 452–467. doi:10.2166/aqua.2013.055
11. Martín-Sánchez L *et al.* (2019) Phylogenomic analyses and distribution of terpene synthases among *Streptomyces*. *Beilstein J Org Chem* **15**, 1181–1193. doi:10.3762/bjoc.15.115
12. Wood S *et al.* (1983) Factors influencing geosmin production by a streptomycete and their relevance to the occurrence of earthy taints in reservoirs. *Water Sci Technol* **15**, 191–198. doi:10.2166/wst.1983.0143
13. Nielsen JL *et al.* (2006) Detection of activity among uncultured actinobacteria in a drinking water reservoir. *FEMS Microbiol Ecol* **55**, 432–438. doi:10.1111/j.1574-6941.2005.00054.x
14. Lacey J, Crook B (1988) Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. *Ann Occup Hyg* **32**, 515–533. doi:10.1093/annhyg/32.4.515
15. Lanciotti E *et al.* (2003) Actinomycetes, cyanobacteria and algae causing tastes and odours in water of the River Arno used for the water supply of Florence. *J Water Supply: Res Technol—AQUA* **52**, 489. doi:10.2166/aqua.2003.0044
16. Jørgensen NOG *et al.* (2016) Relations between abundance of potential geosmin- and 2-MIB-producing organisms and concentrations of these compounds in water from three Australian reservoirs. *J Water Supply: Res Technol—AQUA* **65**, 504. doi:10.2166/aqua.2016.001
17. Zuo Y *et al.* (2009) Isolation, identification and odour-producing abilities of geosmin/2-MIB in actinomycetes from sediments in Lake Lotus, China. *J Water Supply: Res Technol—AQUA (Print)* **58**, 552–561. doi:10.2166/aqua.2009.018
18. Aleshkin AV *et al.* (2013) Bacteriophages as probiotics and decontaminating agents for food products. *Asia Pac J Life Sci* **7**, 91–107.
19. Moye ZD *et al.* (2018) Bacteriophage applications for food production and processing. *Viruses* **10**, 205. doi:10. 10.3390/v10040205
20. Yamada T (2012) Bacteriophages of *Ralstonia solanacearum*: their diversity and utilization as biocontrol agents in agriculture. In *Bacteriophages* (Kurtböke DI, ed.). pp. 113–138. InTech.
21. Jones JB *et al.* (2021) Crop use of bacteriophages. In *Bacteriophages: biology, technology, therapy* (Harper DR *et al.*, eds). pp. 839–856. Springer International Publishing.
22. Monk AB *et al.* (2010) Bacteriophage applications: where are we now? *Lett Appl Microbiol* **51**, 363–369. doi:10.1111/j.1472-765X.2010.02916.x

23. Chibani-Chennoufi S *et al.* (2004) *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. *Antimicrob Agents Chemother* **48**, 2558–2569. doi:10.1128/AAC.48.7.2558-2569.2004
24. Bruttin A, Brüssow H (2005) Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob Agents Chemother* **49**, 2874–2878. doi:10.1128/AAC.49.7.2874-2878.2005
25. Lacey J (1988) Actinomycetes as biodeteriogens and pollutants of the environment. In *Actinomycetes in biotechnology* (Goodfellow M *et al.*, eds). pp. 359–432. Academic Press.
26. Koch R (1883) Über die neuen Untersuchungsmethoden zum Nachweis der Mikrokosmen in Boden, Luft und Wasser (Schwalbe J, ed.). pp. 276–284. Robert Koch-Institut.
27. Kurtböke Dİ (2017) Ecology and habitat distribution of actinobacteria. In *Biology and biotechnology of actinobacteria* (Wink J *et al.*, eds). pp. 123–149. Springer International Publishing.
28. Ackermann HW (2001) Frequency of morphological phage descriptions in the year 2000. *Arch Virol* **146**, 843–857. doi:10.1007/s007050170120
29. Ackermann HW (2006) Classification of bacteriophages. In *The Bacteriophages, General Background of Phage Biology* (Calender R, ed.). pp. 8–16. Oxford University Press.
30. Ackermann HW *et al.* (1985) New actinophage species. *Intervirology* **23**, 121–130. doi:10.1159/000149602
31. Sanz-Gaitero M *et al.* (2019) Structure and function of bacteriophages. In *Bacteriophages: Biology, Technology, Therapy* (Harper DR *et al.*, eds). pp. 1–73. Springer International Publishing.
32. Kurtböke Dİ (2006) From culture collections to biological resource centres. *Microbiol Aust* **27**, 4–5.
33. NSW Food Authority (2009) Guideline levels for microorganisms. In *Microbiological Quality Guide for Ready-to-eat Foods* (NSW Food Authority, ed.). p. 7. NSW Food Authority.
34. El-Tarabily KA (2008) Promotion of tomato (*Lycopersicon esculentum Mill.*) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. *Plant Soil* **308**, 161–174. doi:10.1007/s11104-008-9616-2
35. Thomas DTT, Puthur JT (2017) UV radiation priming: a means of amplifying the inherent potential for abiotic stress tolerance in crop plants. *Environ Exp Bot* **138**, 57–66. doi:10.1016/j.envexpbot.2017.03.003
36. Abdelsattar AS *et al.* (2021) How to train your phage: the recent efforts in phage training. *Biologics* **1**, 70–88. doi:10.3390/biologics1020005
37. McKenna F *et al.* (2001) Novel *in vivo* use of a polyvalent *Streptomyces* phage to disinfest *Streptomyces scabies*-infected seed potatoes. *Plant Pathol* **50**, 666–675. doi:10.1046/j.1365-3059.2001.00648.x
38. Ashfield-Crook NR *et al.* (2018) Assessment of the detrimental impact of polyvalent streptophages intended to be used as biological control agents on beneficial soil streptoflora. *Curr Microbiol* **75**, 1589–1601. doi:10.1007/s00284-018-1565-2
39. Ashfield-Crook N *et al.* (2021) Bioactive streptomycetes from isolation to applications: a Tasmanian potato farm example. In *Methods in Molecular Biology*. Humana Press.
40. Kurtböke Dİ *et al.* (2016) Isolation and characterization of Enterobacteriaceae species infesting post-harvest strawberries and their biological control using bacteriophages. *Appl Microbiol Biotechnol* **100**, 8593–8606. doi:10.1007/s00253-016-7651-0

Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. This research did not receive any specific funding.

Acknowledgements. This work was enabled by use of the Central Analytical Research Facility (CARF) at the Queensland University of Technology (QUT), a Microscopy Australia linked lab.

Author affiliation

[A]Centre for Bioinnovation and the School of Science, Technology and Engineering, University of the Sunshine Coast, Maroochydore DC, Qld 4558, Australia.

Biographies

Laura G. Dionysius is one of the USC recent graduates with first class Honours. She also holds a BSc in the Science Program of the USC. She conducted research with Dr İpek Kurtböke over the past 2 years related to the application of various actinophages. While in Grade 11, she began studying at USC through the Head Start program and has since become the first student from her school, Peregian Beach College, to graduate with Honours and achieve first class. She is also a member of the USC Science Society Committee as the Treasurer.

Dr Peter Brooks has a PhD in Chemistry from the University of New South Wales, awarded in 1989. He then moved to the University of Adelaide before taking up a position at La Trobe University, Bendigo. Since 2001 he has taught analytical, organic and general chemistries at the University of the Sunshine Coast. Dr Brooks has extensive research experience in analytical, environmental, and organic chemistry. His research has focused on environmental monitoring and the quantitation of bioactive compounds.

Dr D. İpek Kurtböke is currently a senior lecturer at the University of the Sunshine Coast (USC) in Australia and one of the members of the Centre for Bioinnovation of the USC, conducting research in applied, industrial and environmental microbiology. She is an internationally reputed actinomycetologist and she has been in the field of biodiscovery since 1982 conducting research into discovery of novel and potent therapeutic compounds produced by actinomycetes in Turkey, Italy, the UK, and Australia with leading pharmaceutical companies. She has been an Executive Board member of the World Federation of Culture Collections (WFCC) since 2000, currently serving her second term as the President of the Federation. She is also one of the members of the International Committee on Taxonomy of Viruses (ICTV)'s Bacterial Viruses Subcommittee. She has editorial duties in different journals including *Marine Drugs*, *Diversity* and *Frontiers Marine Science/Marine Biotechnology*.