J Ind Microbiol Biotechnol (2015) 42:759–767
DOI 10.1007/s10295-015-1600-2

ENVIRONMENTAL MICROBIOLOGY

SIMB
Society for Industrial Microbiology and Biotechnology

Temperature effects on biomass, geosmin, and 2-methylisoborneol production and cellular activity by *Nocardia* spp. and *Streptomyces* spp. isolated from rainbow trout recirculating aquaculture systems

Kevin K. Schrader · Marcuslene D. Harries · Phaedra N. Page

Received: 9 December 2014 / Accepted: 11 February 2015 / Published online: 28 February 2015

Abstract Isolates of *Nocardia cummidelens*, *Nocardia fluminea*, *Streptomyces albidoflavus*, and *Streptomyces luridiscabiei* attributed as the cause of "earthy-musty" off-flavor in rainbow trout (*Oncorhynchus mykiss*) raised in recirculating aquaculture systems (RAS) were evaluated for the effect of temperature (10–30 °C) on biomass, geosmin, and 2-methylisoborneol (MIB) production and cellular activity. Cultures of these isolates were monitored over 7 days by measuring culture dry weight, geosmin, and MIB production using solid-phase microextraction-gas chromatography-mass spectrometry (SPME–GC–MS), and ATP production via a luminometer. Compared to the other isolates, *S. luridiscabiei* had significantly ($P < 0.05$) higher biomass (8.17 ± 0.35 mg/mL) at 15 °C (water temperature in the RAS) after 7 days incubation. In addition, *S. luridiscabiei* produced significantly ($P < 0.05$) higher geosmin (69,976 ± 15,733 ng/L) at 15 °C. At 25 °C and 30 °C, *S. albidoflavus* produced significantly ($P < 0.05$) higher geosmin (182,074 ± 60,272 ng/L and 399,991 ± 102,262 ng/L, respectively). All isolates produced MIB at 15 °C, but *S. luridiscabiei* produced significantly ($P < 0.05$) higher MIB (97,143 ± 28,972 ng/L) and ATP after 7 days. Therefore, *S. luridiscabiei* appears to be a likely contributor of geosmin and MIB in the RAS.

Keywords Aquaculture · Geosmin · 2-methylisoborneol · *Nocardia* · *Streptomyces*

K. K. Schrader (✉) · M. D. Harries · P. N. Page
United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, National Center for Natural Products Research, Post Office Box 1848, University, MS 38677-1848, USA
e-mail: kevin.schrader@ars.usda.gov

Introduction

Preharvest "off-flavor" problems continue to hamper the growth of certain segments of the United States of America aquaculture industry including fish raised in recirculating aquaculture systems (RAS). The most common preharvest off-flavors are due to the bioaccumulation of geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol or MIB [(1-*R*-*exo*)-1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol] in the flesh of the cultured fish which causes "earthy" and "musty" taints, respectively, and results in unpalatable and unmarketable products [9]. The off-flavored fish must be held by producers in water free of off-flavor compounds until they lose the earthy and/or musty taints. The transfer of fish to clean water for depuration is time consuming and requires an adequate depuration system/tank. Additional adverse impacts of these common preharvest off-flavors in fish cultured in RAS include the loss of market demand due to inconsistent product quality, inhibition of growth into new markets, and economic losses associated with delays in stocking a new crop while holding the off-flavor fish until flavor quality improves.

The sources of geosmin and MIB in RAS have been attributed to the production by certain species of actinomycetes such as *Nocardia* spp. and *Streptomyces* spp. [2, 3, 7]. Specifically, in one study by Schrader and Summerfelt [7], *Nocardia cummidelens*, *Nocardia fluminea*, *Streptomyces albidoflavus*, and *Streptomyces luridiscabiei* were isolated from biosolids contained within a series of six RAS which were being used to produce rainbow trout (*Oncorhynchus mykiss*), and these bacterial isolates were confirmed to be producers of geosmin. However, the sources of MIB within these RAS were not identified.

Different temperatures have been found to significantly impact biomass and geosmin production by actinomycetes

[1, 11]. During the study by Schrader and Summerfelt [7], the water temperatures of the different RAS were maintained at 15 ± 1 °C, though some portions of the RAS (e.g., drum filter) might have higher temperature conditions. Therefore, the determination of temperature-dependent relative contributions of the geosmin-producing actinomycetes isolated in the study by Schrader and Summerfelt [7] to off-flavor problems in the RAS would identify those isolates for additional physiology studies and potential management strategies.

In the current study, the impacts of different temperatures on cellular activity and biomass, geosmin, and MIB production by four actinomycete isolates obtained from the study by Schrader and Summerfelt [7] were evaluated. A temperature range of 10–30 °C was studied in order to determine which isolate(s) might be contributing to the off-flavor problems within the RAS.

Materials and methods

Microorganisms

Isolates of *Nocardia cummidelens* and *Nocardia fluminea* were originally cultured on yeast dextrose (YD) plates containing 10 g/L yeast extract, 10 g/L dextrose, and 8 mL/L glycerol, with final media adjusted to pH 7.5 before the addition of 20 g/L agar. Isolates of *Streptomyces albidoflavus* and *Streptomyces luridiscabiei* were originally cultured on actinomycete isolation agar (AIA) (Difco™; Becton, Dickinson and Company, Sparks, MD). The isolated cultures were stored separately in sterile solutions of 10 % (w/v) glycerol and 5 % (w/v) peptone at −80 °C until physiology studies were performed at which time the individual isolates were aseptically streaked for confluent growth onto YD plates. The plates were incubated at room temperature (25 °C) for 5–12 d to obtain confluent growth before preparation of separate propagule suspensions. Propagule suspensions of *N. cummidelens*, *N. fluminea*, *S. albidoflavus*, and *S. luridiscabiei* were "harvested" aseptically from YD plates containing confluent growth and transferred to sterile 0.85 % saline solution to obtain final colony forming units (cfu)/mL of 3.8×10^7, 8.8×10^7, 12.2×10^7, and 9.1×10^7, respectively.

Media and culture conditions for physiology studies

For each actinomycete species, 100 µL of propagule suspension (maintained at 4 °C) was aseptically pipetted into separate sterilized 1-L Erlenmeyer flasks with foam enclosures and containing 250 mL of sterile YD broth in triplicate, except for *N. cummidelens* for which 200 µL of propagule suspension (final 7.6×10^7 cfu/mL) was aseptically added per flask. Temperature studies were conducted separately for each species, and triplicate flasks were incubated at temperatures of 10, 15, 20, 25, and 30 °C for 7 d on a rotary shaker (Model Excella E24R; New Brunswick Scientific, Enfield, CT) at 200 rpm. Cell culture material (35 mL/flask per each sampling time) was aseptically removed from each flask at 2, 4, and 7 d for biomass determination, cell viability assay, and analysis of geosmin and MIB concentrations.

Cell viability assay

Cell viability was determined by measuring adenosine 5′-triphosphate (ATP) production levels. An ATP (Sigma-Aldrich; St. Louis, MO) standard curve was created according to the Bac-Titer-Glo™ Microbial Cell Viability Assay (Promega, Madison, WI) protocol and as outlined by Uwins [10]. Specifically, 100 µL of ATP standard was serially diluted (1:10) using sterile YD broth (90 µL per well) in flat-bottom wells of 96-well, white-bottom polystyrene plates (Thermo Scientific™ Nunc™; Wlatham, MA) to obtain final concentrations of 1 µM to 10 pM (triplicate wells per concentration). Additionally, on the same microplate, culture material from each flask was aseptically transferred to the 96-well plate (100 µL per well in triplicate). Bac-Titer-Glo™ reagent was added to each well (100 µL per well) containing culture material and to the ATP standard wells; the microplate was then mixed on a rotary shaker at 100 rpm for 5 min at room temperature. The sample was allowed to incubate in the dark for an additional 5 min before measuring luminescence for 1 s per well using a luminometer (Model Synergy™ 2, Biotek®; Winooski, VT). Control wells containing sterile YD broth (200 µL per well) were used to obtain background luminescence values which were subtracted from the other luminescence readings.

Biomass determination

Glass-fiber filters (dia. 42.5 mm, grade G4; Fisher Scientific, Pittsburgh, PA) were dried at 121 °C for 24 h, and the initial weight was obtained after cooling. For each flask, 10 mL of culture material (in triplicate) was vacuumed filtered. Filtrate was rinsed with 10 mL of 0.85 % saline, dried for 24 h in a drying oven (121 °C), and the final filter weight recorded. The initial weight of the filter was subtracted from the final weight to determine dried biomass (mg/mL).

Analysis of geosmin and MIB levels in cultures

For each sample, 0.6-mL aliquots of culture material were micropipetted into individual 2-mL, glass, crimp-top vials

containing 0.3 g sodium chloride per vial. The method used to quantify levels of geosmin and MIB was similar to the SPME–GC–MS procedure used by Lloyd et al. [4]. Specifically, vials were heated at 40 °C for 20 min before the volatile compounds were absorbed onto a 100 µm polydimethyl siloxane solid-phase microextraction fiber (Supelco, Bellfonte, PA). The fiber assembly was then shaken for 10 min during the absorption period and then desorbed for 2 min at 250 °C in the injection port of a HP 6890 gas chromatograph-mass spectrometer (Agilent, Palo Alto, CA) with a 5973 mass selective detector operated in selected-ion-monitoring mode. The conditions of the gas chromatograph were as follows: (1) initial oven temperature was 60 °C for 0.5 min; (2) the first ramp rate was 30 °C/min to 100 °C; (3) the second ramp rate was 20 °C/min to 300 °C with an isotherm time of 2 min; and (4) the maintenance of flow pressure was at 124 kPa (18 lb/in^2) with helium used as a carrier gas. The molecular ion base peaks were monitored at the ratio of molecular mass to ionic charge (*m/z*) of 168, 95, and 135 for MIB and at *m/z* of 182, 112, and 126 for geosmin. A DB-5 capillary column [(5 %-phenyl)-methylpolysiloxane, 30 m, 0.25 mm inside diameter, 0.25-µm film thickness; J&W Scientific, Folsom, CA] was used. The retention time for geosmin was 6.8 min and for MIB was 5.2 min. Standards for MIB and geosmin were prepared at 0.1, 0.5, 1.0, and 2.5 µg/L in deionized water. The original standards were obtained from Wako Chemicals USA, Inc., Richmond, Virginia., and were included at the beginning, middle, and end of each group of samples analyzed using a CombiPal autosampler (LEAP Technologies, Inc., Carrboro, NC). Each sample was run in triplicate, and detection threshold limits for geosmin and MIB were 1 ng/L [8].

Data analysis

Means and standard deviations (± SD) were calculated from determinations of geosmin and MIB concentrations. Means and standard errors (± SE) were calculated from biomass determinations and cell viability assays. The calculated means and respective standard deviations and standard errors were graphed against time. Analysis of variance was performed for mean comparisons of each sampled variable (e.g., biomass) at the same temperature and the same sampling day using SigmaPlot software, Version 11.0 (Systat Software Inc., San Jose, CA).

Results

Neither discernible biomass nor geosmin and MIB production occurred throughout the incubation period for any of

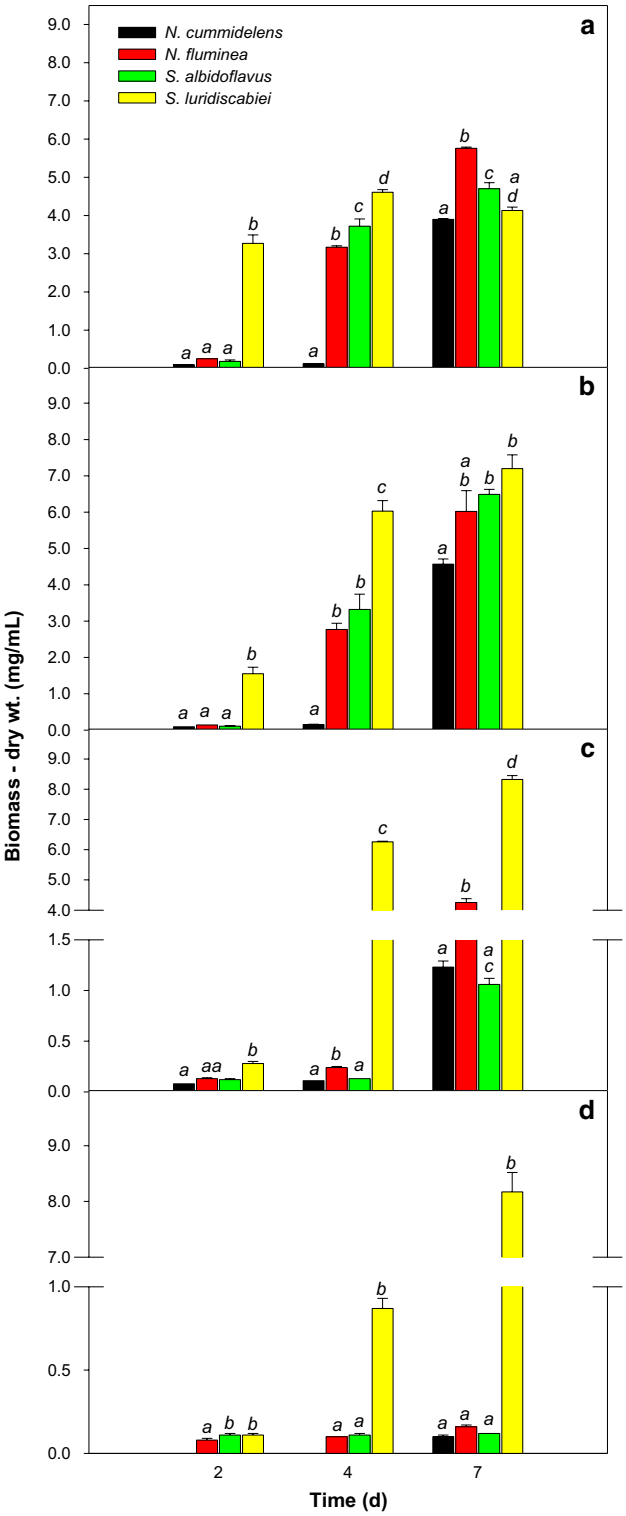

Fig. 1 Effect of different temperatures on biomass production by species of *Nocardia* and *Streptomyces*. **a** 30 °C; **b** 25 °C; **c** 20 °C; **d** 15 °C. *Standard error bars* of the mean ($n = 3$) are included. For each temperature graph, means on the same day with the same letter are not significantly different ($P < 0.05$)

the isolates held at 10 °C. The early stages of growth (e.g., 1–2 days) by the four isolates at the remaining test temperatures may have been partially impacted by temperature adaptation. Biomass production by *N. cummidelens* was highest at 7 days at 30 °C (Fig. 1a) and 25 °C (Fig. 1b), with 3.9 ± 0.02 and 4.57 ± 0.14 mg/mL, respectively. Minimal biomass (0.1 ± 0.01 mg/mL) was produced by *N. cummidelens* at 15 °C after 7 days incubation (Fig. 1d). Incubated cultures of *N. fluminea* also produced minimal biomass (0.16 ± 0.01 mg/mL) at 15 °C after 7 days, while highest biomass production of 5.76 ± 0.03 and 6.02 ± 0.57 mg/mL occurred at 30 °C (Fig. 1a) and 25 °C (Fig. 1b), respectively. Cultures of *S. albidoflavus* produced the highest biomass (6.49 ± 0.14 mg/mL) at 25 °C after 7 days incubation and the lowest biomass production of 0.12 ± 0.00 mg/mL at 15 °C after 7 days incubation. Compared to the other three isolates, *S. luridiscabiei* produced significantly ($P < 0.05$) the greatest biomass of 8.17 ± 0.35 mg/mL at 15 °C after 7 days incubation (Fig. 1d), and *S. luridiscabiei* also produced significantly ($P < 0.05$) greater biomass than the other isolates after 4 and 7 days incubation at 20 °C (Fig. 1c), with 6.26 ± 0.02 and 8.32 ± 0.13 mg/mL, respectively. Overall, *S. luridiscabiei* produced the least biomass (4.13 ± 0.09 mg/mL) after 7 days incubation at 30 °C (Fig. 1a).

Geosmin production by *N. cummidelens* was highest at 7 days incubation at 25 °C, with a yield of 2,717 ± 879 ng/L (Fig. 2b). Very low or undetectable levels (<1 ng/L) of geosmin production by *N. cummidelens* occurred at the other three incubation temperatures. For *N. fluminea*, greatest geosmin production of 997 ± 693 ng/L occurred after 2 days of incubation at 30 °C, while there was a decrease in the geosmin presence in the cultures at days 4 and 7, possibly due to a loss from volatilization of the semi-volatile compound along with decreased production (Fig. 2a). The other incubation temperatures resulted in low or undetectable geosmin production by *N. fluminea*. Geosmin production by *S. albidoflavus* was highest at 4 and 7 days of incubation at 30 °C, with yields of 405,644 ± 37,088 ng/L and 399,991 ± 102,262 ng/L, respectively (Fig. 2a). At 25 °C, there was also substantial geosmin production by *S. albidoflavus*, with 182,074 ± 60,272 ng/L at 7 days of incubation (Fig. 2b). The least amount of geosmin production by *S. albidoflavus* after 7 days of incubation was 46 ± 26 ng/L at 15 °C (Fig. 2d). Conversely, *S. luridiscabiei* produced the highest geosmin (69,976 ± 15,733 ng/L) at 15 °C after 7 days incubation, and this geosmin production yield was significantly ($P < 0.05$) higher compared to the other three isolates. The next highest geosmin production by *S. luridiscabiei* occurred at 20 °C after 4 and 7 days incubation, with yields of 42,979 ± 13,427 and 39,412 ± 4,364 ng/L, respectively (Fig. 2c). The least amount of geosmin production by *S. luridiscabiei* after 7 days of incubation was 4,129 ± 993 ng/L at 30 °C (Fig. 2a).

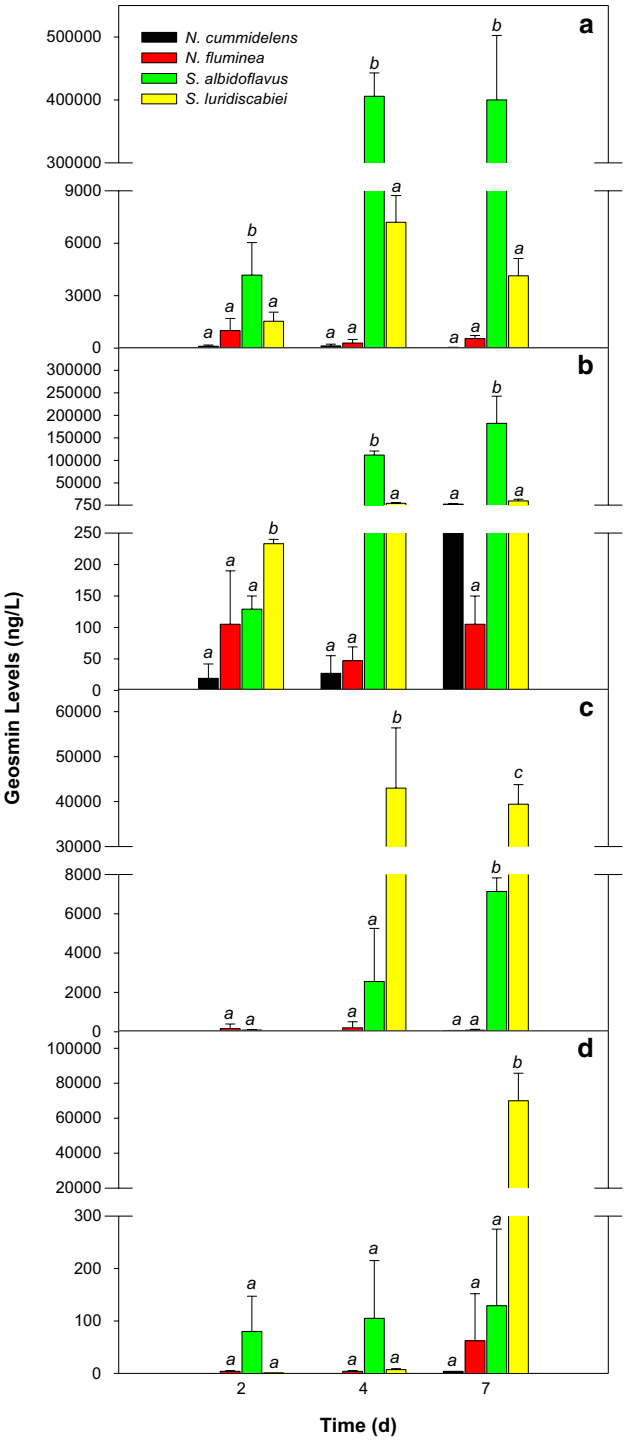

Fig. 2 Effect of different temperatures on geosmin production by species of *Nocardia* and *Streptomyces*. **a** 30 °C; **b** 25 °C; **c** 20 °C; **d** 15 °C. *Standard deviation bars* of the mean ($n = 3$) are included. For each temperature graph, means on the same day with the same letter are not significantly different ($P < 0.05$)

Geosmin/biomass production by *N. cummidelens* was highest at 30 °C after 2 and 4 days incubation, with yields of 1.13 ± 0.75 and 1.1 ± 0.86 ng/mg, respectively, but then

dropped to 0.01 ± 0.01 ng/mg after 7 days incubation, possibly due to a loss from volatilization of the semi-volatile compound along with decreased production (Fig. 3a). Overall, geosmin/biomass production by *N. cummidelens* over the entire 7-day incubation period was lowest at 15 °C, with undetectable levels at 2 and 4 days of incubation and 0.04 ± 0.01 ng/mg after 7 days of incubation (Fig. 3d). For *N. fluminea*, the highest geosmin/biomass production of 3.91 ± 2.68 ng/mg occurred at 30 °C after 2 days incubation, while the lowest geosmin/biomass production of 0.02 ± 0.01 ng/mg occurred at 25 °C (Fig. 3b) and 20 °C (Fig. 3c) after 7 days of incubation. Cultures of *S. albidoflavus* had the significantly ($P < 0.05$) highest geosmin/biomass production at 25 and 30 °C and at 4 and 7 days incubation when compared to the other three isolates, with highest production of 109.8 ± 15.05 ng/mg at 30 °C after 4 days of incubation (Fig. 3a). Conversely, *S. luridiscabiei* had very low geosmin/biomass production (0.46 ± 0.10 to 1.56 ± 0.30 ng/mg) at 30 °C throughout the incubation period, but it had the highest geosmin/biomass production of 8.66 ± 2.40 ng/mg at 15 °C after 7 days incubation which was significantly ($P < 0.05$) higher compared to the other isolates (Fig. 3d).

2-Methylisoborneol (MIB) production by *N. cummidelens* was highest at 7 days incubation at 15 °C, with a yield of 11 ± 5 ng/L (Fig. 4d). However, there was no detectable (<1 ng/L) MIB production by *N. cummidelens* at 2 and 4 days incubation at 15 °C, and <5 ng/L MIB was detected as being produced during incubation at any sampling time at the other three temperatures (Fig. 4a–c). Cultures of *N. fluminea* and *S. albidoflavus* were also not prolific producers of MIB at any of the incubation temperatures. The highest production of MIB by *N. fluminea* was 9 ± 3 ng/L at 15 °C after 2 days of incubation (Fig. 4d), while the highest production of MIB by *S. albidoflavus* was 12 ± 2 ng/L at 20 °C after 4 days of incubation (Fig. 4c). Cultures of *S. luridiscabiei* produced significantly ($P < 0.05$) higher levels of MIB than any of the other cultures at all test temperatures after 7 days of incubation. The greatest amount of MIB production by *S. luridiscabiei* occurred at 15 °C after 7 days of incubation, with a yield of 97,143 ± 28,972 ng/L (Fig. 4d). The next highest level of MIB production by *S. luridiscabiei* occurred at 20 °C after 4 days of incubation, with a yield of 44,829 ± 1,121 ng/L (Fig. 4c). The least amount of MIB production by *S. luridiscabiei* after 7 days of incubation was at 30 °C, with only 10 ± 0 ng/L yield (Fig. 4a).

2-Methylisoborneol/biomass production by *N. cummidelens* was highest at 15 °C after 7 days incubation, with a yield of 0.11 ± 0.05 ng/mg (Fig. 5d). At the other

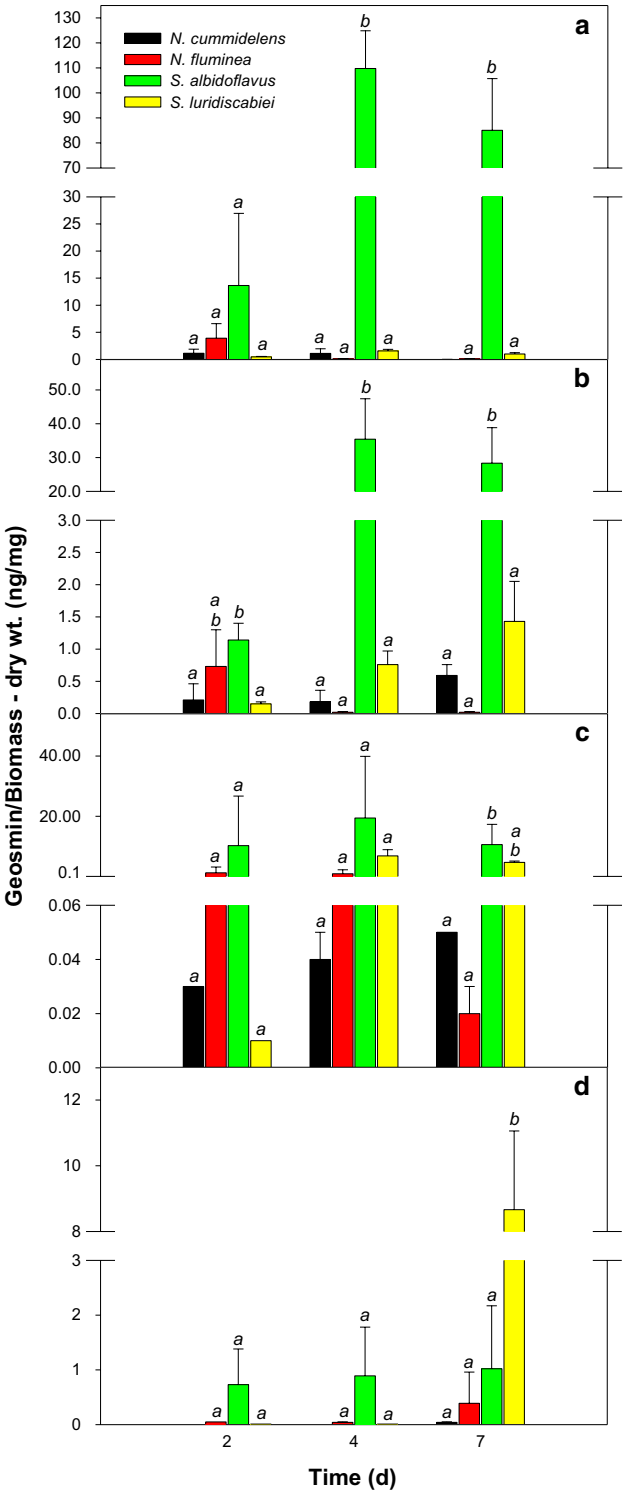

Fig. 3 Effect of different temperatures on geosmin/biomass production by species of *Nocardia* and *Streptomyces*. **a** 30 °C; **b** 25 °C; **c** 20 °C; **d** 15 °C. *Standard deviation bars* of the mean ($n = 3$) are included. For each temperature graph, means on the same day with the same letter are not significantly different ($P < 0.05$)

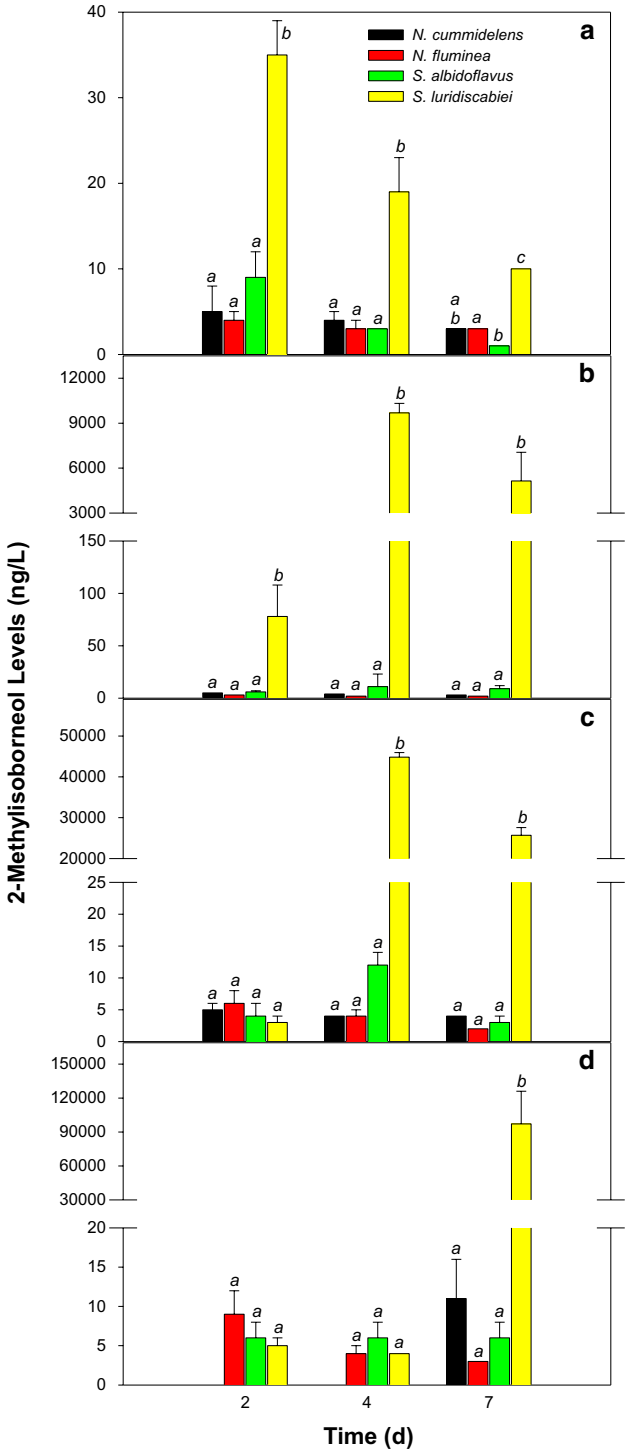

Fig. 4 Effect of different temperatures on 2-methylisoborneol production by species of *Nocardia* and *Streptomyces*. **a** 30 °C; **b** 25 °C; **c** 20 °C; **d** 15 °C. *Standard deviation bars* of the mean ($n = 3$) are included. For each temperature graph, means on the same day with the same letter are not significantly different ($P < 0.05$)

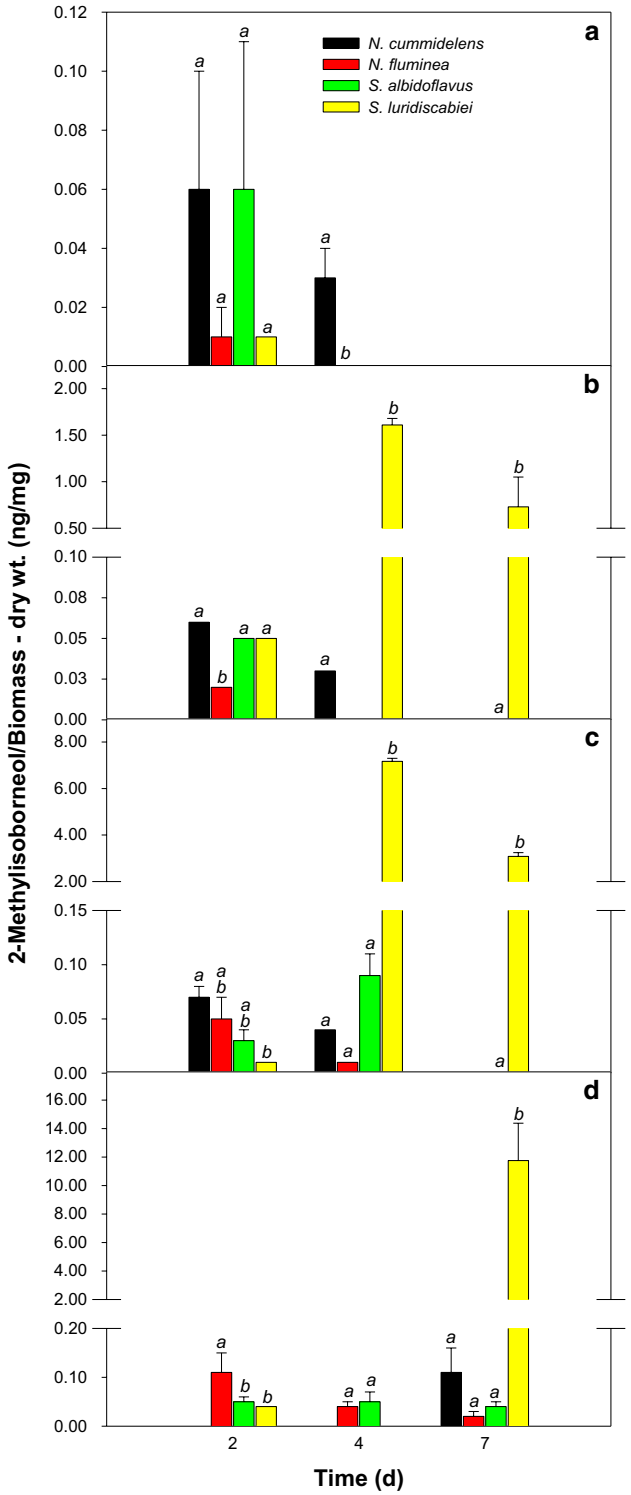

Fig. 5 Effect of different temperatures on 2-methylisoborneol/biomass production by species of *Nocardia* and *Streptomyces*. **a** 30 °C; **b** 25 °C; **c** 20 °C; **d** 15 °C. *Standard deviation bars* of the mean ($n = 3$) are included. For each temperature graph, means on the same day with the same letter are not significantly different ($P < 0.05$)

three temperatures, MIB/biomass production by *N. cummidelens* was highest after 2 days of incubation but then dropped after 7 days incubation, possibly due to a loss from volatilization of the semi-volatile compound along with decreased production. The greatest MIB/biomass production by *N. fluminea* was at 15 °C after 2 days of incubation, with a yield of 0.11 ± 0.04 ng/mg (Fig. 5d). A decrease of MIB production along with an increase in biomass production by *N. fluminea* resulted in decreasing MIB/biomass at the other three temperatures during the incubation period (Fig. 5a–c). For *S. albidoflavus*, the highest MIB/biomass of 0.09 ± 0.02 ng/mg occurred at 20 °C after 4 days of incubation (Fig. 5c). At 15 °C, MIB/biomass production by *S. albidoflavus* remained at 0.04 ± 0.01 to 0.05 ± 0.01 ng/mg throughout the incubation period (Fig. 5d). However, MIB/biomass production by *S. luridiscabiei* at 15 °C increased dramatically to 11.76 ± 2.63 ng/mg after 7 days of incubation and was significantly ($P < 0.05$) higher than the other isolates. The next highest MIB/biomass yield by *S. luridiscabiei* was 7.17 ± 0.13 ng/mg at 20 °C after 4 days of incubation (Fig. 5c), while the lowest MIB/biomass production was at 30 °C throughout the incubation period (Fig. 5a).

Cellular activity by *N. cummidelens* was highest after 7 days incubation at 20 °C, with an ATP level of 134,217 ± 3,797 pg/mL (Fig. 6c), thereby indicating sustained cell viability under those conditions, while the lowest cellular activity was at 30 °C after 7 days of incubation, with an ATP level of 4 ± 0 pg/mL (Fig. 6a). For *N. fluminea*, cellular activity was highest after 4 days incubation at 25 °C, with an ATP level of 104,346 ± 26,885 pg/mL (Fig. 6b), while the lowest cellular activity was at 30 °C after 7 days of incubation, with an ATP level of 14 ± 0 pg/mL. Conversely, cellular activity by *S. albidoflavus* after 7 days of incubation was highest at 30 °C, with an ATP level of 115,545 ± 54,489 pg/mL, while the lowest cellular activity after 7 days of incubation was at 15 °C, with an ATP level of 31,383 ± 754 pg/mL (Fig. 6d). For *S. luridiscabiei*, the highest ATP levels detected were at 30 °C after 2 and 4 days of incubation, with ATP levels of 170,815 ± 16,029 and 206,768 ± 40,845 pg/mL, respectively. However, after 7 days of incubation at 30 °C, cellular activity had dropped dramatically, with ATP levels of only 7 ± 1 pg/mL. Conversely, the highest ATP levels for *S. luridiscabiei* after 7 days of incubation occurred at 15 °C, with levels of 130,208 ± 14,299 pg/mL. Cultures of *S. luridiscabiei* had significantly ($P < 0.05$) higher levels of cellular activity at 15 °C after 4 and 7 days of incubation compared to the other isolates (Fig. 6d).

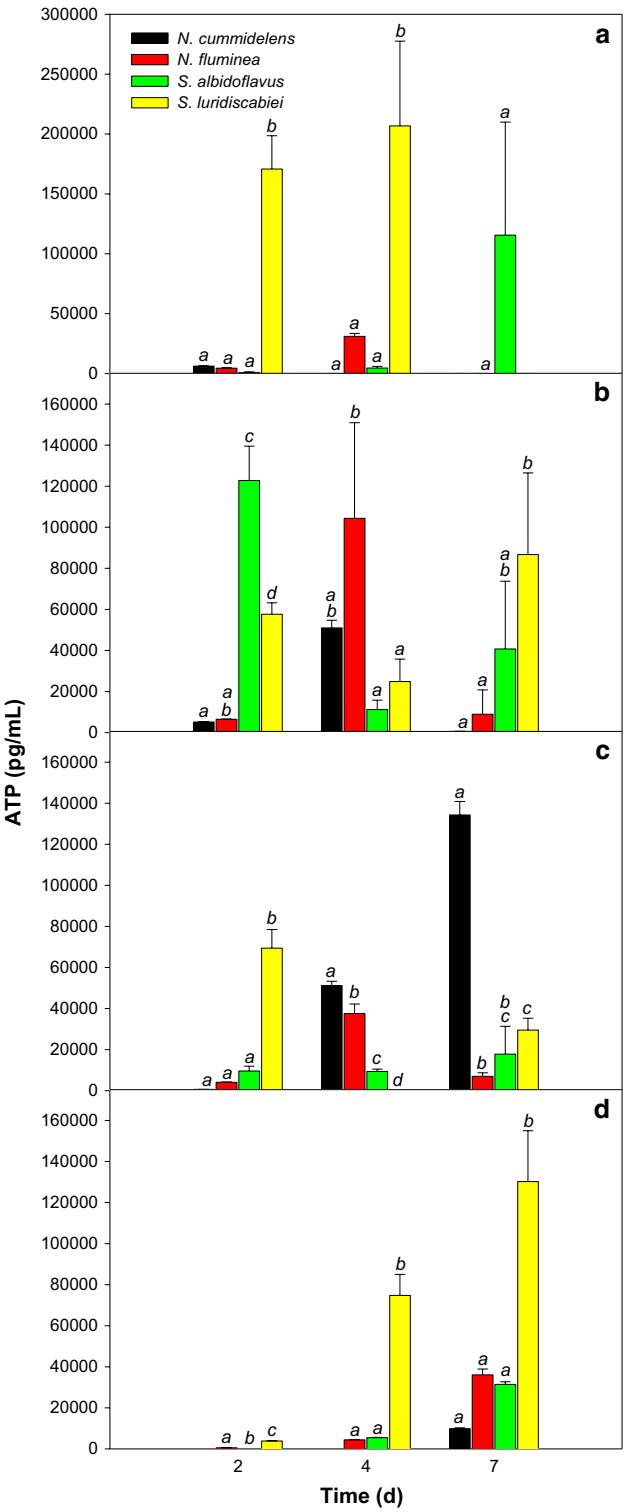

Fig. 6 Effect of different temperatures on cell viability as measured ATP production by species of *Nocardia* and *Streptomyces*. **a** 30 °C; **b** 25 °C; **c** 20 °C; **d** 15 °C. *Standard error bars* of the mean ($n = 3$) are included. For each temperature graph, means on the same day with the same letter are not significantly different ($P < 0.05$)

Discussion

Physiology studies specifically related to geosmin and/or MIB production by *N. cummidelens*, *N. fluminea*, *S. albidoflavus*, *and S. luridiscabiei* have not previously been conducted. In addition, the determination of the contributor(s) among the four isolates to off-flavors in the fish raised in the RAS is imperative for directing future research to focus on the development of management strategies to mitigate the off-flavor problems.

Results from the laboratory studies revealed that *S. luridiscabiei* produced significantly ($P < 0.05$) higher biomass at 15 °C after 4 and 7 days compared to the other three isolates (Fig. 1d). Geosmin production (Fig. 2d) and geosmin/biomass (Fig. 3d) by *S. luridiscabiei* after 7 days incubation were also significantly ($P < 0.05$) higher compared to the other isolates, with geosmin production dramatically higher by over two orders of magnitude compared to the other isolates. Production of MIB (Fig. 4d) and MIB/biomass (Fig. 5d) was also significantly ($P < 0.05$) and dramatically higher by *S. luridiscabiei* at 15 °C after 7 days incubation compared to the other isolates. In addition, production of MIB by *S. luridiscabiei* at the other three incubation temperatures was substantially ($P < 0.05$) higher than the other three isolates (Fig. 4a–c). At 15 °C, cellular activity by *S. luridiscabiei* was also substantially and significantly ($P < 0.05$) higher compared to the other isolates at 4 and 7 days of incubation (Fig. 6d). Overall, among these four isolates, *S. luridiscabiei* can be considered to be a likely contributor of geosmin and MIB presence in the RAS because the water temperature in these systems is maintained at 15 ± 1 °C for the culture of coldwater fish such as rainbow trout.

The biomass production by all four isolates was similar after 7 days incubation at 30 °C (Fig. 1a), while *S. luridiscabiei* produced more biomass than the other three isolates at 25 °C (Fig. 1b) and 20 °C (Fig. 1c) after 7 days incubation. Therefore, *S. luridiscabiei* grew well at all four test temperatures used in the study. Compared to the other isolates, *S. albidoflavus* was the more prolific producer of geosmin at 30 °C (Fig. 2a) and 25 °C (Fig. 2b). If this species were found to be present in other RAS used to culture fish at warmer temperatures, it could also be considered to be a strong contributor to geosmin-related off-flavor problems in those systems. Conversely, *S. luridiscabiei* was a more prolific producer of MIB compared to the other isolates at the warmer test temperatures (Fig. 4a–b). It is interesting that none of these isolates were originally identified as producers of MIB when initially isolated and grown on solid medium (i.e., agar plates) [7], though *N. cummidelens*, *N. fluminea*, and *S. albidoflavus* were not prolific MIB producers while grown even in submerged culture. The greater biomass yield provided by submerged state culture of the actinomycetes compared to growth on agar may have permitted detection of low-level MIB production by the respective isolates. In addition, the incubation temperatures of 25 and 30 °C used by Schrader and Summerfelt [7] to grow isolates on solid media prior to analysis by SPME–GC–MS in order to detect geosmin and/or MIB production were found in the current study to provide substantially lower MIB yields than the other two test temperatures in the case of *S. luridiscabiei* (Fig. 4).

In the study by Schrader and Summerfelt [7], relatively high levels of geosmin and MIB were present in the organic-rich aerobic components of the RAS (e.g., drum filter, heat exchanger). Studies to determine the relative abundance of *S. luridiscabiei* at these sites in the RAS would provide further information on the scope of the contribution of *S. luridiscabiei* to the geosmin and MIB production within the RAS. As demonstrated in previous studies, environmental and nutritional factors may directly impact production of geosmin and/or MIB by streptomycetes [5, 6]. Therefore, physiology studies to determine the impact of certain environmental (e.g., pH) and nutritional factors (e.g., levels of ammonia-N) on geosmin and MIB production by responsible actinomycetes are the first step to provide further insight on conditions within the RAS that may contribute to preharvest off-flavor problems, though nutrient-rich conditions in RAS are more complex than those under in vitro conditions. Understanding such conditions can be useful in the potential prediction of increases or decreases of geosmin and MIB levels within the RAS and may also provide guidance on instituting management practices to reduce the levels of geosmin and MIB. Other future research and management approaches (e.g., biocontrol) also benefit from the knowledge of those noxious microorganisms such as *S. luridiscabiei* to target within the RAS.

Acknowledgments The technical assistance of Michael McCain is greatly appreciated.

References

1. Blevins WT, Schrader KK, Saadoun I (1995) Comparative physiology of geosmin production by *Streptomyces halstedii* and *Anabaena* sp. Wat Sci Technol 31(11):127–133
2. Burr GS, Wolters WR, Schrader KK, Summerfelt ST (2012) Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating system. Aquacult Eng 50:28–36
3. Guttman L, van Rijn J (2008) Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. Aquaculture 279:85–91
4. Lloyd SW, Lea JM, Zimba PV, Grimm CC (1998) Rapid analysis of geosmin and 2-methylisoborneol in water using solid phase micro extraction procedures. Water Res 32:2140–2146
5. Schrader KK, Blevins WT (1999) Effects of selected environmental conditions on biomass and geosmin production by *Streptomyces halstedii*. J Microbiol 37:159–167

6. 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
7. Schrader KK, Summerfelt ST (2010) Distribution of off-flavor compounds and isolation of geosmin-producing bacteria in a series of water recirculating systems for rainbow trout culture. N Am J Aquacult 72:1–9
8. Schrader KK, Nanayakkara NPD, Tucker CS, Rimando AM, Ganzera M, Schaneberg BT (2003) Novel derivatives of 9,10-anthraquinone are selective algicides against the musty-odor cyanobacterium *Oscillatoria perornata*. Appl Environ Microbiol 69:5319–5327
9. Tucker CS (2000) Off-flavor problems in aquaculture. Rev Fish Sci 8:45–88
10. Uwins HK (2011) Triggers for taste and odour events: a study of microbial production of geosmin and 2-methylisoborneol. Doctoral dissertation. Griffith University, Nathan
11. Weete JD, Blevins WT, Wilt GR, Durham D (1977) Chemical, biological, and environmental factors responsible for the earthy odor in the Auburn city water supply. In: Bulletin of the Alabama Agricultural Experiment Station 490, Auburn University, Auburn, AL, p 46