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# Short Note
# The trade-off between antimicrobial production and growth of an Antarctic psychrotroph *Streptomyces* sp. strain INACH3013

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## Introduction

Antarctic microorganisms are known to produce secondary compounds to communicate with each other and/or inhibit their competitors (Montgomery *et al.* 2013).

In our paper on the phylogenetic and physiological characterization of a psychrotolerant *Streptomyces* (strain INACH3013) we reported the capability to inhibit other bacteria (Lavin *et al.* 2016). Secondary compound production occurred primarily during the stationary phase and is generally believed to be a defence mechanism improving bacterial survival. This paper reports the first trade-off, to our knowledge, between antimicrobial production and growth of a *Streptomyces* sp. strain when it is exposed to different temperatures during the exponential phase of growth.

## Materials and methods

In order to determine the effect of media richness on antimicrobial production and growth, the strain was grown in a 1000 ml Erlenmeyer flask containing 500 ml of liquid medium and shaken at 160 rpm at 20°C for 13 days. Five different liquid media were used: Luria Broth (LB; Difco), Oatmeal (OAT; 20 $g\,l^{-1}$; Atlas 2010), Actinomycetes (ACT; Difco), Mueller-Hinton (MH; Difco) and Reasoner's 2A (R2A; Difco). Media formulations are presented in Table S1 found at http://dx.doi.org/10.1017/S0954102017000141. The effect of temperature on antimicrobial production and growth was tested by growing the strain in oatmeal liquid medium with shaking at 160 rpm at 12, 20 and 28°C for nine days. The bacterial growth was measured in colony forming units (log CFU $ml^{-1}$) using three replicates per treatment. Antimicrobial production was evaluated by measuring the diameter of the inhibition zone produced by 10 µl of cell-free culture supernatant against *Staphylococcus aureus* (ATCC 65389). Secondary compound production at different temperatures was analysed using thin layer chromatography (TLC) of the ethyl acetate extract obtained from 200 ml of cell-free supernatant. Statistical analysis was performed using GraphPad Prism demo v6.0 for Windows (GraphPad Software, La Jolla, CA, www.graphpad.com). Statistical differences among treatments were evaluated using a one-way ANOVA, followed by the Tukey multiple comparison test *a posteriori*.

## Result

The results showed no direct relationship between biomass and secondary compound production. On day 3 and day 6 of the culture growth, OAT medium showed the smallest increase in biomass and highest antimicrobial activity (Fig. 1a–d). Considering the nutrient level in the culture media (MH > LB > ACT > R2A > OAT), rich media, such as MH and LB, did not promote higher biomass production and antimicrobial activity. In contrast, temperature had a direct effect on growth and secondary compound production. Lower temperatures had a negative effect on the growth rate and positive effects on secondary compound production and antimicrobial activity (Fig. 2).

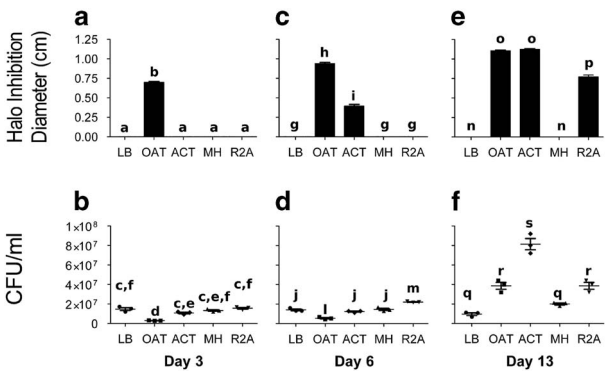


**Fig. 1.** Effect of culture media and time on antibacterial compound production and growth. **a.**, **c.** and **e.** Antimicrobial activity of the cell-free supernatant. **b.**, **d.** and **f.** Growth measured at 20°C in five different media. Data expressed as mean ± standard deviation ($n = 3$). One-way ANOVA, $***P < 0.0001$; same letter represents no significant difference (additional statistical information is available in Table S2 found at http://dx.doi.org/10.1017/S0954102017000141).

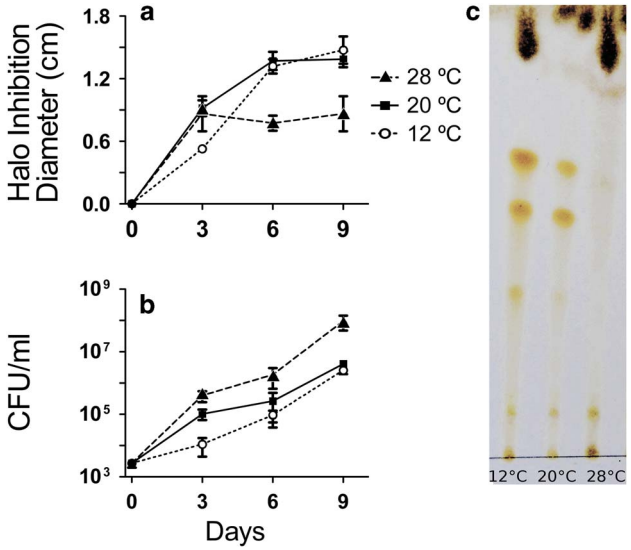


**Fig. 2.** Trade-off between antimicrobial production and growth. **a.** Antimicrobial activity of cell-free supernatant. **b.** Growth curve in oatmeal medium at 12, 20 and 28°C. **c.** TLC-bioautography of ethyl acetate extract of the cell-free supernatant obtained on the last day of the experiment. Ethyl acetate-methanol (9:1) was used as the mobile solvent.

## Discussion

Under laboratory conditions, production of secondary compounds is usually observed during the stationary phase when one or more resources start depleting (Wentzel *et al.* 2012). This could explain the antibacterial activities observed at the beginning of the cultures in the low nutrient media, such as oatmeal and actinomycetes. Antibacterial activity was also detected in the R2A medium on day 13 as the nutrients reduced (Fig. 1e & f). Since the ACT medium has a composition which favours the growth of *Streptomyces*, it had the highest biomass production on day 13 (twice that of OAT), but the antimicrobial activity was similar to that in OAT medium.

Natural environments that support continuous bacterial growth are rare. External factors, such as oligotrophic environments and competition between microorganisms, force bacteria to adapt to harsh and changing situations (Lipson 2015). Rapid growth with high energetic cost, it only occurs in environments with high nutrient availability. A metabolically efficient and high yield strategy is preferable in low nutrient conditions (Molenaar *et al.* 2009).

Moreover, it was observed that temperatures lower than the optimal temperature for growth (30°C; Lavin *et al.* 2016) triggered earlier production of secondary compounds by *Streptomyces* sp. strain INACH3013 at the exponential phase (Fig. 2). Similar results were observed in the actinomycetes medium (data not shown). This shows that at temperatures lower than the optimal temperature for growth the strain had a lower growth rate due to decreased substrate affinity (Nedwell 1999), which led to a trade-off between secondary compound production and growth as a strategy to compete and survive at low temperatures.

Further study will be carried out to understand the molecular mechanisms underlying the trade-off response to low temperatures.

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## Author contributions

PLL, STY and CMVLW conceived the study. PLL and STY performed the research and analyses of the data, and wrote the initial draft of the article. CMVLW, ARGP and CD edited the article.

## Supplemental material

Two supplemental tables will be found at http://dx.doi.org/10.1017/S0954102017000141.

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