Chapter 81
Effect of Salinity on Growth and Cytotoxicity of Extracts from a Marine-Derived *Penicillium* sp. (LY1L5)

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Introduction

Marine-derived fungi may be defined as fungi – be they obligate or facultatively marine – that are isolated from the marine environment [1]. It is not always clear, however, whether all these isolates actually grow in this saline environment or are itinerants. For those who are active in the marine world, then osmoregulatory mechanisms must be in place. Osmoregulation is energetically costly, and it has been postulated that such fungi exhibit decreased amounts or rates of secondary metabolite production in the presence of salt [2]. Salt-dependent strains of marine-derived fungi for metabolite production have been described for *Penicillium* spp. [2]. Some studies have indicated that the production of metabolites from marine-derived fungi is sensitive to seawater concentration [3, 4]. This would have implications in drug discovery programmes using marine-derived organisms. In this study, a marine-derived *Penicillium* sp. (isolate LY1L5) showing cytotoxicity was assessed for their tolerance to salinity (0, 2, 4, 6, 8 and 10 % sea-salt concentration) with respect to their growth and cytotoxic metabolite production.

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Materials and Methods

Isolate LY1L5 was obtained from an unidentified marine invertebrates from Lyttelton Harbour, New Zealand, using adapted isolation techniques [4]. The original cultivation conditions on peptone yeast glucose agar (PYGA) medium, with temperature 25 °C, pH 7.0 ± 0.2 and 4 % sea salt that produced the cytotoxic activity, were used as a basis to study the response of salinity to growth and production of metabolites in isolate LY1L5. Salinity in this study is expressed as sea-salt concentration (%) incorporated in the media.

The isolate was cultivated on PYGA medium (pH 7.0 ± 0.2), and a salinity range of 0, 2, 4, 6, 8 and 10 % was used. Cultures were incubated at 25 °C, and the colony diameter (three replicates) was measured every 2 days for 30 days. Cultures were harvested after 30 days' incubation. Resultant cultures were extracted with ethyl acetate (EtOAc), and the dried extracts were dissolved in the HPLC grade methanol (MeOH) at a concentration of 1 mg/mL prior to the submission for bioassay. The extracts (1 mg/mL) were assayed for cytotoxicity against P388 cells and their metabolite production assessed by HPLC. The presence of cycloaspeptide A and α-cyclopiazonic acid was confirmed by their UV characteristics using the in-house HPLC-UV/Rt library database [5].

Results and Discussions

Cultural Characteristics and Morphology

Isolate LY1L5 was identified as *Penicillium* sp. based on the colony and morphological characteristics shown in Fig. 81.1. Cultures on Czapek medium 20–25 mm diameter on 10 days at 25 °C, mycelium white, reverse yellow or brownish. Cultures on PYGA (4 % sea salt) medium 30–35 mm diameter after 10 days at 25 °C, mycelium black, reverse greyish. Cultures on PYGA (0 % sea salt) medium 25–30 mm diameter after 10 days at 25 °C, mycelium white, reverse yellow or brownish. Stipes and metulae with smooth walls, conidiophores with two-stage-branched, biverticillate on PYGA (4 % sea salt) medium. Phialides four to eight per metulae, ampulliform, 4.0–6.0 × 2.0–2.5 μm; conidia spheroidal, spinose, 1.5–2.0 μm diameter, in chains with small connectives between the conidia.

Metabolite Profile

Penicillium sp. (LY1L5) produced both cycloaspeptide A and α-cyclopiazonic acid as confirmed by their UV characteristics. Its metabolite profile was compared with those *Penicillium* spp. reported to produce cycloaspeptide A (five species) and α-cyclopiazonic acid (six species) (see Table 81.1). The comparison showed that none of the 11 *Penicillium* spp. produced both cycloaspeptide A and α-cyclopiazonic acid [6, 7].

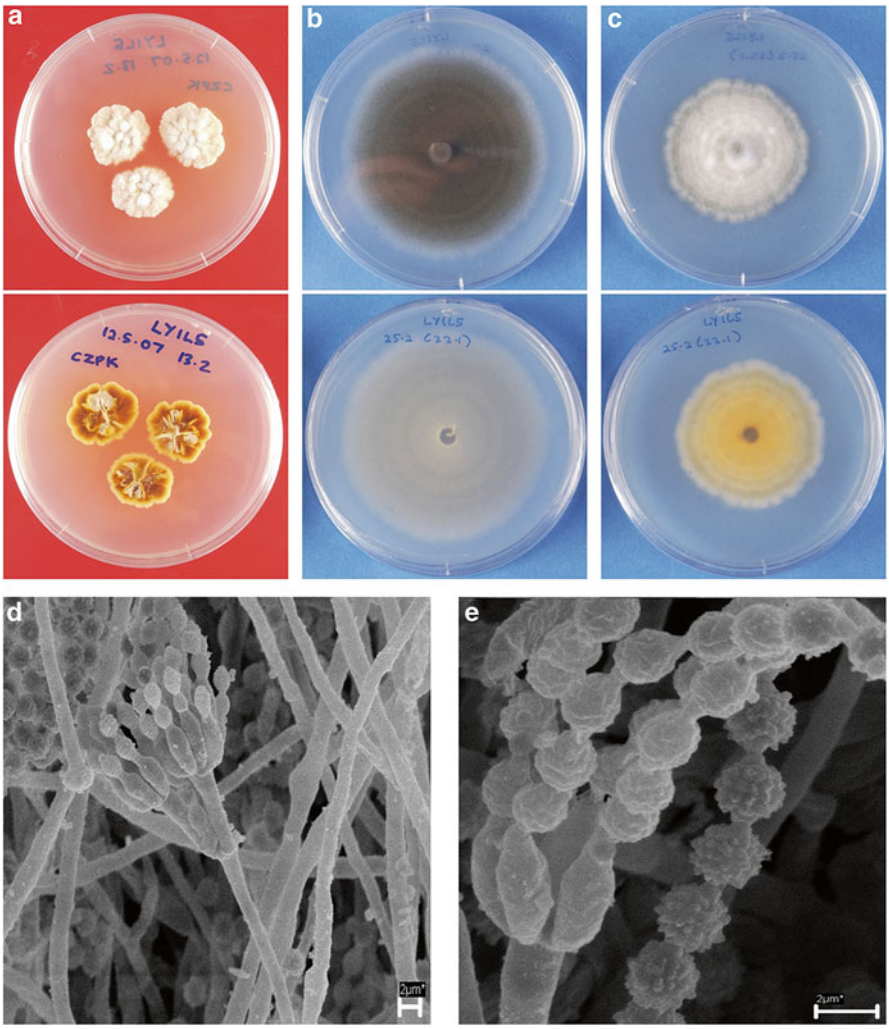

Fig. 81.1 Colony and morphological characteristics of *Penicillium* sp. (LY1L5); (**a**) colonies on Czapek medium at 25 °C, 10 days; (**b**) colonies on PYGA (4 % sea salt) medium at 25 °C, 30 days; (**c**) colonies on PYGA (0 % sea salt) medium at 25 °C, 30 days; (**d–e**) SEM of conidiophores and conidia on PYGA (4 % sea salt) medium at 25 °C, 10 days; (**d**) conidiophores; (**e**) conidia

Effect of Salinity on Growth and Production of Metabolites

The growth of *Penicillium* sp. (LY1L5) was enhanced in the presence of salt (Fig. 81.2), but the growth decreased at 10 % salinity. Growth rate declined after 18–20 days' incubation. The cytotoxicity of extracts is shown in Table 81.2. This isolate showed better IC_{50} values at salinity of 4 and 6 %, but was not active in

Table 81.1 Comparison of secondary metabolite profile of *Penicillium* sp. (LY1L5) with the reported *Penicillium* spp. producing cycloaspeptide A and α-cyclopiazonic acid

Penicillium spp.	Cycloaspeptide A	α-cyclopiazonic acid
Penicillium sp. (LY1L5)	+	+
P. jamesonlandense	+	
P. ribeum	+	
P. lanosum	+	
P. soppii	+	
P. algidum	+	
P. camemberti		+
P. commune		+
P. dipodomyicola		+
P. griseofulvum		+
P. patulum		+
P. palitans		+

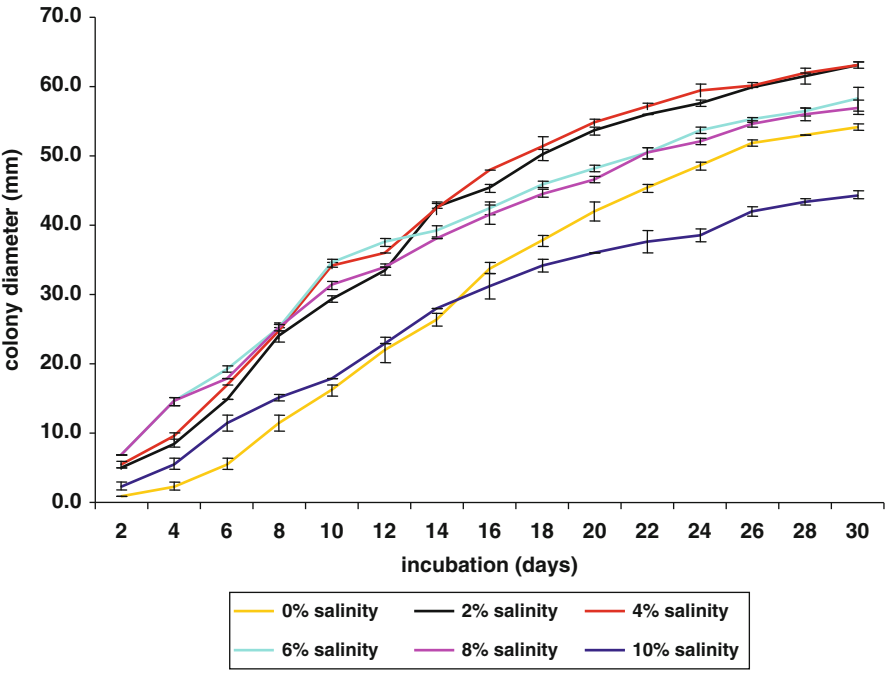

Fig. 81.2 Effect of salinity on growth of *Penicillium* sp. (LY1L5) on PYGA medium (pH 7.0 ± 0.2; 25 °C)

the absence of salt. All extracts showed traces of cycloaspeptide A. Traces of the cytotoxic α-cyclopiazonic acid were detected only in five active extracts (F5975-B, F5975-C, F5975-D, F5975-E and F5975-F) but none in the inactive extract (F5975-A).

Table 81.2 Effect of salinity on cytotoxicity of *Penicillium* sp. (LY1L5) on PYGA medium (pH 7.0 ± 0.2; 25 °C; 30 days)

Salinity (%)	Extracts	Cytotoxicity against P388 cells IC_{50} (ng/mL)	ELSD traces of cycloaspeptide A	ELSD traces of α-cyclopiazonic acid
0	F5975-A	>12,500	+	–
2	F5975-B	7,976	+	+
4	F5975-C	6,664	+	+
6	F5975-D	6,664	+	+
8	F5975-E	7,741	+	+
10	F5975-F	9,837	+	+

Discussions

The effect of salinity on the growth of a marine-derived *Penicillium* sp. (LY1L5) reflects the observations made on an algal-derived *P. dravuni* [8] that also showed greater growth with increasing salinity up to a level of 10 % NaCl. A study on a sponge-derived isolate of *Penicillium* sp. showed that growth was not affected by seawater concentrations [3]. The effect of salinity on bioactivity of marine-derived *Penicillium* spp. has been described where three *Penicillium* spp. exhibited increasing antimicrobial activity with increasing concentrations of artificial seawater [2]. A few studies have been reported on the effect of salinity on bioactivity of other marine-derived fungi. Three marine-derived *Aspergillus* spp. and an unidentified marine fungus showed enhanced antibacterial activity in the presence of seawater [3].

Cycloaspeptide A has only been found in five species of psychrotolerant *Penicillium*, namely, *P. ribeum* [7, 9], *P. algidum* [10], *P. jamesonlandense*, *P. lanosum* and *P. soppii* [7]. It was noted in this study that the ELSD traces of cycloaspeptide A in isolate LY1L5 were more prominent than the cytotoxic α-cyclopiazonic acid at all ranges of salinity (0–10 %). The consistency in cycloaspeptide A production by this isolate was similar to those reported for the cold-tolerant strains of *Penicillium*. Based on this, isolate LY1L5 is suggested to have a closer association with the groups of *Penicillium* that produced cycloaspeptide A. Isolate LY1L5 has small, spinose conidia unlike the α-cyclopiazonic acid producers that have bigger, smooth-walled conidia. Similarly, isolate LY1L5 could not be matched with other cycloaspeptide A-producing *Penicillia*. Hence, further work needs to be conducted to identify the species of isolate LY1L5.

Conclusion

The results from this study showed that salinity affected the growth of *Penicillium* sp. (LY1L5); however, a more extensive study needs to be done to obtain more comprehensive results because growth may also be dependent upon other parameters

such as temperature whether or not adaptation by the fungi to salinity has occurred. The findings also showed that naturally occurring metabolites of marine-derived microorganisms have a great potential to produce metabolites enabling the discovery of new bioactive metabolites and hence merit future studies.

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