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SHORT COMMUNICATION

Antibacterial activity of cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) from *Streptomyces* sp. strain 22-4 against phytopathogenic bacteria

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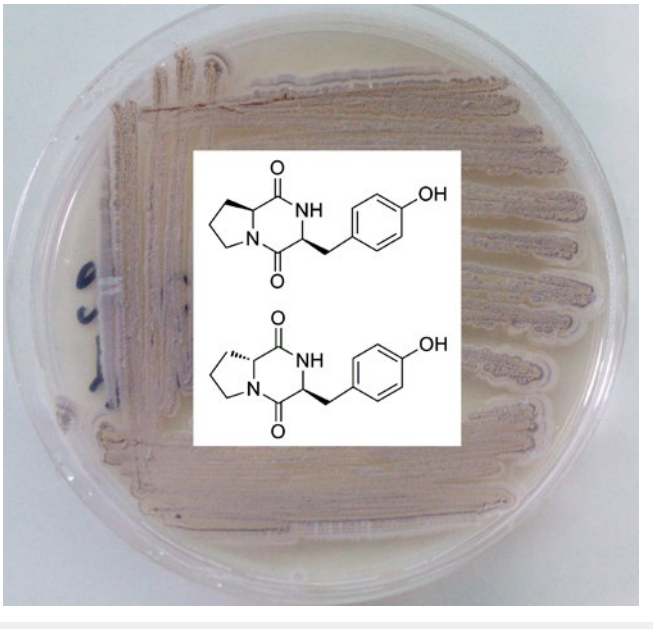
ABSTRACT

Two bioactive cyclic dipeptides, cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr), were isolated from the culture broth of *Streptomyces* sp. strain 22-4 and tested against three economically important plant pathogens, *Xanthomonas axonopodis* pv. citri, *Ralstonia solanacearum* and *Clavibacter michiganensis*. Both cyclic dipeptides were active against *X. axonopodis* pv. citri and *R. Solanacearum* with MIC of 31.25 μg/mL. No activity could be observed against *C. michiganensis*.



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1. Introduction

Screening for bioactive compounds from natural resources to use for medical and agricultural purposes is one of the major goals in medicinal chemistry. One of the major sources of these compounds is *Streptomyces,* a large genus bacteria that produce a vast diversity of secondary metabolites (Lucas et al. 2013), including cyclic dipeptides (Zhou et al. 2014). This class of metabolites contains a diketopiperazine core built with D and L amino acids, that displays a broad spectrum of biological activities including antimicrobial activity (Martins & Carvalho 2007).

Two promising cyclic dipeptides exhibiting potent antimicrobial activity are based on the cyclo(L-Pro-L-Tyr) **(A)** and cyclo(D-Pro-L-Tyr) **(B)** scaffolds (Figure 1). To date, only cyclo(L-Pro-L-Tyr) has been isolated from *Streptomyces* and here we report the isolation and molecular characterisation of both diastereomers produced by *Streptomyces* sp. strain 22-4. Furthermore, we also report the first antibacterial studies of both cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) against three economically important phytopathogenic bacteria.

2. Results and discussion

2.1. Isolation and structure determination of cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr)

From the culture broth of *S.* sp. strain 22-4, a mixture containing cyclo(L-Pro-L-Tyr) and cyclo (D-Pro-L-Tyr) was isolated. Separation of cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) proved to be difficult by HPLC as both diastereoisomers eluted at 25% MeOH/H_2O. However, their structures were elucidated by HR-MS (Figure S1) and NMR (Figures S2–S7) in combination with Marfey's method (Figure S8). The structures were further confirmed by comparison

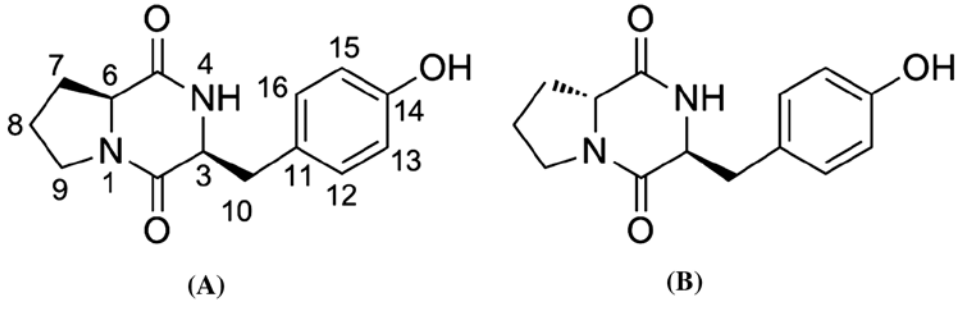

Figure 1. Structures of **(A)** cyclo(L-Pro-L-Tyr) and **(B)** cyclo(D-Pro-L-Tyr).

Table 1. Antibacterial activity of cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr).

	Test bacteria with MIC (μg/mL)	
Compound	*X. axonopodis* pv. citri	*R. solanacearum*
Cyclo(L-Pro-L-Tyr)	31.25	31.25
Cyclo(D-Pro-L-Tyr)	31.25	31.25
Chlortetracycline	0.12	–
Streptomycin sulphate	–	3.91

with the ^{1}H spectra of synthetic standards (Figure S2). To our knowledge, this is the first time that both compounds have been isolated from *S*. sp strain 22-4. As several cyclic dipeptides have been previously reported from production broths (Prasad 1995), the medium alone was extracted and carefully investigated by HPLC to confirm that neither cyclo(L-Pro-L-Tyr) or cyclo(D-Pro-L-Tyr) were present.

2.2. Antibacterial activity against plant pathogenic bacteria

Previously, both cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) have been isolated from several micro-organisms including *Haloterrigena hispanica* and *Bacillus* sp. N strain with antifungal and antibacterial activities (Nishanth Kumar et al. 2012; Tommonaro et al. 2012; Kumar et al. 2013). To the best of our knowledge, this is the first time that the cyclo(D-Pro-L-Tyr) has been isolated from *Streptomyces*. Due to the antimicrobial activity of both cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr), this led us to assess the biological activity on untested phytopathogenic bacteria. Bacterial wilt and canker in particular are important diseases in commercial crop plants, causing significant economic losses worldwide. In this work, both cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) were tested against *Xanthomonas axonopodis* pv. citri, *Ralstonia solanacearum* and *Clavibacter michiganensis*. *X. axonopodis* pv. citri is an extremely persistent causative agent of bacterial canker in citrus (Brunings & Gabriel 2003), whilst *R. solanacearum* and *C. michiganensis* cause bacterial wilt in plants of the *Solanaceae* family (Hayward 1991; Gleason et al. 1993). Both compounds exhibited activity against *X. axonopodis* pv. citri and *R. solanacearum,* albeit weak (MIC 31.25 μg/mL) when compared to standard antibacterial agents (Table 1). No antibacterial activity was observed against *C. michiganensis* even at relatively high concentrations (up to 500 μg/mL).

Although there is currently a small trade-off with potency, the broad spectrum of antimicrobial activity of cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) however means these compounds may have potential for use as agricultural biocontrol agents. This can be seen from a chitinase-producing *Streptomyces glauciniger* WICC-A03 (Awad et al. 2014) or a related compound, cyclo(4-hydroxy-L-Pro-L-Trp), which has already been deployed as a non-toxic biopreservative agent to inhibit the growth of *A. flavus* and *A. niger* on peanut kernels (Kumar et al. 2014). With further toxicity testing and structure modification to improve the biological activity of cyclo(Pro-Tyr), this cyclic dipeptide may be a promising biocontrol agent for use in the agricultural industry.

3. Experimental

Experimental information is provided in Supplementary material.

4. Conclusion

This is the first time that both cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) were isolated from *S*. sp. strain 22-4 and we are the first to show their antibacterial activity against economically phytopathogenic bacteria *X. axonopodis* pv. citri and *R. Solanacearum*. With the use of toxic chemicals in agricultural industry to prevent the crop damage, the use of cyclic dipeptides as biological control agents may provide a sustainable approach to improve the crop quality without damaging the environment or human health.

Disclosure statement

No potential conflict of interest was reported by the authors.

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