Secondary metabolites produced by endophytic *Pantoea ananatis* derived from roots of *Baccharoides anthelmintica* and their effect on melanin synthesis in murine B16 cells

Nigora Rustamovaa,b, Khayrulla Bobakulovac, Nurmirza Begmatovac, Ablajan Turakaa, Abulimiti Yilii, and Haji Akber Aisaa

aKey Laboratory of Plant Resources and Chemistry in Arid Regions, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, Xinjiang, PR China; bUniversity of Chinese Academy of Sciences, Beijing, PR China; cInstitute of the Chemistry of Plant Substances, Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

**ABSTRACT**

Five indole derivatives, 1H-indol-7-ol (1), tryptophol (2), 3-indolepropionic acid (3), tryptophan (4), 3,3-di(1H-indol-3-yl)propane-1,2-diol (5) and two diketopiperazines, cyclo(L-Pro-L-Tyr) (6), cyclo[L-(4-hydroxyprolynly)-L-leucine (7) along with one dihydrocinnamic acid (8) were isolated from *Pantoea ananatis* VERA8, that endophytic bacteria derived from *Baccharoides anthelmintica* roots. This is a first report towards an isolation of endophytic strains (funji or bacteria) from the *B. anthelmintica* herb. The synergistic properties of the total extract compositions, as well as effects of the pure isolated secondary metabolites evaluated on their melanin synthesis in murine B16 cells towards for vitiligo treatment.

**CONTACT** Abulimiti Yili abu@ms.xjb.ac.cn

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1. Introduction

*Baccharoides anthelmintica* (also named *Vernonia anthelmintica*) is a known medicinal plant that only grows in high-altitude areas of southern Xinjiang province of PR China (Liu, Wang, et al. 2018), and small regions in Pakistan and India (Turak et al. 2017). This medicinal plant metabolites has gained much attention due to its use as antidiabetic (Arumugam et al. 2013), anthelmintic (Jaiswal 2010), antibacterial (Hua et al. 2012) potentials and also used in ethnoveterinary medicine to control GIT infections, halitosis, indigestion and pneumonia (Muhammad et al. 2005). In particular, the confirmation of the antidiabetic properties of this plant *in vitro* and *in vivo*, a clinical trial to determine the efficacy in humans is recommended. Except of antidiabetic properties, the isolates of *B. anthelmintica*, especially terpenes and flavonoids demonstrating perspective effects on melanin synthesis on murine B16 cells towards for vitiligo treatment (Liu, Wang, et al. 2018; Maimaiti et al. 2017; Turak et al. 2017). A microbiological and biochemical aspect of such as medicinal plants seems an interesting approach in order to understanding their biosynthesis, as well as could be suggest us an alternatively bio-sources. In this way, bacterial and fungal endophytes are one of the rich sources in order to produce bioactive natural metabolites from medicinal plants (Jin et al. 2017; Li et al. 2018). Besides, bacterial endophytes ubiquitously colonize the internal tissues of plants, being found in nearly every plant worldwide (Santoyo et al. 2016), sometimes in high numbers, without damaging the host or eliciting strong defense responses (Reinhold-Hurek and Hurek 2011). Recently, a lot of new and known secondary metabolites from bacteria or fungi have been isolated and reported to provide lead compounds for new drug discovery (Martinez-Klimova et al. 2017; Palanichamy et al. 2018; Wang et al. 2018). To our knowledge there are no any reports regarding metabolite produced by microbial endophytes isolated from *B. anthelmintica* genus.

Herein, an endophytic bacterium *Pantoea ananatis* VERA8 was isolated from freshly *B. anthelmintica* roots collected from the Hotan region of the Xinjiang Province, PR China. All in all, a crude extract of the VERA8 strain in ethyl acetate fraction revealed that after fermentation, an isolated strain lead to two types of known alkaloids, along with one 3-phenylpropanoic acid. The synergetic properties of the total extract compositions, as well as effects of the individually isolated secondary metabolites evaluated on their melanin synthesis in murine B16 cells.

2. Results and discussion

2.1. Identification of isolated bacterial endophyte *P. ananatis* VERA8

The endophytic bacteria was isolated from the roots of *B. anthelmintica* and collected from Hotan region of the Xinjiang Province, PR China (plant identified by professor Feng Ying, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences). This plant brought to our laboratory Basis of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences. The purified strain was identified as *P. ananatis* VERA8 (GenBank accession number MK478842) on the basis of the morphological method
and reinforced by 18S rDNA and internal transcribed spacer (ITS) sequences with 98.5% identity to the known *P. ananatis* 1846 (NR_026045.1).

### 2.2. Structure characterization of the isolated compounds

The bacterial culture (after fermentation) was extracted with ethyl acetate to afforded total 7 g of a crude extract. The extract was column chromatographed over silica gel (200–300 mesh, 0.3 kg), using dichloromethane and methanol (from 1:0 to 0:1 v/v) as eluents, to give fractions E1-E11. Further purifications were carried out by octadecylsilyl silica gel column chromatography (ODS-A 120c), Sephadex LH-20, and semi-preparative HPLC to afford eight compounds. The chemical structures of all pure compounds were elucidated by analyzing their spectroscopic data (LC-MS, $^1$H NMR, $^{13}$C NMR and 2D NMR) in addition by comparison with the reported literatures. Thus, isolated compounds were identified as five indole alkaloids: $1H$-indol-7-ol (1) (van den Berg et al. 1990), tryptophol (2) (Sugiyama et al. 2009), 3-indolepropionic acid (3) (Chyan et al. 1999), tryptophan (4) (Akita et al. 2014; Bradbury and Norton 1973; Liu, Xu, et al. 2018), 3,3-di($1H$-indol-3-yl)propane-1,2-diol (5) (Wu et al. 2017), two diketopiperazines: cyclo(L-Pro-L-Tyr) (6) (Sugiyama, Ito, Suzuki and Hirota 2009), cyclo[L-(4-hydroxyprolinyl)-L-leucine (7) (Cronan et al. 1998) and one dihydrocinnamic acid (8) (Filho et al. 2004) (Figure 1). These secondary metabolites (1–8) have isolated from *P. ananatis*, which bacterium endophyte derived from the *B. anthelmintica* herb for the first time.

### 2.3. Biological evaluation

#### 2.3.1. Effect of secondary metabolites of endophytic bacteria in melanin synthesis on B16 melanoma cells

An ethyl acetate fraction of the crude extract by bacterium endophyte *P. ananatis* was screened on melanin synthesis in murine B16 cells, and synergetic effects showed that secondary metabolites influenced the activity on melanin synthesis ($117.22 \pm 6.93\%$ at 1 µM, $121.76 \pm 8.18\%$ at 100 µM and $144.60 \pm 8.95\%$ at 50 µM), compared with reference drug 8-methoxypsoralen (8-MOP) ($126.31 \pm 7.41\%$ at 50 µM) (Table S1). In order to understanding which metabolite influenced on activity, we further decided to evaluate...
each isolated secondary metabolites effect on melanin synthesis in murine B16 cells using a previously published method (Nie et al. 2017; Nie, Bozorov, et al. 2018; Nie, Huang, et al. 2018). According to the results shown in Figure S1 (SI, Figure S1), in general, indole isolates 1–4 exhibited lower activity (from 98.41 ± 5.53 to 108.95 ± 6.37%), however 3,3-di(1H-indol-3-yl)propane-1,2-diol (5) satisfactorily increased the activity in percentage 117.27 ± 7.57. Similar result showed cyclo(L-Pro-L-Tyr) (6, 113.19 ± 5.30%) and dihydrocinnamic acid (8, 115.19 ± 6.53%). It was observed that the activity of cyclo[L-(4-hydroxyprolinyl)-L-leucine (7) (139.66 ± 3.53%) was stronger than 8-MOP (128.73 ± 4.49%) on melanin synthesis in murine B16 cells.

3. Conclusion
Medicinal plant such as B. anthelmintica’s endophytic strains did not studied until now and herein we have identified P. ananatis VERA8, an endophytic bacterium isolated from B. anthelmintica roots for the first time. An ethyl acetate fraction of the endophytic P. ananatis, yielded secondary metabolites, such as indole compounds (1–7) and dihydrocinnamic acid (8), respectively. In addition, a crude extract of bacterium endophyte and its isolates influenced on melanin synthesis in murine B16 cells. Metabolite cyclo[L-(4-hydroxyprolinyl)-L-leucine (7) increased a melanin contents than 8-MOP. These results suggest that the biological role of the B. anthelmintica derived microbes, in particular fungal endophytes and its metabolites remains to be investigated in future studies.

Disclosure statement
No potential conflict of interest was reported by the authors.

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ORCID
Nigora Rustamova http://orcid.org/0000-0002-5731-9819
Khayrulla Bobakulov http://orcid.org/0000-0001-8924-4279
Abulimiti Yili http://orcid.org/0000-0002-3435-8372
Haji Akber Aisa http://orcid.org/0000-0003-4652-6879

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