Journal of Microbiology, Immunology and Infection (2016) 49, 8–15

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Anti-quorum sensing activity of flavonoid-rich fraction from *Centella asiatica* L. against *Pseudomonas aeruginosa* PAO1

H.S. Vasavi, A.B. Arun, P.D. Rekha*

Yenepoya Research Centre, Yenepoya University, Deralakatte, Mangalore, India

Received 21 February 2014; received in revised form 11 March 2014; accepted 26 March 2014
Available online 22 May 2014

KEYWORDS
Centella asiatica;
Pseudomonas aeruginosa;
quorum sensing;
virulence

Background/Purpose: Inhibition of quorum sensing (QS), a cell-density dependent regulation of gene expression in bacteria by autoinducers is an attractive strategy for the development of antipathogenic agents.
Methods: In this study, the anti-QS activity of the ethanolic extract of the traditional herb *Centella asiatica* was investigated by the biosensor bioassay using *Chromobacterium violaceum* CV026. The effect of ethyl acetate fraction (CEA) from the bioassay-guided fractionation of ethanol extract on QS-regulated violacein production in *C. violaceum* ATCC12472 and pyocyanin production, proteolytic and elastolytic activities, swarming motility, and biofilm formation in *Pseudomonas aeruginosa* PAO1 were evaluated. Possible mechanism of QS-inhibitory action on autoinducer activity was determined by measuring the acyl homoserine lactone using *C. violaceum* ATCC31532. Anti-QS compounds in the CEA fraction were identified using thin layer chromatography biosensor overlay assay.
Results: Ethanol extract of *C. asiatica* showed QS inhibition in *C. violaceum* CV026. Bioassay-guided fractionation of ethanol extract revealed that CEA was four times more active than the ethanol extract. CEA, at 400 μg/mL, completely inhibited violacein production in *C. violaceum* ATCC12472 without significantly affecting growth. CEA also showed inhibition of QS-regulated phenotypes, namely, pyocyanin production, elastolytic and proteolytic activities, swarming motility, and biofilm formation in *P. aeruginosa* PAO1 in a concentration-dependent manner. Thin layer chromatography of CEA with biosensor overlay showed anti-QS spot with an R_f value that corresponded with that of standard kaempferol.
Conclusion: The anti-QS nature of *C. asiatica* herb can be further exploited for the formulation of drugs targeting bacterial infections where pathogenicity is mediated through QS.

* Corresponding author. Yenepoya Research Centre, Yenepoya University, University Road, Deralakatte, Mangalore 575018, Karnataka, India.
E-mail addresses: dydirectoryrc@yenepoya.edu.in, rekhapd@hotmail.com (P.D. Rekha).

http://dx.doi.org/10.1016/j.jmii.2014.03.012

Introduction

Bacterial populations coordinate communal behavior through a process of cell-to-cell signaling mediated by diffusible signal molecules.[1] This process, termed quorum sensing (QS), is known to control gene expression responsible for diverse physiological functions including virulence, antibiotic production, and biofilm formation.[2] Gram-negative bacteria use a QS system mediated by diffusible signaling molecules of the *N*-acyl homoserine lactones (AHL) family.[3] Many pathogenic bacteria use a QS system to regulate genes required for the expression of virulence, thus, inhibition of the QS system is considered as a novel strategy for development of antipathogenic agents, especially for combating bacterial infections caused by antibiotic-resistant strains.[4]

In the past few years, inhibition of QS has become an intense area of research because of its applications in medicine, industry, and biotechnology. In the quest for QS inhibitors, studies have demonstrated that many eukaryotes, particularly plants, and even bacteria themselves produce anti-QS substances.[5,6] Ajoene from garlic, catechin from *Combretum albiflorum*, and iberin from horseradish specifically inhibit QS in reporter strains.[7,8]

As an adaptive evolution, many plant species produce metabolites that can control the growth of microbes and have traditionally been used to treat human diseases, particularly microbial infections. *Centella asiatica* (L.) Urban is used as a medicinal herb in Ayurvedic medicine, traditional African medicine, and traditional Chinese medicine. *C. asiatica* is one of the chief herbs for treating skin problems, healing wounds, as well as being an antibacterial and antiviral agent.[9] The therapeutic substances in *C. asiatica* are saponin-containing triterpene acids and their sugar esters, of which asiatic acid, madecassic acid, and asiaticosides are considered to be the most important.[10] In this study, anti-QS potential of *C. asiatica* was investigated using *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1. In *C. violaceum*, the LuxR homolog, CviR regulates the production of a purple pigment violacein.[11] *P. aeruginosa* PAO1, an opportunistic pathogen, utilizes two interrelated QS systems, LasI/R regulates the production of LasB elastase, LasA protease, Apr alkaline protease, and exotoxin A through 3-oxo-C_{12}-homoserine lactone and RhlI/R which regulates pyocyanin production, rhamnolipids, hydrogen cyanide, and cytotoxic lectins through C_4-homoserine lactone.[3]

Methods

Bacterial strains, media, and culture conditions

Bacterial strains used in this study were *C. violaceum* ATCC12472, *C. violaceum* ATCC31532, a mini-Tn5 mutant *C. violaceum* CV026, and *P. aeruginosa* PAO1. All the bacterial strains were grown in Luria—Bertani (LB) medium at 32°C for 24 hours. When required, the medium for *C. violaceum* CV026 was supplemented with hexanoyl homoserine lactone (C_6-HSL; Sigma—Aldrich, St Louis, MO, USA). For pyocyanin assay, *P. aeruginosa* PAO1 was grown in glycerol alanine minimal medium. Tryptone broth was used for biofilm assays. For all the experiments, inocula were prepared by growing the bacteria in 10 mL LB broth under shaking (130 rpm) for 24 hours, and the cell density was measured spectrophotometrically (UV-1800; Shimadzu, Kyoto, Japan).

Plant materials and extract preparation

Fresh plants of *C. asiatica* were collected from the areas around the paddy farms of Mangalore, Karnataka, India. Samples were authenticated in the Department of Botany, Mangalore University, Mangalore, India. Leaves were washed in sterile water, shade dried, and powdered using an analytical mill (IKA, Staufen, Germany). For the preparation of ethanol extract, 100 g powdered leaves was extracted with 1 L 90% ethanol in a soxhlet extractor at 70°C for 16 hours and concentrated by vacuum evaporation to obtain a viscous residue.

Biosensor bioassay for the detection of anti-QS activity

Anti-QS activity of *Centella asiatica* extract was detected by bioassay using the reporter strain *Chromobacterium violaceum* CV026.[12] Different concentrations of ethanol extract of *Centella asiatica* (100—1000 μg/disc) were loaded onto 6-mm sterile discs (Himedia, Mumbai, India) and placed on the surface of *C. violaceum* CV026-inoculated LB agar plates supplemented with 50 μL 1 μg/mL C_6-HSL and incubated for 24 hours. Discs loaded with ethanol were included as negative controls. Inhibition of QS was detected by the presence of a zone of colorless but viable cells around the disc.

Bioassay guided fractionation

The ethanol extract of *Centella asiatica* was exhaustively treated with *n*-hexane. The hexane layer was recovered and the remaining extract was simultaneously treated with water, mixed well to resolve into water-soluble and water-insoluble parts. The water-soluble fraction was then exhaustively partitioned with ethyl acetate and the final ethyl acetate fraction was separated from the water-soluble fraction. The ethyl acetate fraction was concentrated by vacuum evaporation and the remaining aqueous portion was lyophilized and collected as an aqueous fraction. All the fractions were concentrated and subsequently tested for anti-QS activity by *Chromobacterium violaceum* CV026 biosensor bioassay. Ethyl acetate fraction showing strong anti-QS activity (CEA) was used for further experiments at the concentration of 25—400 μg/mL and its effect on bacterial growth was also tested by standard plate count method.

Quantitative QS inhibition assay

The effect of CEA on the QS-controlled violacein production in *C. violaceum* ATCC12472 was determined as described previously with some modifications.[13] Briefly, 10 mL LB broth containing different concentrations of CEA was inoculated with 100 μL *C. violaceum* ATCC12472 (10^6 CFU/mL). Solvent control was prepared similarly and all the