ELSEVIER

Archives of Medical Research 42 (2011) 658–668

Archives of Medical Research

ORIGINAL ARTICLE

Antiquorum Sensing and Antibiofilm Potential of *Capparis spinosa*

Sybiya Vasantha Packiavathy Issac Abraham,[a] Agilandeswari Palani,[a] Babu Rajendran Ramaswamy,[b] Karutha Pandian Shunmugiah,[a] and Veera Ravi Arumugam[a]

[a]*Department of Biotechnology, Alagappa University, Karaikudi, Tamil Nadu, India*
[b]*Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India*

Received for publication July 26, 2011; accepted December 8, 2011 (ARCMED-D-11-00362).

Background. Emergence of antibiotic resistance among bacterial pathogens often leads to the failure of existing antibiotics to treat bacterial infections; thus, there is a need to seek alternative treatment measures. The aim of this study was to evaluate the anti-quorum sensing (anti-QS) and antibiofilm potential of *Capparis spinosa* to prevent the onset of bacterial infections as an alternate to antibiotics.

Methods. The methanolic extract of the dried fruits of *C. spinosa* was assessed for its activity in inhibiting QS-depedent phenomenon such as violacein pigment production in *Chromobacterium violaceum,* biosurfactant production in *Pseudomonas aeruginosa* PAO1, swimming and swarming motility, exopolysaccharide production (EPS) and biofilm formation in *Escherichia coli*, *Proteus mirabilis, Serratia marcescens* and PAO1.

Results. Extract of *C. spinosa* showed a higher degree of anti-QS activity in a dose dependent manner without affecting the bacterial growth. At 2 mg/mL, this extract significantly ($p \leq 0.005$) inhibited the biofilm formation to 79, 75, 73, 70% and EPS production to 58, 46, 66 and 67% in *S. marcescens,* PAO1, *E. coli* and *P. mirabilis,* respectively. It also exhibited inhibition in swimming and swarming motility of bacterial pathogens. The non-enzymatic nature of the anti-QS compound in *C. spinosa* was confirmed by proteinase K and heat treatment.

Conclusions. Because the methanolic extract of *C. spinosa* demonstrated anti-QS and antibiofilm activity at 0.5–2 mg/mL, it could be further exploited for novel molecules to treat the emerging infections of antibiotic resistant bacterial pathogens.

Key Words: Capparis spinosa, Antiquorum sensing, Biofilm, Exopolysaccharides, Motility, Vanillic acid.

Introduction

Biofilm formation and antibiotic resistance among bacterial pathogens represent a major hurdle in human health (1). A report from the U.S. National Institutes of Health states that >80% of microbial infections are biofilm based (2). Biofilms are structural communities encased in a self-secreted exopolymeric substance (EPS). It has been reported that bacteria living within biofilm are 1,000-fold more resistant to antibiotics and are inherently insensitive to the host immune response (3). Several studies have shown that continuous treatment with conventional antibiotics lead to the development of resistance among the bacteria because they possess a broad-range efficacy via toxic or growth-inhibitory effects on target organisms (4). Increasing occurrence of such resistance among pathogenic bacterial strains has gradually rendered traditional antibiotic treatment ineffective. As a consequence, researchers have attempted to propose a number of alternatives to synthetic antibiotics including: phage therapy (5,6), probiotics (7) and human antimicrobial peptides (8). Unfortunately, most of these alternatives are based on the mechanism of killing or terminating the target bacteria. Instead, searching for biofilm control by means of signal molecule-based drugs seems to be an attractive goal that

Address reprint requests to: Veera Ravi Arumugam, Dr. Assistant Professor, Department of Biotechnology, Alagappa University, Karaikudi-630 003, Tamil Nadu, India; Phone: +91-4565-225215; FAX: +91-4565-225202; E-mail: aveeraravi@rediffmail.com

0188-4409/$ - see front matter.
doi: 10.1016/j.arcmed.2011.12.002

mainly relies on the interference of bacterial quorum sensing (QS) through which the development of biofilm and virulence are being accomplished.

QS is a widespread prokaryotic intercellular communication system based on the signal molecules (autoinducers) relative to cell density (9). QS plays a vital role in biofilm formation and virulence factor production in several bacterial species (10). Consequently, compounds that interfere with the QS system to attenuate bacterial pathogenicity are termed as anti-QS compounds. Such compounds neither kill the bacteria nor stop their growth and are less expected to develop resistance towards antibiotics. In biofilms, the EPS represents the major structural component and is responsible for the interaction of microbes as well as with interfaces (11). It also plays an important role in microcolony formation and maintenance of biofilm architecture. Similarly, flagellar-dependent swimming and swarming motilities of Gram-negative bacterial pathogens have been linked to biofilm formation by initiating cell-to-surface contact and microcolony formation (12). Biosurfactant production also becomes essential in maintaining biofilm architecture and allows flagellum-based propulsion over semisolid surfaces to form dense biofilm (13). Because factors such as EPS, biosurfactants, and swimming and swarming motilities are mediated by QS and act as key players in the development of biofilm, targeting these factors through anti-QS strategies will lead to the prevention of biofilm.

Among all the possibilities to inhibit QS activity, use of anti-QS compounds may be of great interest to avoid bacterial infections. Furanones isolated from the marine red algae *Delisea pulchra* are one of most extensively studied anti-QS compounds for their role in inhibiting biofilm. Plant-derived compounds such as oroidin (14), urosolic acid (15), naringenin (16), cinnamaldehyde (17), salicylic acid (18), methyl eugenol (19) and extracts from garlic (20) and edible fruits (21) have shown various extents of antibiofilm properties against several pathogens.

Thus, plant-derived dietary products have attracted widespread interest in the search of alternatives for microbial control. However, these compounds are widely considered due to their safety and have a long history of use in traditional medicine for the prevention and treatment of diseases and infections (19,21). In this context, the present study was initiated with the aim of screening anti-QS compounds from *Capparis spinosa*. This spice is reported to have a number of potentially useful medicinal attributes including antioxidative (22), antifungal (23), anti-inflammatory (24), anti-diabetic (25) and anti-obesity (26). In India, buds and roots of *C. spinosa* are being used in the regular diet and the plant is known for its medicinal properties in the treatment of boils. Leaves are used as a counter-irritant and as a cataplasm in swellings. Roots are used to treat fever, rheumatism, paralysis, toothache and kill ear worms. The bark is used in the treatment of coughs, asthma and inflammation (27). But so far, investigations have not yet been reported about its anti-QS and antibiofilm activity. This study was therefore undertaken to investigate for the first time the *in vitro* antibiofilm potential of *C. spinosa* by targeting the QS mechanisms of Gram-negative bacterial pathogens.

Materials and Methods

Bacterial Strains and Culture Conditions

All the bacterial strains used in this study such as *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* PAO1, *Proteus mirabilis* (ATCC 7002), *Serratia marcescens* (FJ584421), *Chromobacterium violaceum* (ATCC 12472) and *C. violaceum* CV026 were cultivated and maintained in Luria−Bertani (LB) medium. All strains were maintained at 37°C except *S. marcescens* and *C. violaceum*, which were maintained at 30°C.

Extraction of Anti-QS Compound from C. spinosa

Dried fruits of *C. spinosa* used in the present investigation were purchased from local outlets. Extraction was carried out by following the method of Choo et al. (28) with little modifications. Five grams of powdered dried fruits from *C. spinosa* was soaked in 50 mL of methanol overnight; then the methanol phase was collected and dried at 55°C. Residues were redissolved with deionized water and stored at −20°C until further use.

Violacein Inhibition Assay

C. violaceum (ATCC 12472) was used in qualitative screening and further quantification of violacein inhibition was carried out using *C. violaceum* (CV026), a mini-Tn5 mutant enabling detection of violacein in response to addition of exogenous N-hexanoyl-L-homoserine lactone (C6-HSL). In qualitative screening, 10 µL of overnight culture of *C. violaceum* ATCC 12472 (OD adjusted to 0.4 at 600 nm) was added into wells of sterile microtiter plates (MTPs) containing 1 mL of LB broth and incubated in the presence and absence of various concentrations of test extracts (0.5−2 mg/mL) at 30°C for 16 h and observed for inhibition in violacein pigment production.

Quantification of violacein production was carried out by performing the same experiment in *C. violaceum* CV026 with the exogenous supply of C6-HSL (Sigma-Aldrich, Steinheim, Switzerland) at a working concentration of 5 µM. One mL culture from each well was centrifuged at 10,000 rpm for 10 min to pelletize the insoluble violacein along with bacterial cells. The culture supernatant was discarded and the cell pellet was resuspended in 1 mL of dimethyl sulfoxide to extract the violacein. The suspension was vortexed vigorously for 30 sec and centrifuged at 10,000 rpm for 10 min to remove the cells and the extracted violacein was quantified spectrophotometrically (Hitachi U-2800, Japan) at 585 nm (28).