Selected Phytochemical Bioactive Compounds as Quorum Sensing Inhibitors

P. Truchado, F.A. Tomás-Barberán and A. Allende
Research Group on Quality, Safety and Bioactivity of Plant Foods
CEBAS-CSIC
Espinardo, Murcia
Spain

A. Ponce
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
Buenos Aires
Argentina

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Abstract

Bacterial infections remain an important problem for human health. The control of bacterial infections has been traditionally treated by inhibiting microbial growth using different types of antibiotics. However, the ability of different bacteria to resist the inhibitory action of antibiotics has become a global problem. In fact, there is an important need for the development of new antimicrobials that act on novel bacterial targets. Many pathogenic bacteria control their population and regulate gene expression in response to their cell population density using diffusible signalling compounds. This type of communication has been referred to as "quorum sensing" (QS). This phenomenon can be essential for the synchronization of the virulence production factors, which make it an attractive therapeutic target. Therefore, the search of non-toxic compounds, which inhibit QS and so, the virulence of pathogenic bacteria can bring new alternatives for the treatment of bacterial infections in humans.

In this work, we made an attempt to screen the anti-QS activity of 11 bioactive compounds extracted from fruits and vegetables using the biosensor strain, *Chromobacterium violaceum*. The anti-QS activity was determined quantifying the violacein production of the biosensor strain at three concentrations (50, 100 and 200 µg/ml). At least five of the tested compounds (cinnamaldehyde, pomegranate extracts, ellagic acid resveratrol and rutin) showed anti-QS activity against the biosensor strain. The obtained results showed the potential of bioactive compounds extracted from fruits and vegetables to be used as a new category of anti-pathogenic compounds against bacterial infections.

INTRODUCTION

Quorum sensing (QS) or cell to cell communication is a mechanism in which bacteria coordinate the expression of certain genes in response to their population density (Fuqua et al., 1997). This bacteria communication system is mediated by numerous signalling molecules of low molecular-mass called autoinducer that spread to environment. In Gram negative bacteria, these autoinducer are N-acyl-homoserine lactones (AHLs) (Parsek and Greenberg, 2000). AHLs coordinate gene expression and in some cases, controls a wide variety of prokaryotic phenotypes including enzyme secretion, virulence factor production, bioluminescence, swarming and biofilm development (Whitehead et al., 2001; Rudrapa et al., 2008). For these reasons, the search of anti-QS compounds offers a new alternative on the application of natural extracts as therapeutic agents. Phytochemical compounds could be used as potential QS interrupters to reduce pathogenicity and control bacterial infections (Fig. 1).

In most of the cases, different biosensor bacteria are used to determine the potential of phytochemical bioactive compounds as QS inhibitor, among them *Chromobacterium violaceum* is the most commonly used (Bodini et al., 2009; Steindlerl and Venturi, 2006). *C. violaceum* is a Gram negative bacteria which synthesizes the violet pigment violacein as a response of QS system (Lichstein and van de Sand, 1945). The QS

in *C. violaceum* is regulated by N-hexanoyl homoserine lactone (C6-HSL). C6-HSL is produced by the autoinducer synthase CviI, released to the environment and diffused back into the bacteria when a quorum has been reached. The molecular signal then binds to the transcriptional regulator CviR and participates in the expression of specific genes such as the gene, which regulates violacein production (McClean et al., 1997).

In a recent work, we determined that natural food as honey influenced in the QS system of different pathogenic and spoilage bacteria (Truchado et al., 2010). Taking into account these results, the objective of the present study was to determine the potential use of different phytochemical bioactive compounds as QS inhibitors of the biosensor bacteria *C. violaceum*.

MATERIALS AND METHODS

Strains and Culture Conditions

Chromobacterium violaceum (CECT 494) was obtained from the Spanish Type Culture Collection (Valencia, Spain). Stocks of the strains were stored at -80°C in Luria-Bertani broth (LB broth acc. to MILLER) (Scharlau Chemie, S.A. Barcelona, Spain) with 30% glycerol. When required, strain was routinely grown aerobically with shaking in LB broth. The strain was incubated at 30°C for 24 h. The pH of the culture media was 7.1.

Quorum Sensing Inhibitors

Resveratrol, ellagic acid, gallic acid, cholorogenic acid, kinurenic acid and rutin were obtained from Sigma Aldrich (Sigma Chemical, St. Louis, MO). Cinnamaldehyde acid, daidzein, dimethylesculetin, were purchased from Extrasynthèse (Genay, France) and vanillic acid from Merck (Darmstadt, Germany). The pomegranate extract (PE) "Nutragranate" was obtained from Nutracitrus S.L. (Elche, Spain). Dimethyl sulfoxide (DMSO)-d6 was obtained from Merck (Darmstadt, Germany). Milli-Q water was used throughout. Phenolic compounds and PE were initially dissolved in DMSO (maximum concentration of 0.1%) and filtered through a sterile Millex-GP 0.22 µm filter (Millipore Corp., USA) (Table 1). A control solution containing only DMSO was made in MilliQ water. The selected compounds were added to the culture medium to obtain concentrations of 50, 100 and 200 µg/ml, depending on the bioactive phytochemical. The stock solutions of each compound were stored at -20°C for further analysis.

Anti-QS Activity Assays

Phenolic compounds and PE were tested for their ability to inhibit the production of purple pigment violacein in *C. violaceum* as previously described (Truchado et al., 2009). The strain was routinely cultured aerobically in LB at 30°C with or without the addition of different concentrations of the bioactive phytochemicals.

Quantification of Violacein Production

One ml of overnight culture of the reported strain was centrifuged (13000 rpm, 10 min) to precipitate the insoluble violacein. The culture supernatant was discarded and 1 ml of DMSO was added to the pellet. The solution was centrifuged (13000 rpm, 10 min) to remove the cells and then, the violacein was quantified at OD585 using a UV-Vis spectrophotometer (Hewlet Packard 8453).

Statistical Analysis

The differences in the growth, violacein inhibition with and without the addition of bioactive phytochemicals were calculated using ANOVA and Brown-Forsythe tests, depending on the homogeneity of the variances. When significant differences were observed, Tukey's Multiple Range Test or Dunnett T3 was performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). A significance level of $P \leq 0.001$ was selected to determine differences between samples. All experiments were performed in triplicate.

RESULTS

The quantification of violacein production by the biosensor strain *C. violaceum* was carried out using a quantitative method to assess the anti-QS activity of natural bioactive compounds isolated from different fruits and vegetables. A total of 11 bioactive compounds were tested and compared to a positive control, where the violacein production was not affected by the presence of any compound (Table 1). It was observed that most of the tested compounds, including the pomegranate extract, were effective reducing or degrading the violacein produced by the biosensor strain at the minimum tested concentration (50 µg/ml) (Table 2). In fact, at 50 µg/ml, resveratrol, cinnamaldehyde ellagic acid and PE significantly reduced the violacein production. However, rutin (50 µg/ml) was the less effective compound as it only decreased the violacein production by 2%. On the other hand, all the tested compounds were able to reduce the production of violacein at the maximum tested dose (200 µg/ml) (Table 2). Thus, they can be potentially used as QS inhibitors of *C. violaceum*.

The reduction in the amount of violacein extracted from an overnight culture of the biosensor strain could be also due to a reduction in bacterial growth. Thus, to determine the main reason of the violacein inhibition, the antimicrobial activity of these compounds at the tested concentrations was determined. Using a broth assay at different concentrations (50, 100 and 200 µg/ml) it was observed that the use of 50 and 100 µg/ml did not affect growth of *C. violaceum*, while in some cases, the use of the highest concentration (200 µg/ml) slightly reduced growth of this biosensor strain. Therefore, the main reason of violacein reduction when different phytochemical compounds were added to an overnight culture was due to an inhibition of the QS system. Thus, it could be concluded that the tested compounds were able to block QS regulation processes.

DISCUSSION

Fruits and vegetables are an important source of antimicrobial compounds that play a key role in the natural defence of living organisms. However, these compounds not only have bacteriostatic and bactericidal action against pathogens, they also can act as an antipathogenic agent inhibiting the cell-cell communication system or QS. The results of the present study reveal that bioactive compounds from fruits and vegetables significantly reduced violacein production, inhibiting the production of C6-HSL by *C. violaceum* at sub-lethal concentrations. Among all the tested compounds, resveratrol, cinnamaldehyde ellagic acid, pomegranate extracts and rutin showed the highest anti-QS activity. So far others work have also found QS inhibitors, including synthetic N-acyl-homoserine lactone analogues, halogenated furanone natural products and plant derived compounds such as polyphenols, showing inhibitory activity against *C. violaceum* (Ni et al., 2009). However, further studies are necessary to determine the ability of natural extracts to modulate QS in pathogenic and spoilage bacteria. Taking into account the obtained results it is possible to think that natural extracts could be used as anti-pathogenic natural agents, which could be applied in the development of novel non-antibiotic drugs, for treating bacterial infections.

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Tables

Table 1. Phytochemical compounds and natural extracts.

	Compound
1	Cinnamaldehyde
2	Cholorogenic acid
3	Daidzein
4	Dimethylesculetin
5	Ellagic acid
6	Gallic acid
7	Kinurenic acid
8	Pomegranate extracts
9	Resveratrol
10	Rutin
11	Vanillic acid

Table 2. Production percentage of violacein by *C. violaceum*.

	50 µg/ml	100 µg/ml	200 µg/ml
Control	100±1.06e	100±6.43f	100±6.15f
Cinnamaldehyde	5.87±1.77a	3.28±1.34a	1.46±0.35a
Chlorogenic A	95.94±1.83d	97.97±5.73f,e	61.71±3.11e,f
Daidzein	62.84±4.45c	62.31±9.33c	46. 38±3.18d,e
Gallic A	79.84±2.33d	71.97±4.24a,b	56.54±14.40d,e,f
Ellagic A	14.14±9.50b	6.08±1.48a	5.31±0.1a,b
Gallic A	83.28±13.43d	83.88±3.4d,e	79.04±6.95f,h
Kinurenic A	100±7.64e	84.18±1.91e,f	76.68±0.71f,h
Pomegranate	23.27±6.85b	23.19±2.82b	16.84±1.55a,b,c
Resveratrol	2.13±0.28a	0.00±0.00a	0.00±0.00a
Rutin	98.45±1.48e	87.01±5.44d,e,f	30.94±2.40a,b,c
Vanillic A	89.92±1.84e	75.03±6.4d	43.28±5.16c,d,e

Figures

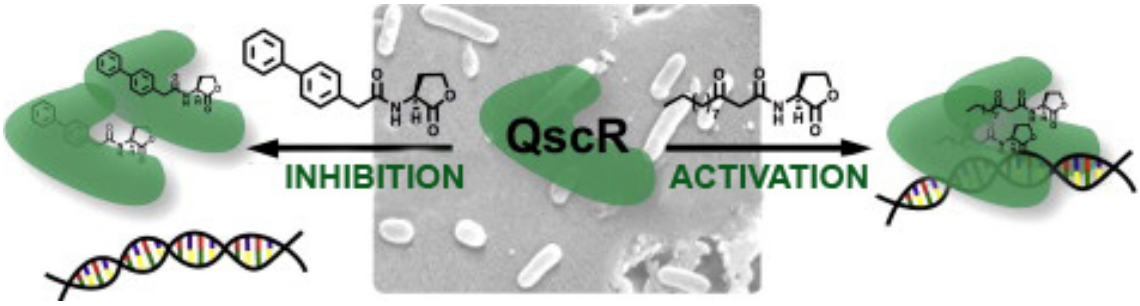

Fig. 1. Diagram of QS inhibition using phytochemical bioactive compounds.