Microbiology (2016), **162**, 1708–1714 **DOI** 10.1099/mic.0.000342

Inhibition of quorum-sensing-mediated biofilm formation in *Cronobacter sakazakii* strains

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Received 2 June 2016
Accepted 25 July 2016

The present study investigated plant extracts for their anti-quorum-sensing (QS) potential to inhibit the biofilm formation in *Cronobacter sakazakii* strains. The bioassay based on loss of pigment production by *Chromobacterium violaceum* 026 and *Agrobacterium tumefaciens* NTL4 (pZLR4) was used for initial screening of the extracts. Further, the effect of extracts on the inhibition of QS-mediated biofilm in *C. sakazakii* isolates was evaluated using standard crystal violet assay. The effect on biofilm texture was studied using SYTO9 staining and light and scanning electron microscopy. Among the tested extracts, *Piper nigrum* and *Cinnamomum verum* at 100 ppm resulted in 78 and 68 % reduction in the production of violacein as well as blue-green colour in both biosensor strains. A higher inhibitory activity (>50 %) on biofilm formation in *C. sakazakii* was observed for *Pip. nigrum* and *Cin. verum,* whereas the other extracts possessed moderate (25–50 %) and minimal (<25 %) inhibitory activities. Further, the fluorescent and scanning electron microscopic images indicated a major disruption in the architecture of biofilms of tested strains by *Pip. nigrum*. This study points to the possibility of using *Pip. nigrum* and *Cin. verum* as inhibitor of QS-mediated biofilm formation by *C. sakazakii* that could be further explored for novel bioactive molecules to limit the emerging infections of *C. sakazakii.*

INTRODUCTION

Cronobacter sakazakii is an emerging Gram-negative opportunistic pathogen that has been associated with invasive infections such as meningitis, bacteraemia and necrotizing enterocolitis in adults and neonates (Joseph *et al.*, 2012). Although the incidence of disease is low, the fatality rate has been reported to be up to 80 % and infants' cases have been epidemiologically related to the ingestion of contaminated powdered infant formula (PIF) (Kalyantanda *et al.*, 2015; Singh *et al.*, 2015a). The organism is reported to form biofilms on different substrates ranging from silicon, latex, polycarbonate, stainless steel, glass and polyvinyl chloride (Iversen *et al.*, 2004; Lehner *et al.*, 2005; Kim *et al.*, 2006) and is able to produce extracellular polysaccharides (Lehner *et al.*, 2005). Furthermore, there are also reports that *Cronobacter* spp. have the ability to survive under desiccated conditions for several weeks and are resistant to osmotic stresses that may be due to formation of biofilms (Iversen & Forsythe, 2003). Numerous literature studies have indicated that the ability to form biofilm among different isolates of *Cronobacter* spp. is generally strain specific (Lehner *et al.*, 2005; Kim *et al.*, 2006; Hartmann *et al.*, 2010; Du *et al.*, 2012; Lee *et al.*, 2012; Jung *et al.*, 2013; Hu *et al.*, 2015). The studies from Lehner *et al.* (2005) demonstrated the ability of biofilm-producing strains of *C. sakazakii* to synthesize the cell signalling molecules acyl homoserine lactones (AHLs), which mediate quorum sensing (QS). This cell-to-cell signalling along with other genetic and environmental factors have been reported to be involved in biofilm formation in bacteria (Waters & Bassler, 2005; Shrout *et al.*, 2011). Therefore, the interference with this phenomenon by means of quorum-sensing inhibitor (QSI) could be an attractive approach to prevent or to reduce biofilm-based infections. Several natural and synthetic anti-QS compounds have been reported where extracts of various natural products (e.g. bean sprout, chamomile, carrot and garlic) (Rasmussen *et al.*, 2005) and essential oils of several plants (e.g. lavender, eucalyptus and citrus) (Szabó *et al.*, 2010) have shown anti-QS effects; however, the studies on effect of these plant extract against biofilm formation and inhibition of QS in *C. sakazakii* have not been reported. Earlier reports on anti-biofilm ability of trans-cinnamaldehyde (TC) from cinnamon indicated that

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Abbreviations: AHL, acyl homoserine lactone; CV, crystal violet; MTP, microtitre plate; PIF, powdered infant formula; QS, quorum sensing; QSI, quorum-sensing inhibitor; SEM, scanning electron microscopy; TC, trans-cinnamaldehyde.

the compound at sub-inhibitory concentration of TC was able to inhibit biofilm synthesis and inactivate mature biofilms of *C. sakazakii* on different abiotic substrates (Amalaradjou & Venkitanarayanan, 2011); however, no investigation was conducted on QS mechanisms.

The plant-derived extracts are widely considered due to their safety and are being used traditionally for prevention and treatment of infections. Therefore, the present study was undertaken to investigate for the first time the anti-QS potential of the plant extracts in *C. sakazakii* and their role in inhibition of biofilm formation.

METHODS

Strains, media and culture conditions

In the previous study, 38 isolates of tentatively identified *Cronobacter* spp. from different commodities were identified based on 16S rRNA (Singh *et al.*, 2015b). Confirmed *C. sakazakii* isolates along with the standard strains *C. sakazakii* ATCC 12868 and E604 (kindly gifted by Dr Ben Davies Tall, Food and Drug Administration) were used in the present study. The two biosensor strains of *Chromobacterium violaceum* CV026 (kindly gifted by Dr Paul Williams, University of Nottingham) and *Agrobacterium tumefaciens* NTL4(pZLR4) (kindly gifted by Dr Stephen K. Farrand, University of Illinois) were used for the detection of AHLs. The *Chr. violaceum* CV026 was cultured in Luria–Bertani (LB) broth supplemented with 100 μg ml^{-1} ampicillin and 30 μg ml^{-1} kanamycin, whereas *Agr. tumefaciens* NTL4(pZLR4) was cultured in nutrient broth (NB) medium containing gentamicin (50 μg ml^{-1}) at 28 °C for 24 h. For all experiments, bacteria were grown in 10 ml trytone soy broth (TSB) medium under shaking (130 r.p.m.) for 24 h.

Plant products and extract preparation

The nine plant products (*Piper nigrum*, *Trigonella foenum-graecum*, *Coriandrum sativum*, *Cuminum cyminum*, *Syzygium aromaticum*, *Myristica fragrans*, *Zingiber officinale*, *Allium sativum* and *Cinnamomum verum*) used in this study were purchased from local outlets in the Solan province of Himachal Pradesh, India, and the extractions were made by following the method of Choo *et al.* (2006), with slight modifications. Each of the plant products was washed in sterile water, dried and powdered using a mixer grinder. For the preparation of methanolic extract, powdered samples (50 g) were soaked in 300 ml of methanol for overnight under shaking at 100 r.p.m. at 30 °C. The methanol phase was collected and concentrated by rotary evaporator to obtain the dried residue. The residues were re-dissolved in 10 % dimethyl sulfoxide (DMSO) and stored at −20 °C until further use.

Anti-QS activity of plant extracts

The anti-QS activity of the extracts was evaluated using indicator strains with well diffusion as well as test tube assay.

The methanolic plant extracts were screened for anti-QS activity using an AHL-based *in vitro* QS assay using two biosensor strains, *Chr. violaceum* CV026 and *Agr. tumefaciens* NTL4(pZLR4) by the agar well diffusion test. A 50 μl sample of overnight broth culture of *Chr. violaceum* CV026 (10^5 c.f.u. ml^{-1}) was added to 10 ml of LB soft agar along with 1.25 μM C6-AHL (99 %, Cayman Chemicals) as QS standard molecule and the mixture was overlaid onto LB agar plates. After solidification of overlay layer, wells of 6 mm diameter were dug using a sterile corkborer. The methanolic plant extract (50 μl) was added to respective wells and the plates were incubated overnight at 28 °C in upright position (McLean *et al.*, 2004; Mukherji & Prabhune, 2015). The testing for QS inhibition against *Agr. tumefaciens* NTL4(pZLR4) was performed in a way similar as supplementing the agar with 50 mg ml^{-1} X-Gal along with 1.25 μM 3-oxo-C8 AHL (99 %, Cayman Chemicals) as standard AHL. The inhibition of QS was detected by the presence of colourless zone with viable cells around the well.

The qualitative effect of extracts on the QS-controlled production in *Chr. violaceum* CV026 and *Agr. tumefaciens* NTL4(pZLR4) was determined as described previously with some modification (Choo *et al.*, 2006). Briefly, 10 ml LB and NB medium containing 50 μl of each plant extracts was inoculated with *Chr. violaceum* CV026 and *Agr. tumefaciens* NTL4(pZLR4) along with 1.25 μM C6 AHL and 3-oxo-C8 AHL, respectively. The tubes were incubated for 24 h at 28 °C in a shaking incubator (130 r.p.m.). Next, violacein was extracted in DMSO from the cells and was quantified at an optical density of 580 nm in a UV– Vis spectrophotometer. Simultaneously, absorbance of the supernatant containing blue-green colouration was measured at a wavelength of 630 nm using DMSO as the blank. The percentage of inhibition was calculated by following the formula: percentage of inhibition = $(\text{control}_{OD} - \text{test}_{OD}/\text{control}_{OD}) \times 100$.

Antibacterial activity of plant extracts

The non-antibacterial activity of all plant extracts was also determined to confirm that the halos produced on lawns of the biosensor strains resulted from QSI rather than the antibacterial activity of the plant extracts. Agar well diffusion assay was performed in tryptone soy agar (TSA) by adapting the method specified by the Clinical and Laboratory Standards Institute (CLSI, 2006). Briefly, 100 μl of test *C. sakazakii* strains was uniformly spread over the surface of TSA plate. The wells were punctured and filled with 50 μl of each plant extract. The plates were incubated at 37 °C for 24 h and observed for growth inhibition.

Inhibition of *C. sakazakii* biofilm formation

The effect of extracts on the biofilm formation of *C. sakazakii* isolates was determined by quantifying the biofilm biomass through microtitre plate (MTP) assay (Thenmozhi *et al.*, 2009). In brief, 20 μl overnight grown *C. sakazakii* isolates (10^6 c.f.u. ml^{-1}) and extracts were added in the dose-dependent manner (50, 100 and 150 ppm) into 230 μl of fresh TSB medium. The plates were incubated without agitation for 48 h at 37 °C. After incubation, the planktonic cells in MTPs were removed by washing the wells twice with sterile water. The surface-adhered cells in the MTP wells were stained with 200 μl of 0.2 % crystal violet (CV) solution. After 15 min, the excess CV solution was removed and the CV in the stained cells was solubilized with 250 μl of 33 % glacial acetic acid. The biofilm biomass was then quantified by measuring the intensity of CV at $OD_{570\ nm}$ using UV–Vis spectrophotometer. The sterile TSB was used as negative control. The percentage of biofilm inhibition was calculated by following the formula: percentage of biofilm inhibition = $(\text{control}_{OD570\ nm} - \text{test}_{OD570\ nm}/\text{control}_{OD570\ nm}) \times 100$. The dose of 100 ppm illustrating strong anti-biofilm activity was taken as minimum inhibitory dose and used for further experiments.

Microscopic analysis of *C. sakazakii* biofilms

Light microscopic analysis. The light microscopy analysis of bacterial biofilm was carried out following the method of Musthafa *et al.* (2010). *C. sakazakii* isolates (10^6 c.f.u. ml^{-1}) (50 μl overnight grown) were added into 2 ml of fresh TSB medium containing cover slips 1 × 1 cm in 24-well MTP along with a control well without extracts. After static incubation for 48 h at 37 °C, the cover slips were removed, rinsed with phosphate buffer (pH 6.5) and stained with 0.2 % CV. The stained cover slips with the biofilm were visualized under light microscopy at magnification of ×40 at a numerical aperture of 0.65 (Labomed CxL Monocular, CxLMONO) with ToupView(x86) as imaging system.

Scanning electron microscopy. For scanning electron microscopy (SEM) analysis, the biofilms of *C. sakazakii* isolates were obtained on glass cover slips as described earlier. After 48 h, the dehydrated biofilms were coated with the thin layer of gold and examined under SEM (Hitachi S-3400N) using an accelerating voltage of 10 kV.

Fluorescent microscopy. The biofilms on the cover slips were also visualized by fluorescence microscopy, by means of the LIVE/DEAD *Bac* Light bacterial viability kit (L10316, Invitrogen-Molecular Probes) to stain cells over a 15 min period in the dark as per manufacturer's instructions. The bacteria were observed at ×400 magnification using a fluorescence microscope BX53 (Olympus Microscopy) equipped with imaging system Qiclick (Olympus). The kit is composed of green-fluorescent nucleic acid stain (SYTO 9) and the red-fluorescent nucleic acid stain (propidium iodide). The green stain can label bacteria with intact membranes and with damage membranes. In contrast, the red stain penetrates only bacteria with damaged membranes and has a diminution in the green fluorescence when both dyes are present. The excitation/emission range of the green stain is 470/510–540 nm and 470/620–650 nm for the red stain.

Statistical analysis

All the experiments were run in triplicates on a single plate on three different days. The mean values were calculated for biofilm formation and percent biofilm inhibition and the comparison between the means was performed by ANOVA and Tukey's multiple comparison test ($P<0.05$) by SPSS software.

RESULTS

Inhibition of QS by plant extracts

The present study investigates the anti-QS activity of methanolic extracts of nine plant products (*Pip. nigrum*, *T. foenum-graecum*, *Cor. sativum*, *Cum. cyminum*, *S. aromaticum*, *M. fragrans*, *Z. officinale*, *All. sativum* and *Cin. verum*) for their potential to inhibit biofilm formation in *C. sakazakii strains*.

The qualitative analysis of the extracts (dissolved in 10 % DMSO) against biosensor strains exhibited QS inhibitory activity. The formation of the halo zone around the well was observed for *Pip. nigrum* and *Cor. sativum* with 15 and 13 mm zone of violacein inhibition against *Chr. violaceum* CV026 (data not shown). Similar results were observed for the QSI using *Agr. tumefaciens* NTL4(pZLR4) in which production of blue-green colour was inhibited in the presence of plant extract. These results demonstrated that extracts might have obstructed the interaction between the added AHLs (e.g. C-6 and 3-oxo-C8 AHL) and their receptors [e.g. CviR for *Chr. violaceum* CV026 and TraR for *Agr. tumefaciens* NTL4(pZLR4)]. The loss of blue-green colour in *Agr. tumefaciens* NTL4(pZLR4) (with added AHL) was detected mainly in *Cin. verum* and *Cum. cyminum* with a zone of pigment inhibition ranging from 10 to 12 mm (data not shown). However, the other plant extracts did not show anti-QS activity. No inhibition was observed with 10 % DMSO used as control.

The plant extracts resulted in a decrease in violacein production in *Chr. violaceum* CV026. A maximum of 78 % inhibition in violacein production was observed with *Pip. nigrum* alone (Fig. 1). However, a 49.0 and 34.5 % reduction in violacein production was observed with extracts from *Cor. sativum* and *All. sativum*, respectively. However, significant loss (68 %) of blue-green colour was detected in *Agr. tumefaciens* NTL4(pZLR4) with extracts from *Cin. verum,* while *Cum. cyminum* and *Pip. nigrum* resulted in a reduction of 21.5 and 16.2 %, respectively (Fig. 1). Negligible effect on inhibition was noted by other plant extracts.

No zone of growth inhibition was observed for plant extracts against the test strains of *C. sakazakii* confirming that the halo effect created in bioassays was due to non-pigmentation (QS-mediated process) in cells adjacent to the well indicating that the inhibitions caused by the plant extracts were solely due to anti-QS activity (data not shown).

Plant extracts influence *C. sakazakii* biofilm formation

The confirmation of the biofilm inhibitory activity of the nine plant extracts was determined at different doses of plant extracts. An increase in percent biofilm inhibition was observed with the increase in dose of the plant extract as compared to the negative control, and the significant inhibition was observed when the extracts were used at a dose of 100 ppm ($P<0.05$). However, higher inhibition was measured at a dose of 150 ppm than at a dose of 100 ppm, but the increase was not significant (data not shown). Therefore, a dose of 100 ppm showing strong anti-biofilm activity was considered as minimum inhibitory dose and used for further experiments.

The extracts were divided into three groups depending on percent biofilm inhibition (high, >50 %; moderate, 50–25 % and minimal, <25 %) against *C. sakazakii* isolates. Four (*Pip. nigrum, S. aromaticum, T. foenum-graecum* and *Cin. verum*) of the nine extracts investigated possessed higher anti-biofilm activity against the strains (Table 1), while the remaining five extracts show moderate or minimal inhibitory effect on the pathogens. *Pip. nigrum* and *Cin. verum* showed a maximum reduction in biofilm biomass ranging from 55 to 75 % against all the tested strains. Moderate inhibitory activity was exhibited by *Cor. sativum* (36–53 %) and *All. sativum* (23–56 %), while *Cum. cyminum*, *M. fragrans* and *Z. officinale* showed minimal activity against biofilm forming *C. sakazakii* isolates.

The results of light and SEM microscopic images revealed clear differences in biofilm structure between biofilms treated with *Pip. nigrum* extract and the untreated control (Fig. 2). The control slides showed a well-developed biofilm growth of the test strain, whereas, on treatment with plant extract, scattered cell growth was observed on the glass slide. Further, fluorescent microscopic images indicated

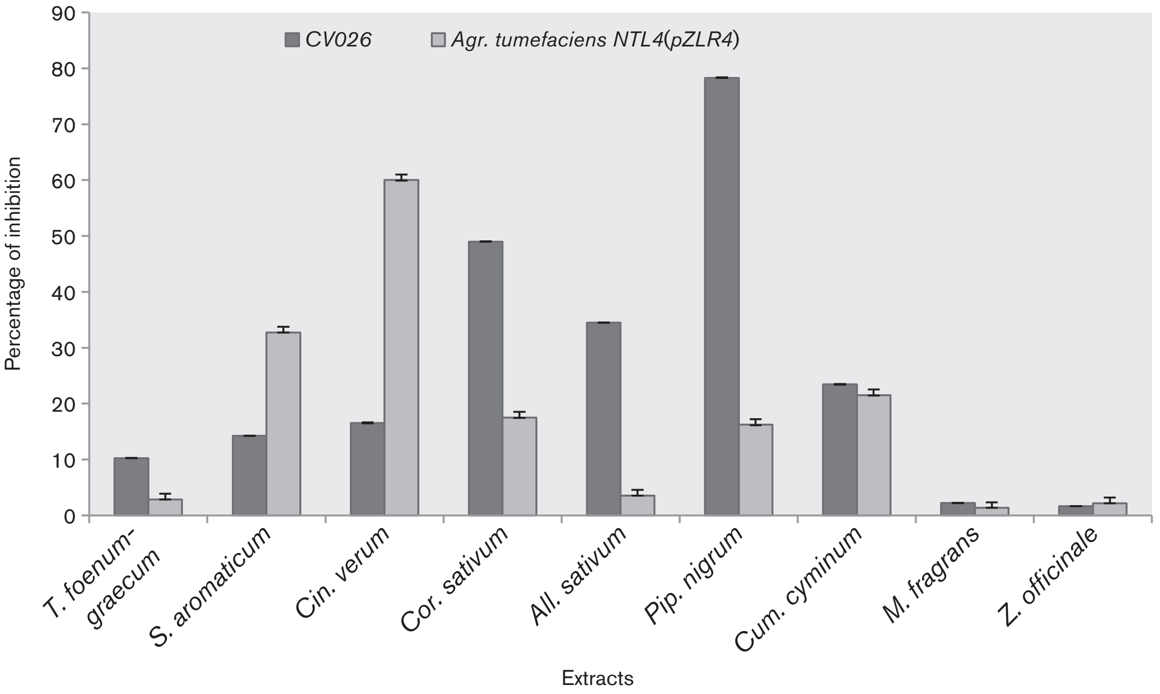

Fig. 1. Quantitative analysis of inhibition of violacein production and blue-green colour in *Chr. violaceum* CV026 and *Agr. tumefaciens* NTL4(pZLR4), respectively, by plant extracts.

well-developed biofilm in control, whereas the strains were treated with extract-developed poor biofilm (Fig. 3). These observations clearly reveal the ability of *Pip. nigrum* extract to disturb the mature biofilms.

DISCUSSION

The present study investigated the anti-QS activity and biofilm inhibitory effect of plant extracts in *C. sakazakii* strains. First, the anti-QS potential of plant extracts was analysed using *Chr. violaceum* CV026 and *Agr. tumefaciens* NTL4 (pZLR4) as biosensor strains. The preliminary screening among the different plant extracts indicated a strong quorum quenching activity in the methanolic crude extract of *Pip. nigrum* and *Cin. verum* by inhibiting pigment production in both biosensor strains. Additionally, the extracts used in the study did not have any antibacterial activity against all test strains. The present data is supported well with the findings of Tan *et al.* (2013) who reported the anti-QS activity of methanolic extracts of *Pip. nigrum* against *Chr. violaceum* CV026. The eugenol, a major component of *Pip. nigrum*, has been previously reported for its QSI activity at the concentration of 150 and 200 μM that inhibited violacein production in *Chr. violaceum* CV026 by up to 48 %

Table 1. Effect of plant extracts on inhibition of biofilms (%) for *C. sakazakii* strains

Test strains	Total biofilm ($OD_{570\ nm}$)	Biofilm inhibition (%)								
		T. foenum-graecum	*S. aromaticum*	*Cin. verum*	*Cor. sativum*	*All. sativum*	*Pip. nigrum*	*Cum. cyminum*	*M. fragrans*	*Z. officinale*
E604	1.79	47.16^{e}	25.47^{b}	73.85^{h}	48.83^{e}	56.61^{f}	68.85^{g}	31.6^{c}	36.54^{d}	11.67^{a}
ATCC 12868	1.65	35.15^{a}	55.09^{de}	56.36^{e}	50.9^{cd}	56.36^{e}	72.12^{f}	40.6^{b}	49.81^{c}	44.84^{b}
N15	2.33	48.67^{b}	53.42^{c}	59.79^{d}	54.4^{c}	47.39^{b}	60.65^{d}	39.26^{a}	41.57^{a}	40.5^{a}
N112	1.76	59.38^{d}	65.34^{ef}	69.93^{f}	53.03^{c}	45.54^{b}	61.99^{de}	47.36^{b}	43.44^{b}	38.06^{a}
N13	1.11	58.34^{f}	23.24^{c}	41.65^{e}	36.17^{d}	23.69^{c}	65.88^{g}	27.2^{c}	2.87^{a}	14.18^{b}

$^{a-g}$Means in the column with same superscript letter are not significantly different as measured by 2 sided Tukey's – post-hoc range test between replications.

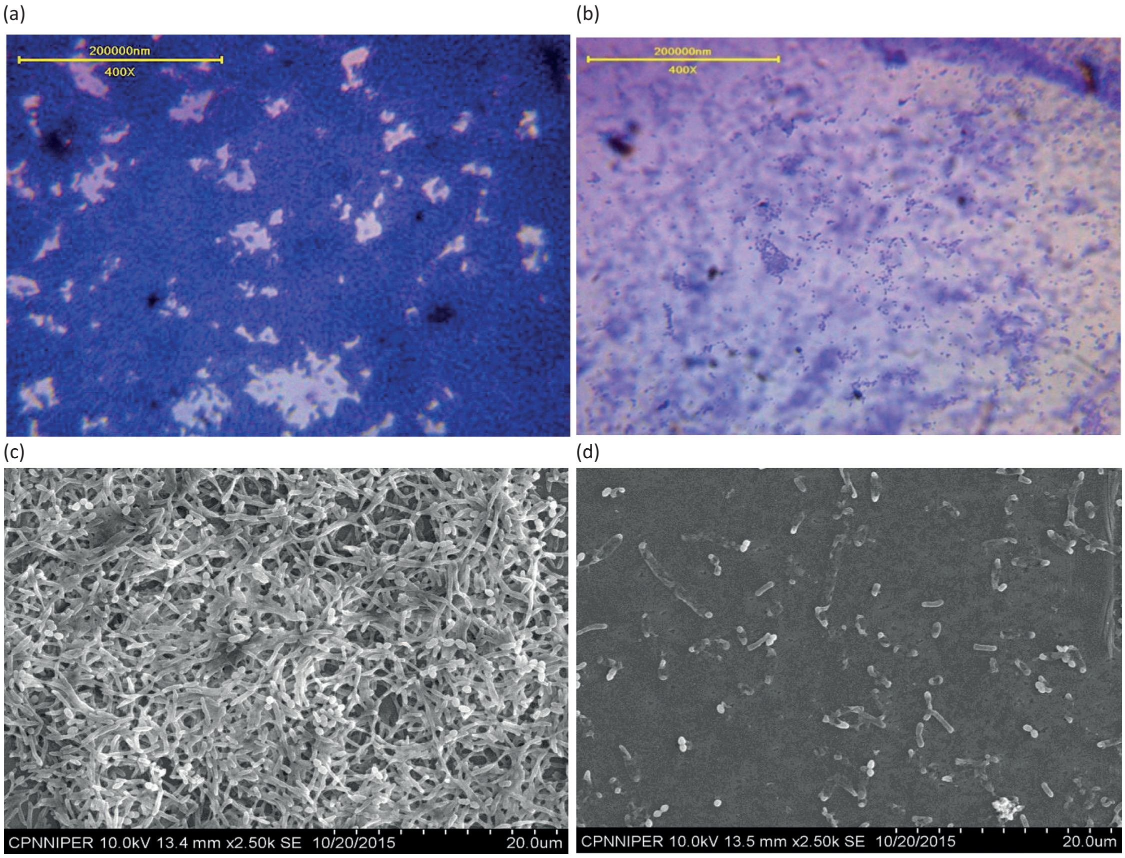

Fig. 2. Light and SEM images of biofilms of *C. sakazakii* isolate grown in the absence (a and c) and presence of methanolic extract of *Pip. nigrum* (100 ppm) (b and d), respectively.

and 56.5 %, respectively (Zhou *et al.*, 2013). Niu *et al.* (2006) reported that cinnamaldehyde (compound of *Cin. verum*) is a potential inhibitor of 3-oxo-C6 AHL in the biosensor strain *E. coli* and was also found to inhibit QS in *Vibrio harveyi* by inhibiting the 3-hydroxy-C4 AHL QS signalling molecule at sub-inhibitory concentrations. Earlier, the spices such as thyme, *Z. officinale* and *S. aromaticum* have been described to reduce violacein production in *Chr. violaceum* to different extents (Vattem *et al.*, 2007; Khan *et al.*, 2009).

Screening for anti-QS activity using multiple biosensor strains removes artefact effects. If plant extracts showed activity with a *Chr. violaceum* CV026 strain and not *Agr. tumefaciens* NTL4(pZLR4), this activity might be limited to an aspect of violacein production. In contrast, if a plant extract sample had activity against *Agr. tumefaciens* NTL4 (pZLR4) only, it might be circumscribed to an issue on long-chain signalling molecules. The data represented that four out of nine extracts were efficiently effective at inhibiting QS in both the biosensor strains. However, moderate or negligible QS inhibitory effect was observed upon exposure to *Cum. cyminum*, *M. fragrans* and *Z. officinale* extracts. This may be due to either low concentration of crude extracts used in the present study or the mechanism by which the compounds affect the QS system. Various mechanisms have been suggested for QS inhibitions such as disruption of competition of the AHLs binding to the receptors by degradation of AHLs, blocking AHLs from forming AHL–receptor complex and changing the structures of the enzymes that are involved in AHL syntheses (Manefield *et al.*, 1999; Dong & Zhang, 2005).

The biofilm formation plays a significant part in the pathogenesis of *C. sakazakii* and developments of these biofilms are based on the signal-mediated QS system. Therefore, an interference with QS may prevent the development of bacterial biofilms and further infections. Biofilm inhibition experiments showed that all the extracts inhibited the biofilm formation of *C. sakazakii* isolates, in a dose-dependent manner. From the data obtained, it is evident that *Pip. nigrum* and *Cin. verum* were able to inhibit the biofilm formation in *C. sakazakii* isolates (Table 1). Very few studies have been conducted to inhibit the biofilms by *C. sakazakii* using various physico-chemical and biological approaches. Earlier study by Amalaradjou & Venkitanarayanan (2011) investigated the efficacy of TC (a principal component of bark extract obtained from *Cin. verum*). They reported the

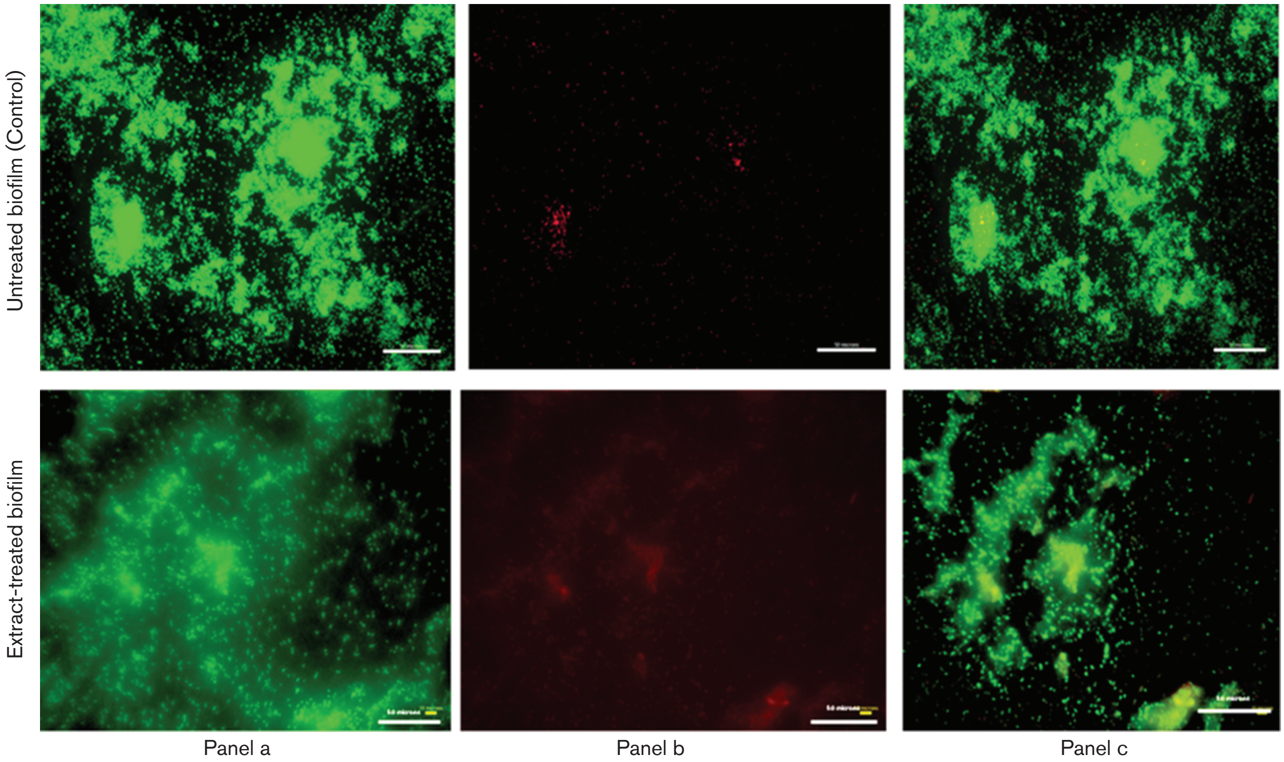

Fig. 3. Fluorescent microscopic images of biofilms of *C. sakazakii* grown in the absence and presence of methanolic extract of *Pip. nigrum* (100 ppm). (a, left) Image obtained from the green channel and (b, centre) from the red channel and (c, right) is a merged image. The excitation/emission range of the green stain is 470/510–540 nm and 470/620–650 nm for the red stain.

efficacy of sub-inhibitory concentration of TC for inhibiting biofilm synthesis (560 and 750 μM) and inactivating mature biofilms (23 and 38 mM TC) of *C. sakazakii* at 24 and 12 °C in the presence and absence of reconstituted PIF on different abiotic surfaces. Another study by Yang *et al.* (2013) reported the inhibition of biofilms in *Cronobacter* spp. with cell-free culture supernatant (100 μl ml^{-1}) of *Paenibacillus polymyxa*. Among the chemical approaches, quaternary ammonium and phenolic disinfectants and a combination of peroxyacetic acid and hydrogen peroxide were used and reported to exhibit different levels of lethality toward *Cronobacter*, depending on time of exposure (1–10 min) and whether the bacterium is present in a food matrix (reconstituted PIF) or as biofilm (Kim *et al.*, 2007). Recently, the effectiveness of a chlorine sanitiser solution against *Cronobacter* biofilms (decrease by 2–3 log cycles) was reported to be affected by the concentration of chlorine solution (100–5000 ppm), its pH (7.0–9.0) and type of surface of conveyer belt (Buna-N or polyvinyl chloride) (Song *et al.*, 2014). However, the disinfectants that are regularly used in the hospital, day care and food service kitchens are reported to be ineffective in eradicating biofilms composed of *Cronobacter* spp. (Kim *et al.*, 2007).

The results obtained in this present investigation indicated that the extracts not only reduced the biofilm biomass (as quantified by CV staining) but also reduced the microcolony formation, which was more evident from the light microscopic (Fig. 2) and fluorescent microscopic images (Fig. 3). Herein, we observed that when using SYTO9, a diminution in fluorescence was viewed in treated biofilms. The fluorescence of dead cells was more in comparison to that of living cells; this reduction proposes that treatment with QSI resulted in lower cell numbers through reduced attachment and/or increased detachment. These data suggest that the QSI may exert their effect during initial stages of attachment or promote detachment at later stages with reduction in AHL activity indicating interference with the bacterial QS system.

In summary, the tested plant extracts reported in the present investigation efficiently inhibited AHL-based QS mechanisms in *C. sakazakii* along with their ability to disrupt the biofilms of the pathogen. In the interest of food safety, this study introduces the QSI and anti-biofilm potential of plant extracts that can be easily incorporated as food ingredient to limit the biofilm forming ability in *C. sakazakii* from different processing environments.

ACKNOWLEDGEMENTS

The authors wish to thank the Department of Science and Technology (SB/FT/LS-426/2012), Government of India, and Jaypee University of

Information Technology, Solan, India, for providing financial support and essential facilities required for this research work.

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Edited by: D. Demuth