DOI: 10.1002/cbic.200800606

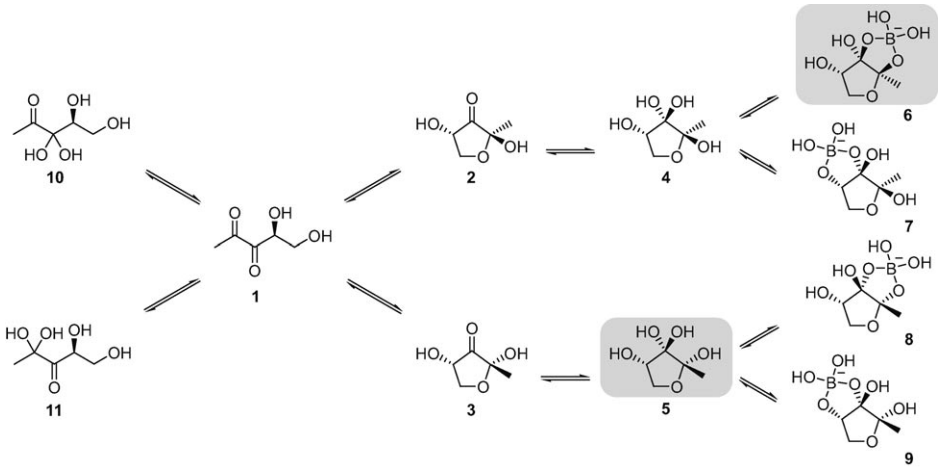
Identification, Quantification, and Determination of the Absolute Configuration of the Bacterial Quorum-Sensing Signal Autoinducer-2 by Gas Chromatography–Mass Spectrometry

Verena Thiel,[a] Ramiro Vilchez,[b] Helena Sztajer,[b] Irene Wagner-Döbler,[b] and Stefan Schulz*[a]

*Autoinducer-2 (AI-2) is an important, small extracellular signaling molecule that is used by many bacteria. It is part of the AI-2 pool, a group of equilibrium-connected compounds derived from (S)-4,5-dihydroxy-2,3-pentanedione [(S)-DPD, **1**]. Currently, these compounds are analyzed by indirect methods relying on the luminescence of sensor strains, the fluorescence of receptor proteins modified with fluorophores, or by isolation procedures not practical for quantitative analysis. Herein, we report a direct analytical procedure that allows for the unambiguous identification and quantification of molecular species by mass spectrometry. Phenylenediamine reacts readily and quantitatively with **1** to form the quinoxalinediol **12** under aqueous conditions. The extraction and silylation of this compound results in the formation of a silyl ether (**13**), which is amenable for analysis by gas chromatography–mass spectrometry. The use of an isotopically labeled variant (**16**) of **12** as an internal standard opens the possibility for the accurate quantification of samples containing AI-2 or its equilibrium products. The analysis of cell-free culture supernatants of* Vibrio harveyi *and* Streptococcus mutans *allowed for the accurate quantification of the AI-2 concentration above the limit of detection (0.7 ng mL^{-1}). No compounds were detected in mutants lacking the capability to produce AI-2. In addition, the absolute configuration of **1** can be analyzed using the derivative **13** by chiral gas chromatography.*

Introduction

Cell–cell communication in bacteria, also termed "quorum sensing," has been shown to regulate numerous important traits, including biofilm formation, virulence, the production of antibiotics, swarming, fruiting body formation, and gene transfer.[1] These effects are mediated by small, diffusible compounds, the so-called autoinducers. Among the known autoinducers, autoinducer-2 (AI-2) plays a unique role.[2] Produced by both Gram-positive and Gram-negative bacteria, it is the only non-species specific, quorum-sensing molecule known that mediates intra- and interspecies communication among bacteria.[3] AI-2 is biosynthesized by the LuxS protein in the *S*-adenosylhomocysteine pathway. LuxS cleaves the intermediate *S*-ribosylhomocysteine into homocysteine and (*S*)-4,5-dihydroxy-2,3-pentanedione [(*S*)-DPD, **1**], the metabolic precursor of AI-2.[4] Through the processes of cyclization (**2**, **3**), hydration (**4**, **5**, **10**, **11**), and borate ester formation if enough borate is present in solution (**6**–**9**),[5] DPD exists as an equilibrium mixture of several compounds (Scheme 1), some of which function as signaling molecules.



Scheme 1. The AI-2 pool. Complex equilibria involving (*S*)-DPD (**1**) and its derivatives are possible in water and in the presence of borate. The boxed compounds have been described as signaling molecules.

[a] *V. Thiel, Prof. Dr. S. Schulz*
Institut für Organische Chemie, Technische Universität Braunschweig
Hagenring 30, 38106 Braunschweig (Germany)
Fax: (+49) 531-3915272
E-mail: stefan.schulz@tu-bs.de

[b] *Dr. R. Vilchez, Dr. H. Sztajer, Prof. Dr. I. Wagner-Döbler*
Helmholtz-Zentrum für Infektionsforschung
Inhoffenstrasse 7, 38124 Braunschweig (Germany)

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

Different bacterial species recognize different signals within this AI-2 pool. For example, the LuxP receptor protein of *Vibrio harveyi* binds the 2,3-borate diester of the hydrated α-anomer (**6**),[6] whereas *Salmonella typhimurium* recognizes the hydrated β-anomer (**5**) with the receptor protein LsrB.[7] These two forms are linked through a chemical equilibrium, as depicted in Scheme 1.[8]

The analysis of DPD and its various forms is currently difficult. Until recently, the common method for AI-2 detection has been a bioassay based on transposon mutants of *V. harveyi*,[9] in which changes in the luminescence of the sensor strain are measured. However, this bioassay is sensitive to assay conditions,[10] including pH, growth conditions, and borate concentration and is susceptible to interference from unknown compounds in the culture supernatant as well as to AI-2 indigenously produced by the reporter strain. Recently, a new method for the identification and quantification of AI-2 has been developed.[11] The two receptor proteins LuxP and LsrB were converted into specific AI-2 biosensors by coupling them with fluorophores near their ligand-binding sites. The binding of AI-2 to the sensor proteins induces a conformational change, and fluorescence is emitted in a concentration-dependent manner. The detection limit is about 1 μM, and the response is linear only up to 20 μM, making quantification difficult. Moreover, the purification and labeling of the proteins are time consuming and expensive. In addition, the possibility that compounds other than the one of interest (AI-2) are also detected cannot be totally excluded, which is a general drawback of indirect analytical methods.

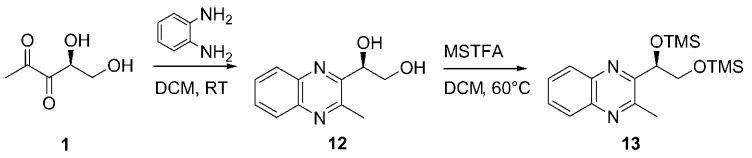
The direct identification of the active forms of AI-2, DPD, or other components of the AI-2 pool by chemical methods using HPLC, LC-MS, or gas chromatography-mass spectrometry (GC–MS) is not possible at present. The low concentrations found in biological samples, and the high polarity and water solubility of DPD makes extraction difficult and limits the use of common derivatization techniques requiring lipophilic media.

DPD has been identified as a single, stable, quinoxaline derivative (**12**) using the quantitative reaction of 1,2-phenylenediamine with 1,2-dicarbonyl compounds in water.[12,13] This product was identified by LC–MS methods or NMR,[14,15] but no quantitative results were obtained. Based on this derivatization step, we developed a highly sensitive and rapid method for the unambiguous identification and quantification of DPD in biological extracts. Because of its high separation power and high sensitivity, GC–MS was the method of choice. The development and validation of this approach will be presented in this paper, as well as its application to quantify DPD in culture supernatants of *Vibrio harveyi* BB152 and *Streptococcus mutans* UA159. In addition, the absolute configuration of DPD can be determined using the described approach.

Results and Discussion

Method development

The analysis of DPD by GC–MS requires a two-step derivatization procedure (Scheme 2). First, the AI-2 pool components shown in Scheme 1 were transformed via DPD into a single,



1 12 13

Scheme 2. Derivatization of DPD for GC analysis. DPD (**1**) represents the whole AI-2 pool shown in Scheme 1. DCM: dichloromethane; MSTFA: *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide.

stable, quinoxaline derivative, as described previously.[12,13] The addition of excess basic 1,2-phenylenediamine directly to the supernatants of the bacterial cultures led to the formation of the corresponding 1-(3-methylquinoxaline-2-yl)ethane-1,2-diol (**12**). This Maillard reaction was nearly complete within 30 min, as monitored by ^{1}H NMR spectroscopy.[12] With the addition of basic 1,2-phenylenediamine to the supernatant, a change in pH from 6.5 to 8.5 was observed; this induced side reactions with components from the medium. Base-catalyzed epimerization, isomerization, and rearrangement reactions of carbohydrates via the Lobry de Bruin–van Ekenstein rearrangement[16] took place, leading to the formation of DPD, and thus influencing the analysis with false positive results. In an approximately neutral milieu, these side reactions did not occur, so that the adjustment of the pH to 7.2 with a potassium phosphate buffer prevented the transformation of carbohydrates into DPD. No formation of **1** was observed in control experiments carried out with different culture media buffered at pH 7.2. Quantitative derivatization was achieved by the addition of 0.5 mL of buffer solution to 1 mL of the culture supernatant, the addition of excess 1,2-phenylenediamine, and a reaction time of 2 h at room temperature.

In order to make the polar quinoxaline derivative accessible for GC–MS analysis, modification of the polar hydroxyl groups was necessary. Therefore, water had to be removed from the samples. Initially, the extracts were evaporated to dryness, and the residue was resuspended in dichloromethane, but this simple and direct approach did not yield satisfying results due to matrix interferences. Similarly, neither the extraction of **12** with ethyl acetate nor dichloromethane led to an improvement. The best results were obtained with liquid-liquid extraction, carried out with an EXtrelut® NT 1 column with dichloromethane as the eluent for the simultaneous elimination of water and filtration of some of the insoluble matrix material; the use of this system resulted in better peak resolution in the GC analysis, and consequently, in a higher sensitivity.

In the second derivatization step, the dichloromethane extracts were treated with *N*-methyl-*N*-(trimethylsilyl)trifluoroace-

tamide (MSTFA), which is an effective and strong trimethylsilyl donor, furnishing the trimethylsilylated quinoxaline **13**. The silylation was complete after 30 min at 60 °C. Finally, the resulting residue was taken up in 20 μL of dichloromethane and analyzed by the injection of 2 μL of the dichloromethane extract into the GC with the detector operated in full-scan EI mode (70 eV). Thus, the derivative formed could be easily identified by characteristic key fragments in the mass spectrum, such as *m/z* 73 [$(CH_3)_3Si^+$], a significant molecular ion [*m/z* 348], a typical fragment ion resulting from the loss of a methyl group, *m/z* 335 [$M^+ - CH_3$], and the characteristic ion at *m/z* 245 [$M^+ - CH_2OSi(CH_3)_3$] (Figure 1 B). Furthermore, a comparison of the retention time and mass spectrum of **13** derived from synthetic **1** with those of natural **13** from the biological extract confirmed the success of the derivatization.

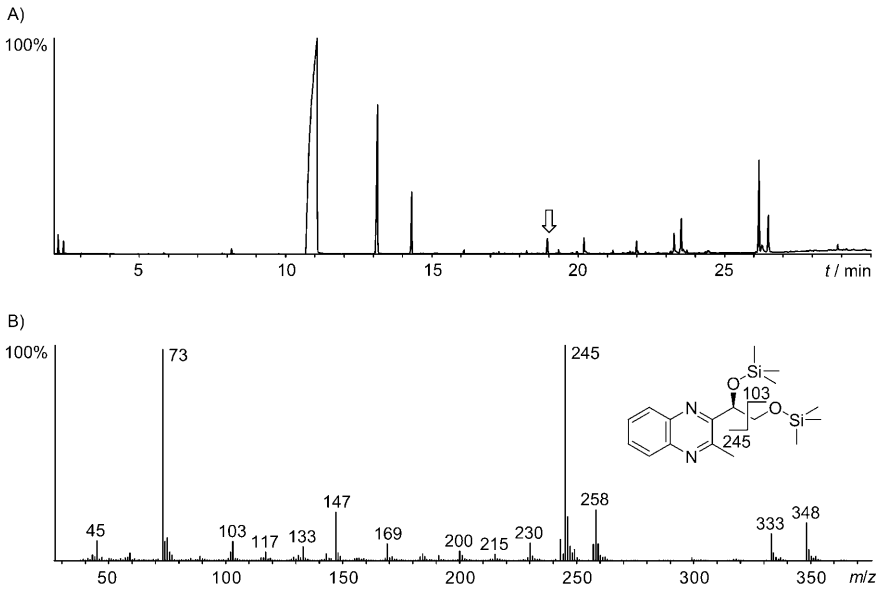


Figure 1. Analysis of the quinoxaline **13** by GC–MS. A) Total ion chromatogram of a derivatized extract of *Vibrio harveyi* BB152 supernatant. Large peaks originate from phenylenediamine and carbohydrates present in the culture medium. The arrow indicates the peak of **13**. *V. harveyi* is a strain that produces large amounts of AI-2. B) MS and fragmentation of compound **13**. The base peak is found at *m/z* 245 and the molecular ion at *m/z* 348.

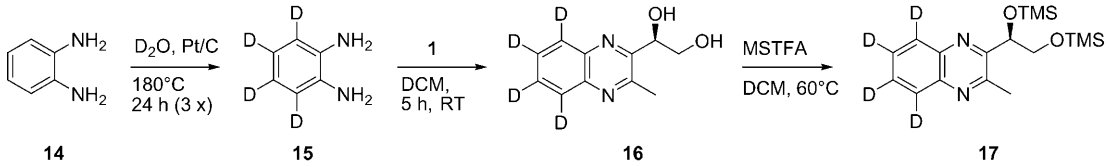
A typical chromatogram of the derivatized culture supernatant of *Vibrio harveyi* BB152 and the mass spectrum of the silylated DPD derivative **12** are presented in Figure 1. In this sample, the concentration of DPD was high compared to that of other samples. Compound **12** was positively identified by a comparison of the retention time and mass spectrum with those of the synthetic reference compound. For extracts containing only low concentrations of DPD, exhibiting no good full-scan mass spectra, specificity can be improved by monitoring only the *m/z* 245 and *m/z* 348 ions from the total ion current.

Quantification

In addition to the detection, the determination of the DPD concentration in biological samples is of special interest. Usually, internal standards that are chemically different to the analyte are used; this has several disadvantages that can lead to shifts in analyte/standard ratios, resulting in inaccurate quantifications. Therefore, we chose to perform quantification by isotope dilution using GC–MS, a method successfully used for the quantitative analysis of dioxins and PCBs, for example.[17] In this method, a chemically similar, isotopically labeled variant of the analyte is added, thus effectively avoiding any shifts in analyte/standard ratios during the analytical process. In the method described herein, the isotopically labeled derivative **16** was added to the extract after the first derivatization step but before the extraction of the analyte. During the following analytical manipulations, the ratio of internal standard and analyte remained constant, independent of any potential loss of the compounds during the following extraction and derivatization steps. As a further advantage, the isotopically labeled standard can also act as sample-specific marker for the identification of the native analyte.

We used the deuterium-labeled standard [5,6,7,8-^{2}H]1-(3-methylquinoxalin-2-yl)ethane-1,2-diol (**16**) because of its availability by a short and convenient synthesis. 1,2-Phenylenediamine (**14**) was deuterated repeatedly in the aromatic ring with Pt/C and D_2O under a hydrogen atmosphere,[18] followed by its transformation into the corresponding deuterated quinoxaline **16** by its addition to synthetic (*S*)-DPD (Scheme 3).

The relative ion-intensities in the MS of the labeled (**17**) and unlabeled (**13**) derivatives can be directly used for absolute quantification, provided that a known amount of the standard



Scheme 3. Synthesis of the deuterated standard **16** with subsequent silylation.

was added. The amount of DPD in the natural sample equals the peak ratio of the standard-specific ion trace of *m/z* 249 and the ion trace of *m/z* 245 of natural DPD in the ion chromatogram, according to Equation 1, in which χ_{DPD} = DPD amount in the sample, α_{245} = peak area of the ion at *m/z* 245, χ_{IS} = amount of standard added, and α_{249} = peak area of the ion at *m/z* 249.

$$\chi_{DPD} = \chi_{IS}\frac{\alpha_{245}}{\alpha_{249}} \quad (1)$$

Figure 2 shows the mass spectra of the silylated internal standard **17** and a sample of *V. harveyi* BB152 analyzed after the addition of the deuterated standard. During the analysis of *V. harveyi*, we routinely added 50 ng (2.4×10^{-10} mol) of **16** to 1 mL of the extract, so that the concentration of the sample shown in Figure 2B can be calculated to be 113 ng mL^{-1} (0.86 μM) DPD. The addition of borate to the supernatants did not alter the results.

We note that the retention time of the deuterated standard **17** was slightly shorter than that of the unlabeled analogue **12**. This well-known phenomenon of deuterium-labeled compounds in GC can be explained by the slightly shorter bond length of a C–D bond compared to that of a C–H bond.[19,20]

Reproducibility, method linearity, and sensitivity

Six aliquots each of a *V. harveyi* BB152 supernatant and a *Streptococcus mutans* UA159 supernatant were analyzed in order to determine the reproducibility of the method. The *V. harveyi* BB152 strain contained DPD in a concentration of 258 ng mL^{-1} (1.95 μM) with an absolute error of 17.6 ng mL^{-1}, resulting in a standard deviation of 6.8%. The concentration of DPD in *S. mutans* was calculated to be 34.2 ng mL^{-1} (0.26 μM) with an absolute error of 1.2 ng mL^{-1}, resulting in a standard deviation of 3.5%. The analysis of the culture media showed no presence of DPD.

The linearity of the method was evaluated on a six-point calibration curve. Therefore, basal metal salt enrichment medium (BMS) was spiked with known amounts of DPD in the range of 2.5–250 ng mL^{-1}, and every sample underwent the full analytical procedure including sample preparation and derivatization followed by GC–MS analysis. The DPD concentrations were calculated as described above, and then the linear correlation coefficient (R^2) was calculated. There was a good correlation between the added and the measured amounts of DPD (R^2 = 0.9977), thus showing the response linearity of the method at different analyte concentrations, as presented in Figure 3.

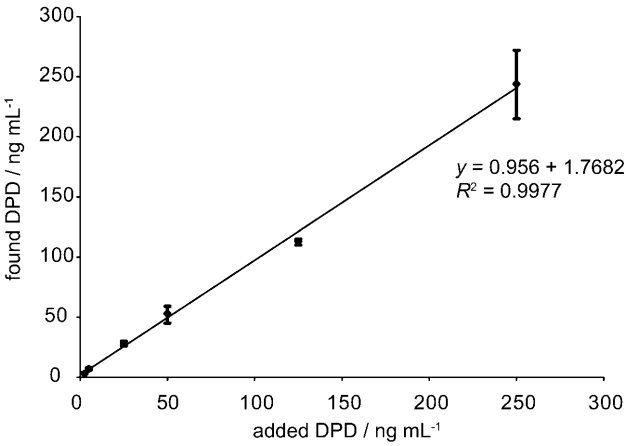


Figure 3. Linearity testing. The correlation coefficient was obtained by plotting the amount of DPD added against the measured concentration.

The limit of detection for this method, defined as a signal-to-noise ratio (*S/N*) of 5, was 0.7 ng mL^{-1} (5.3 nM); the limit of quantification was 2.1 ng mL^{-1} (16.0 nM) at *S/N* = 15. At this concentration, the relative standard deviation of the analyte was below 7.5%.

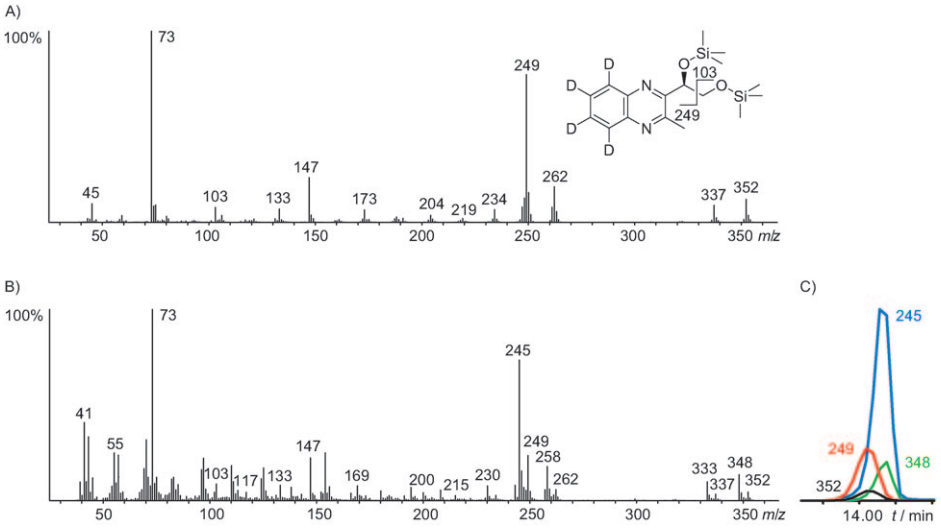


Figure 2. Mass spectra and ion traces for the quantification of DPD. Mass spectra of A) the silylated, deuterated, internal standard **17** and B) the integrated MS of the analytes (whole peak area) in a sample of *V. harveyi* supernatant. C) the ion chromatograms used for quantification. The GC temperature program differed from that used in Figure 1.

Validation of the approach

S. mutans UA159 is a cariogenic bacterium that possesses a functional *luxS* gene, recognized as the enzyme primarily responsible for the biosynthesis of DPD.[21] Although the gene was shown to be expressed constitutively during growth,[22] no AI-2 could be detected with the *V. harveyi* bioassay due to the presence of an inhibitory compound in the culture supernatant (R. Vilchez, personal communication). The method de-

scribed above was applied to sterile culture supernatants from the wild-type *S. mutans* UA159 and *S. mutans* UA159 Δ*luxS*, a *luxS*-deficient strain lacking the ability to synthesize DPD, in order to demonstrate the utility of the approach. In analyzing culture supernatants of both strains for the presence of DPD, we could unambiguously identify DPD in samples of wild-type *S. mutans*, while we could not detect it in the mutant (see Figure S1 in the Supporting Information). Thus, we were able, for the first time, to provide direct evidence that *S. mutans* UA159 is capable of producing and releasing DPD into culture fluids. The amount of DPD secreted into the medium by *S. mutans* UA159 during growth is shown in Figure 4. The DPD concentration increased during the exponential growth phase and reached a maximum value (29.3 ng mL^{-1} or 0.22 μM) at the transition from the late exponential to the stationary growth phase ($OD_{600} = 1.2$). Thereafter, a drastic decline in DPD concentration was observed. A sharp decline in AI-2 concentration in the medium at stationary phase has also been seen in other species including *Shewanella* sp.[23] and *Salmonella typhimurium*, where it is due to AI-2-induced synthesis of a transporter.[24] The analysis of the Δ*luxS* strain of *S. mutans* UA159 at different times of the growth phase showed the absence of DPD (Figure 4).

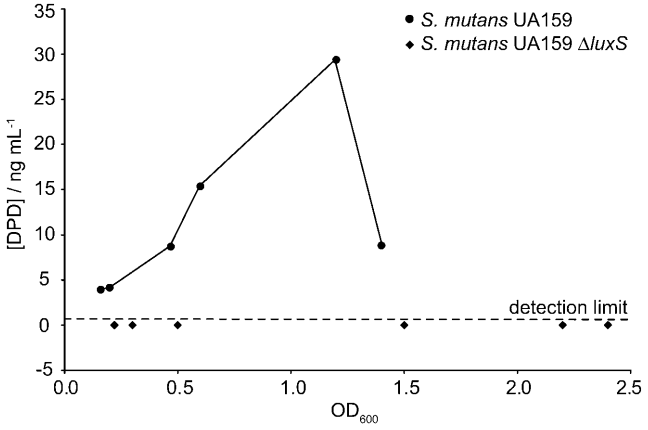


Figure 4. Concentration of DPD during the cultivation of wild-type *S. mutans*.

The results of the analysis of *V. harveyi* BB152 in the late exponential phase (see above) served as a control. Again, in *V. harveyi* MM77, a strain lacking the capability to produce **1**, no DPD was found. These data prove the usefulness of our approach.

Confirmation of the absolute configuration

DPD is a chiral molecule with a stereogenic center at C-4. The first studies employing X-ray analysis gave evidence for DPD as (*S*) configured; this was supported by extensive biosynthetic investigations.[4,6,25] A synthetic verification confirmed (*S*)-DPD as the precursor of the AI-2 group of signaling molecules.[26] With the derivative **13**, the direct determination of the absolute configuration was also possible.

The two DPD enantiomers were synthesized according to published procedures, starting either from methyl (*S*)-isopropylideneglycerate or methyl (*R*)-isopropylideneglycerate.[10,13] The enantiomerically pure products were then transformed into the corresponding silylated quinoxalines **13** and were analyzed by GC on a chiral hydrodex-6-TBDMS GC phase (see Figure S3). The analysis of an extract of *V. harveyi* BB152 showed the presence of only one enantiomer of DPD. The co-injection of the derivatized natural extract with the silylated (*S*)-quinoxaline (*S*)-**13** showed the coelution of both peaks (see Figure S2); thus, this confirmed the (*S*) configuration of the natural DPD.

Conclusions

In this article we report for the first time the direct chemical analysis of AI-2 through isotope dilution analysis in culture supernatants of bacteria; this allows for positive identification through direct observation of the target molecule. This method is reliable in an appropriate buffer and is not influenced by the presence or absence of borate. It allows qualitative and quantitative on-target analysis over a wide range of concentrations with a 5 nM detection limit. It can be adopted to various concentrations with proper amounts of the internal standard. Furthermore, the sensitivity is similar to or even better than currently employed biological sensor systems[10,11] and might be further enhanced with more sensitive mass spectrometric analytical techniques like detection in single-ion-monitoring mode (SIM).

Experimental Section

Chemicals: Chemicals were purchased from Fluka or Sigma–Aldrich and used without further purification. NMR spectra were obtained using a Bruker AMX400 (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) spectrometer with TMS as an internal standard. Column chromatography was carried out using Merck silica gel 60. Thin layer chromatography was carried out using 0.2 mm pre-coated plastic sheets (Polygram Sil G/UV$_{254}$, Marcherey & Nagel, Düren, Germany). Solvents were purified by distillation and dried according to standard methods.

Biological samples: Wild-type *Streptococcus mutans* UA159 (ATCC 700610) and a l*uxS*-null mutant, constructed by the allelic replacement of the *luxS* gene with an erythromycin resistance cassette using the PCR mutagenesis strategy,[27] were grown at 37 °C without agitation under anaerobic conditions (80% N_2, 10% H_2, 10% CO_2) in a defined semisynthetic medium containing 0.5% sucrose.[22] During growth, 5 mL aliquots of the culture were withdrawn, centrifuged at 13000 rpm at 4 °C, and the resulting supernatants were used for the AI-2 analysis. *Vibrio harveyi* BB152 and MM77 were incubated in the AB medium described by Greenberg et al.,[28] and cell-free culture supernatants served as positive and negative controls in the AI-2 analysis, respectively.

GC–MS analysis: GC–MS analyses were carried out on a HP 6890 Series GC System connected to a HP 5973 Mass Selective Detector (Hewlett–Packard Company, Wilmington, USA) fitted

with a BPX5 fused-silica capillary column (25 m × 0.22 mm i.d., 0.25 mm film, SGE Inc., Melbourne, Australia). Conditions were as follows: splitless injection (60 s valve time), inlet pressure of 77.1 kPa, He flowrate of 23.3 mL min^{-1}, injection volume of 2 μL, transfer line temperature of 300 °C, and an electron energy of 70 eV. The GC was programmed as follows: 5 min at 100 °C then to 300 °C at 10 °C min^{-1} or 2 min at 100 °C then to 250 °C at 10 °C min^{-1} and finally to 320 °C at 20 °C min^{-1}. The carrier gas was He at a 1 mL min^{-1} column head pressure.

Chiral GC analysis: Separation of the enantiomers was carried out on a 8000Top GC (ThermoQuest, Toronto, Canada) instrument fitted with a Hydrodex-6-TBDMS column (35 m × 0.25 mm i.d., Macherey & Nagel) and a flame ionization detector. Conditions were as follows: inlet pressure of 30 kPa, H_2 flowrate of 20 mL min^{-1}, and an injection volume of 2 μL. The GC was programmed as follows: 30 min at 150 °C increasing to 220 °C at 1 °C min^{-1}. The carrier gas was H_2 at a 1 mL min^{-1} column head pressure.

Synthesis of standard 16: According to the method of Sajiki et al.,[18] a mixture of 1,2-phenylenediamine (5.0 g, 46.2 mmol) in D_2O (150 mL) and 5% Pt/C catalyst (1.0 g, 20 wt%) were stirred in a closed apparatus under a H_2 atmosphere at 180 °C for 48 h. After being cooled to room temperature, the reaction mixture was extracted with dichloromethane (3×), and the combined organic extracts were washed with water and dried with $MgSO_4$. Removal of the solvent in vacuo furnished [3,4,5,6-^{2}H]1,2-phenylenediamine (**15**). This procedure was repeated two times to ensure complete deuteration. Finally, **15** (3.12 g, 27.9 mmol, 60.5%) was obtained with a ^{2}H incorporation of >95% (determined by GC–MS). EI-MS (70 eV): *m/z* (%) = 112 (100) $[M]^+$, 84 (53), 56 (23), 42 (11).

Similar to a method of DeKeersmaecker et al.,[13] deuterated diamine **15** was transformed into the quinoxaline **16**. Compound **15** (28.0 mg, 0.25 mmol, 2.5 equiv.) was dissolved in dichloromethane (5 mL), and (*S*)-4,5-dihydroxy-2,3-pentanedione (**1**, 13.2 mg, 0.1 mmol) in dichloromethane (2 mL) was added. The mixture was stirred for 2 h at room temperature, the solvent was removed, and the crude product was purified by flash column chromatography to obtain [5,6,7,8-^{2}H]1-(3-methylquinoxalin-2-yl)ethane-1,2-diol (**16**, 7.1 mg, 0.03 mmol, 33%, ^{2}H incorporation of 95.2%, as determined by ^{1}H NMR) as a yellow solid. R_f = 0.28 (dichloromethane/methanol, 19:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): 5.14–5.12 (m, 1H, C*H*OH), 4.05 (dd, *J* = 11.6 Hz, 3.5 Hz, 1H, C*H*H*OH), 3.86 (dd, *J* = 11.6 Hz, 5.5 Hz, 1H, CH*H**OH), 2.82 (s, 3H, CH_3).

Derivatization of DPD in culture supernatants: Potassium phosphate buffer (0.1 M, K_2HPO_4/KH_2PO_4, pH 7.2, 0.5 mL) and 1,2-phenylenediamine (2.0 mg, 18.5 μmol) were added to the culture supernatant (1 mL), and the mixture was allowed to react for 2 h at room temperature. The deuterated internal standard **16** (50.0 ng, 0.24 nmol) was then added, and the sample was applied onto an EXtrelut® NT 1 column (Merck). After 10 min, dichloromethane (1 mL) was added, and after an additional 5 min, the sample was eluted with dichloromethane (4 mL). The resulting dichloromethane extract was concentrated under a gentle stream of nitrogen, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA, 50 μL) was added, and the mixture was heated at 60 °C for 30 min.[29] The mixture was evaporated to dryness under a stream of N_2 to remove excessive reagent, the residue was taken up in dichloromethane (20 μL), and 2 μL of the mixture were injected into the gas chromatograph for GC–MS analysis.

Synthesis of (*S*)- and (*R*)-4,5-dihydroxy-2,3-pentanedione (1): Both enantiomers were synthesized according to the method of DeKeersmaker et al. starting with either methyl (*S*)-isopropylideneglycerate or methyl (*R*)-isopropylideneglycerate.[10,13] The spectroscopic data matched the reported values.

Acknowledgements

Financial support by the Fonds der Chemischen Industrie is gratefully acknowledged. The authors declare that they have no competing financial interests.

Keywords: carbohydrates · gas chromatography · mass spectrometry · pheromones · quorum sensing

[1] N. C. Reading, V. Sperandio, *FEMS Microbiol. Lett.* **2006**, *254*, 1–11.
[2] A. Vendeville, K. Winzer, K. Heurlier, C. M. Tang, K. R. Hardie, *Nat. Rev. Microbiol.* **2005**, *3*, 383–396.
[3] B. L. Bassler, E. P. Greenberg, A. M. Stevens, *J. Bacteriol.* **1997**, *179*, 4043–4045.
[4] S. Schauder, K. Shokat, M. G. Surette, B. L. Bassler, *Mol. Microbiol.* **2001**, *41*, 463–476.
[5] S. Chapelle, J.-F. Verchere, *Tetrahedron* **1988**, *44*, 4469–4482.
[6] X. Chen, S. Schauder, N. Potier, A. Van Dorsselaer, I. Pelczer, B. L. Bassler, F. M. Hughson, *Nature* **2002**, *415*, 545–549.
[7] S. T. Miller, K. B. Xavier, S. R. Campagna, M. E. Taga, M. F. Semmelhack, B. L. Bassler, F. M. Hughson, *Mol. Cell* **2004**, *15*, 677–687.
[8] M. F. Semmelhack, S. R. Campagna, M. J. Federle, B. L. Bassler, *Org. Lett.* **2005**, *7*, 569–572.
[9] B. L. Bassler, M. Wright, R. E. Showalter, M. R. Silverman, *Mol. Microbiol.* **1993**, *9*, 773–786.
[10] R. Vilchez, A. Lemme, V. Thiel, S. Schulz, H. Sztajer, I. Wagner-Döbler, *Anal. Bioanal. Chem.* **2007**, *387*, 489–496.
[11] J. Zhu, D. Pei, *ACS Chem. Biol.* **2008**, *3*, 110–119.
[12] M. M. Meijler, L. G. Hom, G. F. Kaufmann, K. M. McKenzie, C. Sun, J. A. Moss, M. Matsushita, K. D. Janda, *Angew. Chem.* **2004**, *116*, 2158–2160; *Angew. Chem. Int. Ed.* **2004**, *43*, 2106–2108.
[13] S. C. J. De Keersmaecker, C. Varszegi, N. van Boxel, L. W. Habel, K. Metzger, R. Daniels, K. Marchal, D. De Voss, J. Vanderleyden, *J. Biol. Chem.* **2005**, *280*, 19563–19568.
[14] J. Zhu, E. Dizin, X. Hu, A.-S. Wavreille, J. Park, D. Pei, *Biochemistry* **2003**, *42*, 4717–4726.
[15] T. Hauck, Y. Hübner, F. Brühlmann, W. Schwab, *Biochim. Biophys. Acta Gen. Subj.* **2003**, *1623*, 109–119.
[16] C. A. Lobry de Bruin, W. A. van Ekenstein, *Ber. Dtsch. Chem. Ges. B* **1895**, *28*, 3078–3082.
[17] A. K. Djien Liem, *Trends Anal. Chem.* **1999**, *18*, 429–439.
[18] H. Sajiki, N. Ito, H. Esaki, T. Maesawa, T. Maegawa, K. Hirota, *Tetrahedron Lett.* **2005**, *46*, 6995–6998.
[19] J. S. Dickschat, S. C. Wenzel, H. B. Bode, R. Müller, S. Schulz, *ChemBioChem* **2004**, *5*, 778–787.
[20] L. Dehennin, A. Reiffsteck, R. Scholler, *Biomed. Mass Spectrom.* **1980**, *7*, 493–499.
[21] J. Merritt, F. Qi, S. D. Goodman, M. H. Anderson, W. Shi, *Infect. Immun.* **2003**, *71*, 1972–1979.

[22] H. Sztajer, A. Lemme, R. Vilchez, S. Schulz, R. Geffers, C. Y. Y. Yip, C. M. Levesque, D. G. Cvitkovitch, I. Wagner-Döbler, *J. Bacteriol.* **2008**, *190*, 401–415.
[23] A. Bodor, B. Elxnat, V. Thiel, S. Schulz, I. Wagner-Döbler, *BMC Microbiol.* **2008**, *8*, 13.
[24] M. E. Taga, J. L. Semmelhack, B. L. Bassler, *Mol. Microbiol.* **2001**, *42*, 777–793.
[25] J. F. Alfaro, T. Zhang, D. P. Wynn, E. L. Karschner, Z. S. Zhou, *Org. Lett.* **2004**, *6*, 3043–3046.
[26] C. A. Lowery, K. M. McKenzie, L. Qi, M. M. Meijler, K. D. Janda, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2395–2398.
[27] P. C. Y. Lau, C. K. Sung, J. H. Lee, D. A. Morrison, D. G. Cvitkovitch, *J. Microbiol. Methods* **2002**, *49*,193–205.
[28] E. P. Greenberg, J. W. Hastings, S. Ulitzur, *Arch. Microbiol.* **1979**, *120*, 87–91.
[29] S. Schulz, C. Arsene, M. Tauber, J. N. McNeil, *Phytochemistry* **2000**, *54*, 325–336.

Received: September 8, 2008
Published online on December 29, 2008

