LuxS-independent formation of AI-2 from ribulose-5-phosphate
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Abstract
Background: In many bacteria, the signal molecule AI-2 is generated from its precursor S-ribosyl-L-homocysteine in a reaction catalysed by the enzyme LuxS. However, generation of AI-2-like activity has also been reported for organisms lacking the luxS gene and the existence of alternative pathways for AI-2 formation in Escherichia coli has recently been predicted by stochastic modelling. Here, we investigate the possibility that spontaneous conversion of ribulose-5-phosphate could be responsible for AI-2 generation in the absence of luxS.

Results: Buffered solutions of ribulose-5-phosphate, but not ribose-5-phosphate, were found to contain high levels of AI-2 activity following incubation at concentrations similar to those reported in vivo. To test whether this process contributes to AI-2 formation by bacterial cells in vivo, an improved Vibrio harveyi bioassay was used. In agreement with previous studies, culture supernatants of E. coli and Staphylococcus aureus luxS mutants were found not to contain detectable levels of AI-2 activity. However, low activities were detected in an E. coli pgi-eda-edd-luxS mutant, a strain which degrades glucose entirely via the oxidative pentose phosphate pathway, with ribulose-5-phosphate as an obligatory intermediate.

Conclusion: Our results suggest that LuxS-independent formation of AI-2, via spontaneous conversion of ribulose-5-phosphate, may indeed occur in vivo. It does not contribute to AI-2 formation in wildtype E. coli and S. aureus under the conditions tested, but may be responsible for the AI-2-like activities reported for other organisms lacking the luxS gene.

Background
In the marine bacterium Vibrio harveyi, autoinducer 2 (AI-2) is one of three quorum-sensing molecules regulating the production of bioluminescence in a population-density-dependent fashion [1,2]. In recent years, numerous pathogenic and non-pathogenic bacteria have also been shown to produce AI-2 (for a recent review see [3]), and for this reason the molecule has been suggested to function in interspecies communication [1,4-7].

AI-2 is the collective term for a group of signal molecules formed from a common precursor, 4,5-dihydroxy-2,3-pentanedione (DPD). DPD is generated by many bacteria as a by-product of the activated methyl cycle in a reaction.
catalysed by LuxS [4,8]. LuxS acts by cleaving S-ribozyllhomocysteine (SRH) to yield homocysteine and the reactive DPD, which spontaneously cyclises to from a range of furanone derivatives (Fig. 1). Two of these, (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (S-THMF-borate) and (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF), are recognised by specific periplasmic binding proteins in V. harveyi and Salmonella enterica Serovar Typhimurium, respectively [9,10]. Another derivative, 4-hydroxy-5-methyl-3(2H)-furanone (MHF), has also been confirmed as a product of the LuxS catalysed reaction in vitro [8] and shown to have moderate bioluminescence inducing ability in V. harveyi [4,8].

Interestingly, formation of MHF from D-ribulose-5-phosphate (Rul-5-P) has also been reported. This phenomenon was initially witnessed following the action of spinach phosphoriboisomerase on ribose-5-phosphate (Rib-5-P) but was dismissed as an anomalous side-activity of the enzyme [11]. The authors demonstrated a transient accumulation of an unknown intermediate, formed from the sugar phosphate, which subsequently converted to MHF. Later, Hauck et al. demonstrated that MHF arose spontaneously from Rul-5-P, and identified the unknown intermediate as DPD [12,13]. Thus, spontaneous conversion of Rul-5-P may provide a novel route by which Al-2 could form in a luxS-independent fashion. However, whilst it is known that Rul-5-P gives rise to DPD under certain conditions in vitro, and that chemically synthesised DPD can stimulate bioluminescence in V. harveyi Al-2-responsive reporter strains [14-16] the principle of LuxS-independent formation of Al-2 activity from Rul-5-P in biologically relevant quantities has yet to be proved. Furthermore, the possibility and the ramifications of this process occurring in vivo have not been previously addressed. Here we demonstrate that spontaneous con-

**Figure 1**
Pathways of DPD and Al-2 formation. The schematic integrates pathways described for production of 4,5-dihydroxy-2,3-pentanedione (DPD) from D-ribulose-5-phosphate [13] and S-ribozyllhomocysteine (SRH) [4] with the subsequent formation of Al-2 molecules (yellow box) detected by V. harveyi and S. enterica serovar Typhimurium [10]. Ribulose-5-phosphate is formed enzymatically from other sugar phosphates and its reactive open-chain carbonyl form in aqueous solution facilitates DPD generation. DPD cyclisation leads to several products forming via 2,4-dihydroxy-2-methylhydrofuran-3-one intermediates, including two distinct autoinducer molecules, S-THMF-borate ((2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate; detected by V. harveyi) and R-THMF ((2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran; detected by S. enterica serovar Typhimurium) as well as MHF (4-hydroxy-5-methyl-3(2H)-furanone). Intermediates or side products shown are: S-THMF, (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate; detected by V. harveyi and R-THMF, (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran; detected by S. enterica serovar Typhimurium; R-DHMF, (2R,4S)-2,4-dihydroxy-2-methylhydrofuran-3-one; S-DHMF, (2S,4S)-2,4-dihydroxy-2-methylhydrofuran-3-one; Hcy, homocysteine.
version of Rul-5-P does indeed give rise to potent AI-2 activity and investigate various luxS mutants, including an E. coli strain affected in central carbon metabolism, for LuxS-independent AI-2 formation in vivo. In addition, consideration is given to the possible implications of this alternative mechanism for AI-2 production.

Results and discussion
High levels of AI-2 activity arises from ribulose-5-phosphate in vitro
A solution of 5 mM Rul-5-P was incubated at 37°C for 24 h, based on the methods previously described for MHF formation [12,13], and assayed for bioluminescence-inducing activity in V. harveyi BB170, a bioreporter for detection of AI-2 activity [17]. Addition of this solution to the reporter strain induced high levels of bioluminescence, much higher than those observed for a positive control of 5 mM MHF. By comparison, the isomers Rib-5-P and xylulose-5-phosphate (Xyl-5-P) stimulated little or no bioluminescence (Fig. 2A). Since bioluminescence in V. harveyi is also controlled by the signal molecule N-3-hydroxybutanoyl-L-homoserine lactone (AI-1), each compound was also tested with the AI-1 responsive bioreporter, V. harveyi BB886 [17]. No bioluminescence was induced in this strain (Fig. 2A) indicating Rul-5-P-mediated bioluminescence to be induced specifically via the AI-2 detection system.

To demonstrate that the observed AI-2 activity was not caused by impurities present in the commercial preparations, Rul-5-P was also produced enzymatically. Incubation of 5 mM ribose-5-phosphate in the presence of active spinach phosphoribose isomerase (10 U/ml), which isomerised the substrate to Rul-5-P, resulted in the formation of high AI-2 activity, whereas no significant activity was observed after incubation with heat-inactivated enzyme (Fig. 2B). Furthermore, time course experiments performed with freshly prepared 0.5 mM Rul-5-P incubated at 37°C showed that AI-2 activity gradually increased over time, typically displaying a maximum after 5 hours (Fig. 2C). This was consistent with a conversion of Rul-5-P firstly to DPD, and then to the less-active MHF, in agreement with previously observed spectrophotometric data [11,13]. Fig. 2C shows that approximately 20-fold induction of bioluminescence was observed for the sample removed at the beginning of the time course experiment (0 h). This activity most likely originated from Rul-5-P conversion during the several hours of incubation in the V. harveyi BB170 bioassay rather than an intrinsic activity of the compound itself.

The concentration range of Rul-5-P used in these experiments was chosen because it matches the intracellular concentrations reported for some organisms in the literature, such as yeast (approximately 0.5 mM; [18]). How-
ever, much lower values in the range of 1–10 μM can be estimated from metabolite determinations in animal and human tissues, including liver, brain, blood samples [19-22].

Rul-5-P has been previously reported to give rise to MHF with approximately 1.3% of the sugar-phosphate converting to the furanone after incubation at pH 7.5 and 35°C for 15 h [13]. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) confirmed that MHF was also formed under the conditions used in this study (data not shown), with conversion rates below 1% after 24 h of incubation at pH 7.7 and 37°C. Taking into account the low conversion rates, much higher AI-2 activities were observed in Rul-5-P solutions than could be expected solely due to formation of MHF (Fig. 2A and data not shown). This demonstrates that another compound derived from Rul-5-P, but different from MHF, was responsible for the observed activities. Since DPD is known to be generated in Rul-5-P solutions [13] and was shown). This coincided with enhanced recovery of endogenous-induced bioluminescence observed for the luxS mutant culture supernatants (Fig. 3B). Incorporation of HEPES did not affect the detection of true AI-2 activity in culture-fluid samples as shown by the unaffected response to positive control samples. Overall, the data demonstrated that culture-supernatants of the two luxS mutants grown in LB medium did not contain detectable AI-2 activity.

**AI-2 activity is detectable in an E. coli luxS mutant with altered carbon flow**

AI-2 activity was not apparent in the culture-fluids of luxS mutants investigated above. However, in E. coli at least, just 25% of exogenously supplied glucose is believed to be catabolised via the oxidative pentose phosphate pathway (OPPP) to yield the intermediate Rul-5-P [25]. It is therefore possible that LuxS-independent generation of AI-2 from Rul-5-P may be limited under the growth condition used in this study. It is also possible, that Rul-5-P concentrations are generally very low in E. coli and related organisms. In order to maximise the potential for detectable AI-2 production from the sugar-phosphate in vivo, an E. coli MG1655 pgi, edd, eda triple mutant (E. coli pgi-EDP; [25]) was utilised. In this strain, glucose catabolism occurs exclusively via the OPPP as entry of the sugar into the glycolytic and Entner-Doudoroff pathways are blocked ([25]; pgi encodes phosphoglucose isomerase; edd and eda encode phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase, respectively). Production of LuxS-derived AI-2 activity by E. coli pgi-EDP was eliminated through the introduction of a luxS mutation. This was achieved by P1 bacteriophage transduction of the disrupted luxS gene from E. coli BL21 luxS [9] into E. coli pgi-EDP. The mutation was also introduced into the E. coli MG1655 parent strain.

E. coli MG1655 luxS and pgi-EDP luxS were grown in LB containing 0.5% glucose. Cell-free culture-supernatants were harvested and tested for bioluminescence-inducing ability using the optimised (HEPES-containing) V. harveyi BB170 bioassay. E. coli pgi-EDP luxS culture-supernatants induced a response in V. harveyi BB170 that, whilst modest, was clearly not observed for E. coli MG1655 luxS (Fig. 3C). Typically a peak in bioluminescence-inducing activity was observed in E. coli pgi-EDP luxS culture-fluids following 5 hours of culture and displayed 4–6 fold greater bioluminescence induction than sterile LB alone. However, in the absence of glucose, AI-2 activity could not be detected (data not shown).

Several potential sources of false-positive results were investigated to determine whether the observed effects were a result of some non-AI-2-specific influence as witnessed for the unmodified bioassay. Assay medium pH was recorded following addition of all test samples and
verified as being unaffected, thus confirming that elevated bioluminescence-induction by \textit{E. coli} pgi-EDP luxS culture-fluids was not an artefact of acidification of the culture medium. In addition, viable counts were performed for the \textit{V. harveyi} BB170 assay samples used to generate the data in Fig. 3C to ensure that differences in bioluminescence-induction between supernatant samples were not caused by effects on proliferation of the reporter strain (not shown). Furthermore, the possibility of obtaining erroneous results due to the repressive effects of glucose upon bioluminescence, as described by [24], was eliminated through the use of AI-2-negative media control samples with and without added glucose. These data therefore indicated that \textit{E. coli} pgi-EDP luxS culture-fluids contained very low but detectable levels of AI-2 activity.

This suggests that increased flux through the OPPP may indeed lead to increased cellular generation of AI-2-like molecules via the DPD intermediate. However, whether the OPPP represents one of the alternative major AI-2 production pathways predicted to exist in \textit{E. coli} by stochastic modelling [26] remains doubtful, as only very low amounts of AI-2 could be detected for a metabolically crippled \textit{E. coli} MG1655 pgi-edd-eda-luxS quadruple mutant but not \textit{E. coli} MG1655 luxS. Future work will require the development of more stringent physical or chemical methods to unequivocally detect and quantify AI-2 molecules in complex biological samples, particularly when present at low concentrations. Once developed, such methods may allow more robust quantitative detection of, and distinction between, different DPD-derivatives. This would allow conclusive confirmation as to whether the Rul-5-P-dependent mechanism of AI-2 production is operational \textit{in vivo}. An overview of the different pathways leading to DPD and AI-2 formation is given in Fig. 1.

**Implications**

Our finding that the DPD levels formed during the spontaneous conversion of Rul-5-P are sufficient to give rise to measurable AI-2 activity has several important implications. First, given the ubiquitous presence of Rul-5-P in metabolically active cells [27], it seems possible that molecules with AI-2 activity are intrinsically formed as by-products of pentose phosphate metabolism in all organisms and independently of the LuxS enzyme. Indeed, AI-2 activity has been reported in stationary phase culture-fluids of \textit{Streptococcus pyogenes} luxS mutants [28]. Furthermore, some microorganisms, plants, and animals are known to produce MHF [29-33], a compound which, at least in the case of certain yeasts, is believed to be derived from Rul-5-P [12,13].

Production of AI-2-like activity by higher organisms has also been demonstrated for algae of the genera \textit{Chlamydomonas} and \textit{Chlorella} [34] and evidence for the

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**Figure 3**

\textit{E. coli} MG1655 pgi-EDP luxS produces extracellular AI-2 activity. (A) Conventional \textit{V. harveyi} BB170 bioassay [17] with \textit{E. coli} DH5\textsubscript{x} culture supernatants obtained from cultures growing in LB + 0.5\% (w/v) glucose. Turquoise, grey, pink, bright green, dark green, blue, and red lines indicate the bioluminescence observed for \textit{E. coli} DH5\textsubscript{x} culture supernatants at 0, 1, 2, 3, 4, 5, and 6 h of growth, respectively. Open circles, negative control (LB medium + 0.5\% glucose); closed circles, AI-2 containing positive control (\textit{E. coli} MG1655 culture supernatant after 3 h of growth). (B) Modified \textit{V. harveyi} BB170 bioassay with the same \textit{E. coli} DH5\textsubscript{x} culture supernatants as analysed in (A). 25 mM HEPES was present in the bioassay medium to prevent acidification. For figure legends, see (A). (C) Growth (lines) and AI-2 activity profiles (bars) for \textit{E. coli} MG1655 luxS (blue) and \textit{E. coli} MG1655 pgi-EDP luxS (red). Each strain was grown in LB medium + 0.5\% (w/v) glucose and samples removed hourly. For each sample the optical density at 600 nm was recorded and AI-2 activity in culture supernatants recorded using the modified \textit{V. harveyi} BB170 bioassay containing 25 mM HEPES. Results represent the mean of three independent bioassays. For AI-2 activity, error bars represent the standard deviations. The experiments were repeated five times with similar results.
formation of DPD from Rul-5-P in tomato fruits has been provided [13]. Thus, organisms other than bacteria may have developed the machinery necessary to either metabolise or exclude DPD-derived by-products such as AI-2. This may also explain the presence of lsr-type AI-2 uptake systems in bacteria that do not contain a luxS homologue [3]. Sinorhizobium meliloti, for instance, possesses a complete putative lsr AI-2 uptake system and an AI-2 kinase (locus tags SmB12016-21022). Systems like this may have been acquired to minimise the loss of intrinsically produced DPD-derived compounds, or alternatively to scavenge the molecules released from other organisms present in the same niche, either bacteria or plants.

Finally, given the relative ease with which DPD, and thus AI-2, appears to form from Rul-5-P, and the potential advantages currently believed to be gained by the utilisation of AI-2-dependent signalling, it would seem an interesting, but as yet unexplored, possibility that some organisms may have acquired enzymes to direct and enhance this process of AI-2 formation in a controlled fashion.

Conclusion
In conclusion, we have demonstrated that spontaneous degradation of Rul-5-P gives rise to substantial amounts of AI-2 activity in vitro. However, our data suggest that whilst this route may also be operational in vivo, in E. coli its contribution to AI-2 production is negligible. It could, however, be responsible for the AI-2-like signals reported for some higher organisms or bacteria lacking luxS. Given the postulated importance of AI-2-based quorum sensing in many pathogenic bacteria, the generation of DPD via a LuxS-independent route may have important implications.

Methods

Strains and media
E. coli DH 5α, E. coli Bl21 luxS, E. coli MG1655 derivatives, and S. aureus Newman luxS [35] were routinely grown in Luria-Bertani (LB) broth or agar plates at 37°C. V. harveyi BB170 was grown in LB or AB medium [17]. Where required, antibiotics were used at the following concentrations: 30 μg/ml chloramphenicol (for E. coli luxS mutants), 10 μg/ml tetracycline (S. aureus Newman), and 100 μg/ml kanamycin (V. harveyi BB170 and BB886).

Autoinducer bioassays
The V. harveyi BB170 bioassay was used for the detection of AI-2 activity in culture supernatants or in vitro reactions. Initially the procedure outlined by Bassler et al. [17] was followed. For more careful analyses of luxS mutants, the assay was modified so that the AB medium employed contained 25 mM HEPES buffer, pH7.8, as additional component. AI-2 activity was defined as the fold increase in light production in comparison with medium or buffer controls. In vitro reactions were also tested using the Al-1 responsive reporter V. harveyi BB886 [17].

Generation of luxS mutants
The luxS deletion of E. coli Bl21 luxS [9] was transferred into E. coli MG1655 wildtype and an E. coli MG1655 pgil-ede eda triple mutant (E. coli pgil-EDP, [25] by phage transduction using the bacteriophage P1 and a standard protocol [36]. Successful transfer of the locus was established by PCR, using the primer pair LuxS-50-UF (CTCAGCTCGCATGGAAGAAAGG) and LuxS-50-DR (GTGCCGACTAAGTACAACTAAGG).

Preparation of sugar phosphates for the in vitro production of AI-2
Sugar phosphate solutions were prepared in 10 mM sodium phosphate buffer (pH 7.7) and, where indicated, were incubated at 37°C. Following the indicated incubation period each sample was frozen using dry ice and stored at -80°C. Rul-5-P and other sugar phosphates were obtained from Fluka/Riedel-de Haën and Sigma-Aldrich, respectively.

Enzymatic synthesis of Rul-5-P was carried out by incubation of 5 mM ribose-5-phosphate with 10 U/ml spinach phosphoribosilomerase in 10 mM sodium phosphate buffer (pH 7.7). The reaction was incubated at 30°C for 2 h. Catalysis was stopped by chloroform extraction of the enzyme followed by freezing the samples on dry ice. For negative controls, spinach phosphoribosilomerase was inactivated by heat treatment (5 min at 85°C).

Determination of DPD and MHF
DPD was detected after its derivatisation with o-phenylenediamine as described by Hauck et al. [13]. Detection and quantification of MHF was achieved by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS-MS) after chemical derivatisation of the compound as described by Husek [37].

Abbreviations
AI-2: Autoinducer-2; DPD: 4,5-dihydroxy-2,3-pentanediol; LC-MS/MS: liquid chromatography/tandem mass spectrometry; MHF: 4-hydroxy-5-methyl-3(2H)-furanone; OPPP: oxidative pentosephosphate pathway; Rib-5-P: ribose-5-phosphate; Rul-5-P: D-ribulose-5-phosphate; S-THMF-borate: (2R,4S)-(R)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate; R-ThMF: (2R,4S)-(R)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran; Xyl-5-P: xylulose-5-phosphate.

Authors’ contributions
TJT performed the Rul-5-P in vitro experiments and AI-2 bioassays. He also generated the luxS mutants and per-
formed the growth experiments. NMH carried out all chemical analyses, looking at DPD and MHF formation in both culture supernatants and in vitro reactions. KW conceived and designed this study and wrote the manuscript. KRH contributed to the design of this study and sections of the manuscript. All authors contributed to data analysis and interpretation.

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References

1. Bassler BL: How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 1999, 2:582-587.
2. Henke JM, Bassler BL: Three parallel quorum-sensing systems regulate gene expression in Vibrio harveyi. J Bacteriol 2004, 186:6902-6914.
3. Varsudivele A, Winzer K, Heurlier K, Tang CM, Hardie KR: Making 'sense' of metabolosensing-inducers, LuxS and pathogenic bacteria. Nat Rev Microbiol 2005, 3:383-396.
4. Schauer S, Shokat K, Surette MG, Bassler BL: The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum sensing signal molecule. Mol Microbiol 2001, 41:463-476.
5. Xavier KB, Bassler BL: LuxS: quorum sensing more than just a numbers game. Curr Opin Microbiol 2003, 6:191-197.
6. Federle MJ, Bassler BL: Interspecies communication in bacteria. J Clin Invest 2003, 112(9):1291-1299.
7. Kaper JB, Spandrio V: Bacterial cell-to-cell signaling in the gastrointestinal tract. Infect Immun 2005, 73:3197-3209.
8. Winzer K, Hardie KR, Burgess N, Doherty N, Kirke D, Holden MTG, Linforth R, Cornell KA, Taylor AJ, Hill PJ, Williams P: LuxS: its role in central metabolism and the in vitro synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. Microbiology 2002, 148:809-922.
9. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hill PJ, Williams P: Structural identification of a bacterial quorum-sensing signal containing boron. J Biol Chem 2006, 281:259-276.
10. Linforth R, Cornell KA, Taylor AJ, Hill PJ, Williams P: Effects of boron on the expression of Enzyme-1 and Enzyme-2 in the quorum sensing bacterium Vibrio harveyi. J Bacteriol 1999, 181:2321-2327.
11. Knowles FC, Chanley JD, Pan NG: Spectral changes arising from the action of spinach chloroplast ribose-phosphate isomerase on ribose 5-phosphate. Arch Biochem Biophys 1980, 202:106-115.
12. Hauck T, Landmann C, Bruhlmann F, Schwab W: Formation of 4-methyl-4-hydroxy-3(2H)-furanone in cytosolic extracts obtained from Zygosaccharomyces rouxii. J Agric Food Chem 2003, 26:1410-1414.
13. Hauck T, Hubner Y, Bruhlmann F, Schwab W: Alternative pathway for the formation of 4,5-dihydroxy-2,3-pentanedione, the proposed precursor of 4-hydroxy-5-methyl-3(2H)-furanone as well as autoinducer-2, and its detection as natural constituent of tomato fruit. Biochim Biophys Acta 2003, 1623(2-3):109-119.
14. Meijer MM, Hom LG, Kaufmann GF, McKenzie KM, Sun C, Moss JA, Matsushita M, Janda KD: Synthesis and biological validation of a ubiquitous quorum-sensing molecule. Angew Chem Int Ed Engl 2004, 43:2106-2108.
15. De Keersmaecker SC, Varszegi C, van Boxel N, Habel LW, Metzger K, Daniels R, Marchal K, De Vos D, Vanderleyden J: Chemical synthesis of S, -4,5-dihydroxy-2,3-pentanedione, a bacterial signal molecule precursor, and validation of its activity in Salmonella typhimurium. J Bacteriol 2005, 187:4553-4560.
16. Semmelhack MF, Campagna SR, Federle MJ, Bassler BL: An expeditious synthesis of DPD and boron binding studies. Org Lett 2005, 7:569-572.
17. Bassler BL, Greenberg EP, Stevens AM: Cross species induction of luminescence in the quorum sensing bacterium Vibrio harveyi. J Bacteriol 1997, 179(12):4043-4045.
18. Teleman A, Richard P, Toivari M, Penttila M: Identification and quantitation of phosphorus metabolites in yeast neutral PH extracts by nuclear magnetic resonance spectroscopy. Anal Biochem 1999, 272:71-79.
19. Huck JH, Struys EA, Verhoeven NM, Jakobs C, Knaap MS van der: Profiling of pentose phosphate pathway intermediates in blood spots by tandem mass spectrometry: application to transaldolase deficiency. Clin Chem 2003, 49:1375-1380.
20. Karetanr GC, Brown JG, Passonneau JV, Lowry OH: Effects of changes in brain metabolism on levels of pentose phosphate pathway intermediates. J Biol Chem 1969, 244:3647-3653.
21. Casazza JP, Veech RL: The measurement of xylulose 5-phosphate, ribulose 5-phosphate, and combined sedoheptulose 7-phosphate and ribose 5-phosphate in liver tissue. And Biochem 1986, 159:243-248.
22. Boss GR, Pilz RB: Phosphoribosylpyrophosphate synthesis from glucose decreases during amino acid starvation of human lymphoblasts. J Biol Chem 1985, 260(10):6054-6059.
23. Xavier KB, Bassler BL: Regulation of uptake and processing of the quorum-sensing autoinducer Al-2 in Escherichia coli. J Bacteriol 2005, 187:238-248.
24. DeKeersmaecker SC, Vanderleyden J: Constraints on detection of autoinducer-2 (Al-2) signalling molecules using Vibrio harveyi as a reporter. Microbiology 2003, 149:459-470.
25. Sauer U, Canacono F, Heri S, Perrenoud A, Fischer E: The soluble and membrane-bound transhydrogenases UdHA and PntAB have divergent functions in NADPH metabolism of Escherichia coli. J Biol Chem 2004, 279:6613-6619.
26. Li J, Wang L, Hashimoto Y, Tsao C-Y, Wood TK, Valdes JJ, Zafiriou E, Bentley WE: Stochastic model of E. coli Al-2 quorum signal circuit reveals alternative synthesis pathways. Mol Syst Biol 2006, 2:67.
27. Sprunger GA: Genetics of pentose-phosphate pathway enzymes of Escherichia coli K-12. Arch Microbiol 1995, 164:324-330.
28. Lyon WR, Madden JC, Stein J, Caparon MG: Mutation of luxS affects growth and virulence factor expression in Streptococcus pyogenes. Mol Microbiol 2001, 42:145-147.
29. Slaughter JC: The naturally occuring furanones:formation and function from pheromone to food. Biol Rev Camb Philos Soc 1999, 74:259-276.
30. Butterf GK, Takeoka GR, Krammer GE, Ling LC: The aroma of Finnish wild raspberries. Z Lebensm-Unters Forsch 1980, 171:180-182.
31. Farina JP, Le Quere JL, Duffy J, Semon E, Brossat R: 4-Hydroxy-5-methyl-3(2H)-furanone and 4-hydroxy-2,3-dimethyl-3(2H)-furanone, two components of the male sex pheromone of Eucrypta floridana (Walker) (Insecta: Blattidae, Polyzosteriinae). Biosci Biotechnol Biochem 1993, 57:2026-2030.
32. Teplitzki M, Chen H, Rajamani S, Gao M, Merighi M, Sayre RT, Robinson JB, Rolfe BG, Bauer WD: Chlamydomonas reinhardtii: secretes compounds that mimic bacterial signals and interfere with quorum sensing regulation in bacteria. Plant Physiol 2004, 134:137-146.
33. Doherty N, Holden MTG, Qazi SN, Williams P, Winzer K: Functional analysis of luxS in Staphylococcus aureus reveals a role in metabolism but not quorum sensing. J Bacteriol 2006, 188:2885-2897.
34. Sambrook J, Russell DW: Molecular Cloning: A Laboratory Manual Cold Spring Harbour: Cold Spring Harbour Laboratory Press; 2001.
37. Husek P. Chloroformates in gas chromatography as general purpose derivatizing agents. *J Chromatogr B Biomed Sci Appl* 1998, 717:57-91.