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Research Paper

Serotonin Activates Bacterial Quorum Sensing and Enhances the Virulence of *Pseudomonas aeruginosa* in the Host

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ABSTRACT

Bacteria in humans play an important role in health and disease. Considerable emphasis has been placed in understanding the role of bacteria in host-microbiome interkingdom communication. Here we show that serotonin, responsible for mood in the brain and motility in the gut, can also act as a bacterial signaling molecule for pathogenic bacteria. Specifically, we found that serotonin acts as an interkingdom signaling molecule *via* quorum sensing and that it stimulates the production of bacterial virulence factors and increases biofilm formation *in vitro* and *in vivo* in a novel mouse infection model. This discovery points out at roles of serotonin both in bacteria and humans, and at phenotypic implications not only manifested in mood behavior but also in infection processes in the host. Thus, regulating serotonin concentrations in the gut may provide with paradigm shifting therapeutic approaches.

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1. Introduction

There is compelling evidence demonstrating a gut-microbiome-brain connection linking the enteric microbiome to modulations in human health and behavior, as well as regulation of gut motility, nutrient absorption, immune system, and fat distribution (Cryan and Dinan, 2012, Backhed et al., 2005, Lee and Mazmanian, 2010). Further, the microbiome plays a role in several disorders including autism, anxiety, and depression (Cryan and Dinan, 2012, Bravo et al., 2011). Studies in mice elucidated a few of these roles: improved behavior (Hsiao et al., 2013), reduction in levels of hormones linked to stress (Desbonnet et al., 2010, Bravo et al., 2011), increased cognitive performance (Backhed et al., 2005), and modulation of brain control of emotion and sensation (Tillisch et al., 2013). These studies point out to the gut microbiota being a virtual endocrine organ, manipulating and producing hormones and neurotransmitters that influence the host's brain and behavior (Clarke et al., 2014).

Key to unlocking the role of the gut microbiome is to understand the interactions with its environment. More than 90% of the body's monoamine serotonin is synthesized by gut enterochromaffin cells (Berger et al., 2009). However, the molecular mechanism that dictates the levels of serotonin produced and its metabolism is not fully elucidated. This is of utmost importance given that gut-derived serotonin is responsible for regulation of functions such as bone development, immune responses, gut motility, and platelet aggregation (Berger et al., 2009). Perhaps more interesting is the role that dysregulation of serotonin plays in the pathogenesis of certain intestinal diseases such as irritable bowel syndrome (IBS). IBS has been shown to have a microbial etiology (Knights et al., 2013, Jeffery et al., 2012) and as such, the link between the microbiome and serotonin is of interest. Two hypotheses have been formulated regarding the microbiome's contribution to serotonin levels in the host. The first relates to the fact that some species of bacteria, such as *Escherichia coli* and *Streptococcus* species, are capable of *de novo* serotonin synthesis (Lyte, 2013). The second hypothesis states that the microbiome influences the biosynthesis of serotonin by the host (Yano et al., 2015). Indigenous gut bacteria are able to regulate peripheral host serotonin biosynthesis by interactions with the intestinal enterochromaffin cells (Yano et al., 2015). Further, the microbiota plays a role in the regulation of central nervous system serotonergic neurotransmission profiles in a sex-dependent manner (Clarke et al., 2013). It has been also demonstrated that germ free mice synthesize lower levels of serotonin and its metabolites (Marcobal et al., 2013), indicating that the microbiome is an important factor for the synthesis of serotonin.

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Despite all these observations linking bacteria to intrinsic serotonin synthesis, very little is understood regarding why, how, and what are the consequences of the microbiome's influence on the host's neurotransmitter levels and its manipulation, and *vice versa*. Most studies focus on the influence of the microbiome on changes to the host's production of these molecules, while very few examine the effects these molecules have on bacteria. It was observed that serotonin was able to stimulate growth of specific bacteria in culture (Oleskin et al., 1998), and some virulent bacteria can use neurotransmitters such as epinephrine and norepinephrine to activate virulence genes (Clarke et al., 2006). Both of these phenomena are linked to bacterial quorum sensing (QS), which is, in bacteria, a phenomenon when populations reach a specific threshold they communicate with organisms in their surroundings by releasing small diffusible quorum sensing molecules (QSMs). The QSMs then bind to regulatory proteins, causing a conformational change and allowing the protein to bind to DNA and initiate the transcription of virulence factors (Fig. 1). Herein, we hypothesized that bacteria are able to interact with host serotonin molecules and exploit them as a bacterial QSMs. Specifically, through experiments conducted *in vitro* and *in vivo* in animal studies, we demonstrate for the first time that serotonin acts as a signaling molecule for the *las* regulatory QS system of *Pseudomonas aeruginosa* inducing, among other effects, serious pathogenicity in the host. *P. aeruginosa* has a well-studied QS network that relies on multiple QS pathways critical in activating *Pseudomonas* virulence including the *las* and *rhl* systems. This work helps explains how high levels of serotonin found in the gut, produced endogenously or by bacteria, can be linked to the host's health.

2. Materials and Methods

2.1. Plasmids and Bacterial Strains

For the whole-cell bioassays, *E. coli* DH5α cells harboring pSB1075 and pSB904, for LasR and RhlR production, respectively, were used. Native *P. aeruginosa* strain PAO-1 and the *lasI* and *rhlI* double mutant, JP2, were supplied by Dr. Johanna Schwingel.

2.2. Dose-response curves for LasR and serotonin

Dose-response curves to determine response to analyte and competitive assays were generated as previously described (Kumari et al., 2006).

2.3. Elastase Studies

Elastase production was measured using a previously established protocol with minor adjustments (Smith et al., 2003). *P. aeruginosa* PAO1 cells and JP2 cells were grown overnight in LB broth at 37 °C and 250 rpm. The cells were then diluted to an OD_{600} of 0.05 in fresh PTSB media and incubated in the presence of either *N*-3-oxo-C12-HSL or serotonin for 16 h at 37 °C and 250 rpm. After incubation, the OD_{600} of the cells was measured and they were centrifuged to separate the cells from the supernatant. The supernatant was then added to tubes containing 25 mg of Elastin congo red and a 20 mM Tris, 1 mM $CaCl_2$ buffer and incubated overnight at 37 °C and 250 rpm. The reaction was then quenched with EDTA and the tubes were then centrifuged to remove unreacted Congo red-elastin and the absorbance of the supernatant was measured at 495 nm. All absorbance values were normalized to the OD_{600} values in order to correct for the variations of cell growth.

2.4. Protease Studies

To measure proteolytic activity, 3 mL of supernatant from an overnight culture of PAO1 or JP2 cells were added to 10 mM Tris HCl buffer, pH 7.5 and incubated with 15 mg of Hide powder at 37 ° C for 1 h at 250 rpm. Undissolved substrate was removed by centrifugation at 3000*g* for 10 min and the absorbance of the supernatant was measured at 595 nm.

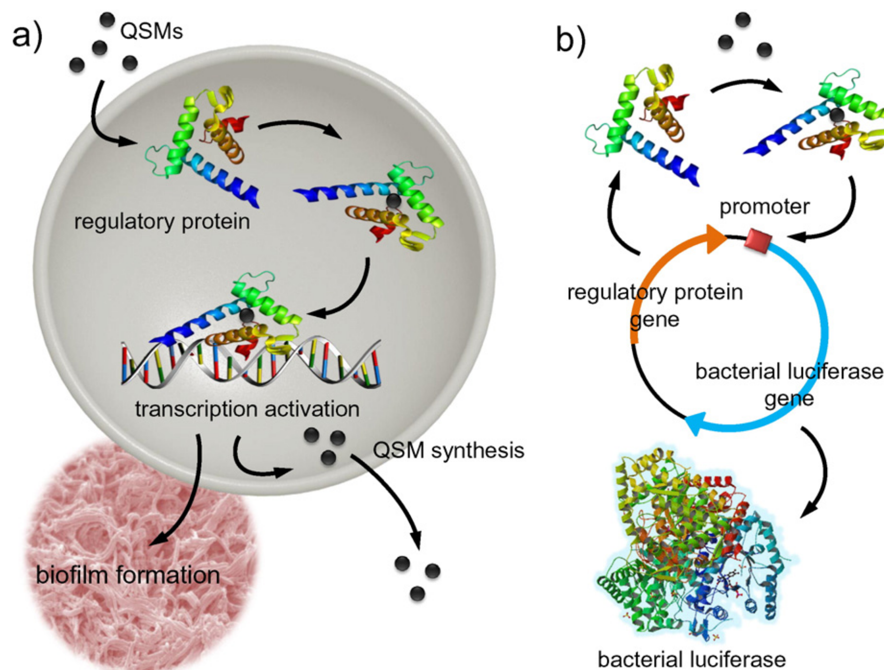

Fig. 1. Bacterial quorum sensing and quorum sensing molecule detection. (a) Quorum sensing molecules (QSMs) enter the bacterial cell and bind to specific regulatory proteins. The QSM-regulatory protein complex activates transcription of virulence factors, such as biofilm formation, as well as production of more QSMs. (b) QSMs can be detected by employing the native regulatory proteins and corresponding promoter regions in a bacterial plasmid based system. The plasmid consists of a regulatory protein that is constitutively expressed and the corresponding promoter region that is fused to a reporter protein, such as bacterial luciferase. This allows for dose-dependent generation of signal when QSMs are present. The plasmid is then expressed in a strain of bacteria that does not generate the QSM of interest.

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