Microbial Small-Talk: Does Quorum Sensing Play a Role in Beer Fermentation?

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ABSTRACT

Inter- and intraspecies communication between microorganisms is recognized to play an influential role across many relevant applications, such as bioethanol production, food preservation, bioremediation, wastewater treatment, and in the clinical environment. At high population densities, the accumulation of small hormone-like molecules in the extracellular environment has been demonstrated to play a role in transcriptional regulation of both prokaryotic and eukaryotic microorganisms. This mechanism enables independent cells, known as the quorum, to trigger coordinated, community-wide gene expression, increasing their prospect of survival in crowded and ever-changing conditions. This density-dependent phenomenon has been shown to influence community-wide behavioral factors, such as biofilm formation, virulence factor secretion, social motility, cellular adhesion, autolysis, secondary metabolite production, and nutrient uptake across a range of organisms. In *Saccharomyces cerevisiae*, aromatic alcohols 2-phenylethanol, tryptophol, and tyrosol have already been demonstrated to act as quorum sensing molecules under certain environmental conditions, such as low-nitrogen availability. This review discusses the underlying mechanisms of quorum sensing, with an emphasis on brewer's yeast, while offering insights into how cell density-dependent signaling could influence the commercial brewing industry.

INTRODUCTION

Microorganisms do not live a cloistered, solitary self-reliant existence. Instead, these microscopic lifeforms cohabitate, establish complex communities, and regularly interact with each other across many different environments (Allen et al., 2019; Butler & O'Dwyer, 2018; Florenza, Tamminen, & Bertilsson, 2019; Fröls, 2013; Kent & Triplett, 2002; Laureys, Britton, & De Clippeleer, 2020; Little, Robinson, Peterson, Raffa, & Handelsman, 2008; Montgomery, Charlesworth, LeBard, Visscher, & Burns, 2013; Mosel, Dumitru, Hornby, Atkin, & Nickerson, 2005; Niemann et al., 2006; Pereira, Cabezas, Etchebehere, Chernicharo, & de Araújo, 2017; Rankin, Gibson, Franzmann, & Burton, 1996; Shade, McManus, & Handelsmana, 2013; Shank, 2018; Wood, 2019; Zengler & Zaramela, 2018). As a means of survival, many microorganisms have evolved the capacity to communicate via the exchange, and detection of molecular cues referred to as autoinducers (AI) or quorum sensing molecules (QSM) (Fuqua et al., 1994). Although diversity exists between the quorum sensing mechanisms discovered in archaea, bacteria, and fungi, particularly the chemical identity of the signaling molecule and the behavior regulated, every quorum-sensing system (QSS) requires the molecular machinery to synthesize, sense, and react to secreted communication molecules (Albuquerque & Casadevall, 2012; Atkinson & Williams, 2009; Avbelj, Zupan, Kranjc, & Raspor, 2015; Bassler, 2002; March & Bentley, 2004; Montgomery et al., 2013; Padder, Prasad, & Shah, 2018; Pour, 2018; Sifri, 2008; Waters & Bassler, 2005). As a population of "quorum competent" microorganisms arises, a proportional increase in these intercellular signaling molecules (ISMs) occurs, and once the "quorate" threshold is achieved, this results in the quorum-associated transcriptional control of specific target genes (Albuquerque & Casadevall, 2012; Avbelj et al., 2015; De Sordi & Mühlschlegel, 2009; Hense & Schuster, 2015; Hogan, 2006a, 2006b; Padder et al., 2018; Schuster, Lostroh, Ogi, & Greenberg, 2003; Sifri, 2008; Verbeke et al., 2017). At the transcriptome level, the coordinated response to a quorum-sensing signal can be substantial, for instance in the bacterium *Pseudomonas aeruginosa* hundreds of genes, representing more than 5% of the entire genome, were identified as either quorum-induced or quorum-repressed (Schuster et al., 2003; Schuster, Sexton, & Hense, 2017; Wagner, Bushnell, Passador, Brooks, & Iglewski, 2014). Although, there is evidence of signal-blind organisms synthesizing, but that are incapable of responding to, QSMs in order to manipulate the behavior of neighboring cells, andexamples of signal eavesdropping, where nearby cells will detect, and respond to, QSMs

not produced by their own genotype (Katzianer, Wang, Carey, & Zhu, 2015; Miller & Bassler, 2001; Özkaya, Xavier, Dionisio, & Balbontín, 2017; Wellington & Greenberg, 2019).

It is now widely accepted that quorum-sensing (QS) mechnisms promote population density-dependent advantages that result from a synchronized cooperative behavioral response, these include biofilm regulation; bioluminescence; sporulation; competence; fruiting body development; social motility; cellular adhesion; population density control; virulence; exopolysaccharide and antibiotic production; secondary metabolite production; stress adaptation mechanisms; cell morphology and dimorphism; spatially heterogeneous behavior, and modifications to nutrient uptake amongst others, which enable them to benefit from collective cooperative behaviors, often these can be in the form of secreted products, such as exopolysaccharide or extracellular enzymes, occasionally refered to as "public goods" (Albuquerque & Casadevall, 2012; An et al., 2014; Darch et al., 2012; Diggle, Crusz, et al., 2007; Diggle, Gardner, et al., 2007; Gómez-Gil et al., 2019; Hense et al., 2012; Hornby et al., 2001; Labbate et al., 2007; Miller & Bassler, 2001; Mosel et al., 2005; Nealson & Hastings, 2006; Nickerson et al., 2006; Padder et al., 2018; Pai et al., 2012; Pena et al., 2019; Ramage et al., 2002; Schuster et al., 2017; Waters & Bassler, 2005; Westwater et al., 2005; Williams et al., 2007; Wongsuk et al., 2016). These diffusible extracellular products provide an advantage to cells co-existing at higher cell density, but little advantage those in less dense populations. However, recent studies have demonstrated several non-diffusible cell-bound products, referred to as "private goods" , such as cytosolic- or surface-bound proteins and metabolites, to also be under QS control, where the beneficial advantage may be associated with “private goods” is either directly or indirectly involved in the cooperative behavior (Schuster et al., 2017). Furthermore, it is not yet apparent why cellularly-bound products are subject to QS regulation, as it was previously assumed, due to their intracellular or membrane-bound nature, that the benefits associated with “private goods” were limited to the individual cell.

Although defined as a density-dependent behavior, it should not be generally assumed that the biological functions of QSMs are exclusively restricted to provoking synchronized behavioral adaptation; conversely, some researchers have proposed a less socially motivated explanation, primarily that AI may be used by cells as a means to assess diffusion efficacy within the local environment (Redfield, 2002; West, Winzer, Gardner, & Diggle, 2012). Presumably, diminished diffusion rates, as assessed by increasing AI concentrations, may be advantageous for the transcriptional expression or repression of particular gene products. Although some experimental evidence supports this hypothesis, the recognition of diffusion sensing as the generalized model for small molecule-based signaling remains to be widely accepted.

Regardless, whether present in nature or a synthetic environment, microorganisms are in constant interaction with themselves (autoinduction) and each other. In principle, commercially fermented products, like beer, are defined by the metabolic activity of a dense population that is likely influenced by the chemical 'small-talk' occurring between these organisms. However, while the metabolic activity related to beer fermentations have been exhaustively investigated for more than two centuries (Barnett, 1998), the impact of density-dependent signaling within the commercial brewing ecosystem remains to be explored. As it is highly plausible that these underlying social mechanisms influence the brewing fermentation and resultant product; this review presents the current understanding of quorum sensing, particularly in the brewer's yeast *Saccharomyces cerevisiae*, while also offering insights on how density-dependent signaling could unknowingly impact the commercial brewing process.

DEFINING QUORUM SENSING MOLECULES

A fundamental problem when studying quorum sensing is the lack of consensus on what defines a QSM explicitly. As a wide-range of microbial metabolites can be found within commercial fermentations, at least in principle, each of these metabolites could potentially act as a QSM. Previously, a few groups have proposed minimum criteria to designate a specific chemical compound as a QSM. These minimal criteria were recently summarized by (Winters, Arneborg, Appels, & Howell, 2019); however, they also offered a more restrictive definition, which proposes that QSM, as a sub-set of ISM, must additionally meet the essential criteria for classification as an ISM. As proposed by Winters and colleagues (2019) and summarized in Table 1, their criteria would require that: **I)** the compound be known, secreted, and accumulate in the external environment in a manner where it is accessible to other cells (Albuquerque & Casadevall, 2012; Monds & O'Toole, 2014), **II)** specific receptors and signaling cascade mechanisms that detect and respond to the compound should be present and identified (Monds & O'Toole, 2014; Winzer, Hardie, & Williams, 2002), **III)** the purified compound reproduces the biological response at concentrations found in the experimental conditions (Monds & O'Toole, 2014), **IV)** the biological response to the compound maximizes the fitness of the community involved in signaling (March & Bentley, 2004; Monds & O'Toole, 2014), **V)** the compound must be synthesized in a concentration proportional to cell density in a diffusion-limited environment, and **VI)** a concerted biological response, other than detoxification or metabolism of the compound itself, is induced once quorate threshold is reached..

Nevertheless, debate still exists on specific criteria needed to classify QSM, mainly whether a compound must be created exclusively for intercellular signaling (IS), or if a compound may also serve a coexisting metabolic function (Monds & O'Toole, 2014). Monds & O'Toole (2014) advocated that metabolites are well-suited to be ISMs, and consequently, QSMs difunctionality is energetically favorable for the organism. Although this has been less thoroughly investigated in fungal systems, as far as the authors are aware, several studies support the suggestion that metabolites, such as antibiotics, serve as QSMs in bacteria without violating the VI criterion (Davies, Spiegelman, & Yim, 2006; Morcia et al., 2017; Struss, Pasini, Flomenhoft, Shashidhar, & Daunert, 2012; Yim, Wang, & Davies, 2007). Certain antibiotics, like erythromycin and rifampin, were shown to even activate transcription at sub-MIC concentrations, despite being well-recognized inhibitors of translation and transcription, respectively (Goh et al., 2002). The divergent effect of antibiotics (hormesis), presenting both a stimulatory and inhibitory effects on transcription, at low and high doses, respectively has been demonstrated in numerous studies (Andersson & Hughes, 2014; Davies et al., 2006; Hoffman et al., 2005; Laureti, Matic, & Gutierrez, 2013; Patel et al., 2006; Rachid, Ohlsen, Witte, Hacker, & Ziebuhr, 2000; Yim et al., 2007).

Therefore, the authors concur with the restrictive definition and criteria established proposed by Winters and colleagues (2019) for QSMs, and do not support the addition of criteria that would restrict the definition to compounds used exclusively for IS, as substantial evidence exists for a difunctional role of QSMs as metabolites in bacteria.

QUORUM SENSING MECHANISMS

Quorum sensing, as a term, was initially introduced by Fuqua et al., (1994), and can be best described as a subset of IS chemical cues that facilitate cell-density dependant gene expression after a critical concentration of the signaling compound has been achieved in the external environment (Begley et al., 2008; Miller & Bassler, 2001; Winzer et al., 2002). Every quorum sensing system can be separated into four distinct phases: **I)** synthesis of autoinducer QS molecules by the cell; **II)** release of the QS molecules, either actively or passively, into the

extracellular environment; **III)** recognition of the QS molecules by a cognate receptor-regulator, and (**IV**) initiating changes in gene regulation, either induction or repression, in receiver cells once the threshold concentration is exceeded (Engebrecht et al., 1983; Sifri, 2008). Consequently, QS provides a significant evolutionary advantage by allowing microbial populations to adapt to swift environmental changes. Miller and Bassler (2001) proposed that QS could be a neo-Darwinian mechanism of evolution that contributed to the emergence of multicellular organisms roughly 600 million years ago.

The diversity of QSMs within microorganisms are wide-ranging in terms of chemical structure and function. Presently, four primary classes of QSMs are widely recognized; these include lactones, alcohols, sesquiterpenes, and peptides (Barriuso, Hogan, Keshavarz, & Martínez, 2018; Winters et al., 2019). However, the recent discovery of QS mechanisms in yeast and dimorphic fungi, employing amino acid-derived aromatic alcohols, have been proposed as an entirely new class of QSMs (Winters et al., 2019).

QUORUM SENSING MECHANISMS IN BACTERIA

In bacteria, quorum-sensing systems can be divided into four primary classes based on autoinducer type and the associated cellular detection machinery; **I)** LuxI/R-type, **II)** autoinducer oligopeptide (AIP) transcription factor binding, **III)** Gram-positive two-component and **IV)** Gram-negative two-component circuits (Miller & Bassler, 2001; Rutherford & Bassler, 2012). Most quorum sensing gene regulatory systems existing in Gram-negative bacteria are regulated via the binding of acylated homoserine lactone molecules (AHL), a LuxI-type synthase that catalyzes a reaction between S-adenosylmethionine (SAM) and an acyl carrier protein (ACP), or an alternate molecule similarly dependant on SAM, to a cognate cytosolic LuxR-type transcription factor that binds DNA and regulates differential gene expression (Ball et al., 2017; Miller & Bassler, 2001; Rutherford & Bassler, 2012; Yong & Zhong, 2012). It is noteworthy to mention that AHLs are not exclusively manufactured by LuxI-type proteins and that AHLs of dissimilar bacteria possess side chains of varying lengths, from C4 – C18, and may include side-chain decorations like carbonyl and hydroxy moieties (Fuqua et al., 2001; Ng & Bassler, 2009). Structural diversity among AHLs may aid in intraspecies-specific communication via interaction with high-specificity cognate LuxR proteins, which are only stabilized to fold, bind DNA, and modulate transcription once bound to QSMs (Engebrecht et al., 1983; Engebrecht & Silverman, 1984; Stevens et al., 1995; Zhu & Winans, 1999, 2001). However, when not bound to QSMs, LuxR-type proteins are undergo swift degradation, likely to inhibit bacteria from side-stepping their own innate QS systems (Rutherford & Bassler, 2012).

Autoinducer oligopeptides (AIPs), or quorum sensing peptides (QSPs), are the signaling molecules, often post-translational modified, used by Gram-positive bacteria for cell-to-cell communication (Ansaldi, Marolt, Stebe, Mandic-Mulec, & Dubnau, 2002; Otto, Süßmuth, Jung, & Götz, 1998; Rajput, Gupta, & Kumar, 2015). In these QS systems, precursor AIPs (pro-AIPs) are secreted, processed by extracellular proteases, and afterwards imported into the cell where their direct binding to a DNA response regulator alters the transcriptional regulation of associated quorum-sensing genes (Slamti & Lereclus, 2002).

Alternatively, Gram-positive bacteria may also rely on two-component QS circuits, where oligopeptide AIPs, or other molecules, are instead are detected by membrane-bound signal transduction systems (Esmaeilishirazifard, De Vizio, Moschos, & Keshavarz, 2017; Håvarstein, Coomaraswamy, & Morrison, 1995; Ji, Beavis, & Novick, 1995; Kleerebezem, Quadri, Kuipers, & De Vos, 1997; Monnet, Juillard, & Gardan, 2016). Within this third class of QS circuits, AIPs are encoded as precursors (pro-AIPs), processed and transported to the external milieau via specialized transporters to yield AIPs, typically 5 to 17 amino acids in length, which afterwards

are detected by membrane-bound two-component histine kinase receptors (Esmaeilishirazifard et al., 2017; Monnet et al., 2016; Rutherford & Bassler, 2012). After binding of the AIP to the two-component kinases, autophosphorylation of conserved histidines occurs, where afterwards the phosphoryl group is transferred from histidine to a conserved aspartate residue on a cognate cytoplasmic response-regulator (Rutherford & Bassler, 2012). Lastly, Gram-negative bacteria, in certain instances, have also demonstrated the capability to detect AIs via two-component histidine kinase receptors that function analogously to those described in Gram-positive bacteria.As LuxI/LuxR, autoinducer oligopeptide (AIP) transcription factor binding, and two-compartment sensing systems in bacteria have already been extensively reviewed; the authors recommend reviewing the publications by Abisado, Benomar, Klaus, Dandekar, & Chandler, (2018); Papenfort & Bassler, (2016); and Rutherford and Bassler (2012) for further information.

QUORUM SENSING MECHANISMS IN FUNGI

In fungi, it has for a long-time been widely accepted that IS is responsible for the regulation of a wide range of critical biological processes controlled by chemical messengers, such as alcohols, small peptides, lipids, small molecules, and volatile compounds (Cottier & Mühlschlegel, 2012; Jones & Bennett, 2011; Palkova et al., 1997; Richard, Bakker, & Teusink, 1996). However, unlike bacteria, the existence of QS in eukaryotic organisms was unknown until farnesol was demonstrated to be a QSM in *Candida albicans*, a well-described human pathogen, less than two decades ago (Hornby et al., 2001). Since then, aromatic alcohols like tyrosol (Chen, Fujita, Feng, Clardy, & Fink, 2004; Gori, Knudsen, Nielsen, Arneborg, & Jespersen, 2011; Rossignol et al., 2007), 2-phenylethanol (Chen & Fink, 2006; Gori et al., 2011; Huang et al., 2020), and tryptophol (Chen & Fink, 2006; Gori et al., 2011; Verbrugghe, Adriaensen, Martel, Vanhaecke, & Pasmans, 2019) have been shown to act as QSM in across many different fungal species. However, the role of farnesol and tyrosol in the regulation of yeast-to-hyphal transition, within the model organism *Candida albicans*, remains the most exhaustively investigated eukaryotic QSS (Chen et al., 2004; Hornby et al., 2001; Lindsay, Deveau, Piispanen, & Hogan, 2012; Polke et al., 2017).

Tyrosol, tryptophol, and 2-phenylethanol are created from the transanimation, decarboxylation, and reduction of the amino acids tyrosine, tryptophan, and phenylalanine, respectively via the Ehrlich pathway (Ehrlich, 1907; Hazelwood, Daran, Van Maris, Pronk, & Dickinson, 2008; SentheShanuganathan, 1960). Tyrosol is generated via the transamination of tyrosine into the α-keto acid p-hydroxy 3-phenylpyruvate, which is then decarboxylated to the higher aldehyde p-hydroxy 3-phenylacetaldehyde and then subsequently reduced by alcohol dehydrogenase to the higher alcohol tyrosol (Mas et al., 2014; SentheShanuganathan, 1960). In a comparable mechanism, tryptophol is synthesized by the transanimation of tryptophan to indol-3-pyruvate, decarboxylated to indole-3-aldehyde, and reduced to the higher alcohol tryptophol (Iraqui, Vissers, Cartiaux, & Urrestarazu, 1998; Mas et al., 2014), while phenylalanine is transaminated to phenylpyruvate, decarboxylated to 2-phenylacetaldehyde, and then reduced to 2-phenylethanol (Etschmann, Bluemke, Sell, & Schrader, 2002; Etschmann & Schrader, 2006; Mas et al., 2014). As it relates to these aromatic alcohols, transamination is catalyzed by aromatic aminotransferases I and II encoded by *ARO8* and *ARO9*, respectively; decarboxylated by aromatic decarboxylase encoded by *ARO10* and pyruvate decarboxylases (*PDC6*, *PDC5*, and *PDC1*); and reduced by alcohol dehydrogenases (*SF1*, *ADH4*, and *ADH5*) (Iraqui et al., 1998; Mas et al., 2014).

Aromatic alcohols in yeast were already shown to influence the differential expression of hundreds of genes (Chen & Fink, 2006; Wuster & Babu, 2009). An investigation by Wuster & Babu (2009) revealed 2-

phenylethanol has the highest impact with the differential expression of 412 genes in *Saccharomyces cerevisiae*, while tryptophol and tyrosol led to the differential expression of 264 and 251 genes, respectively. Similarly, investigations conducted by Jin et al. (2018) demonstrated 580 differentially expressed genes when exposed to 2-phenylethanol. Previously, Chen & Fink (2006) demonstrated that transcription factors Flo8p and Aro80p were regulated in the presence of the aromatic alcohols phenylethanol and tryptophol. Subsequent investigations by Wuster & Babu (2009) suggest that other transcription factors, like Msn2p, Mig1p, Rgm1p, Sip4p, and Cat8p, differentially regulate specific genes in the presence of 2-phenylethanol; similarly, Cat8p and Mig1p were also shown to be involved in differential transcription in the presence of tryptophol and tyrosol, which implies these genes are essential for aromatic alcohol QS in *Saccharomyces cerevisiae*. *Cat8p* has mostly been shown to be an activator of gluconeogenic genes, while *Mig1p* is involved in the regulation of enzymes that participate in response to glucose repression by influencing activators of respiration (Frolova, Johnston, & Majors, 1999; Haurie et al., 2001; Rahner, Hiesinger, & Schüller, 1999). Further transcriptional analysis, also revealed the transcription factor Pdr1p, which regulates the expression of the pleiotropic drug-resistant (PDR) transporters and amino acid metabolic genes, was shown to be regulated by tryptophol and tyrosol (Hlaváček, Kučerová, Harant, Palková, & Váchová, 2009; Nawrocki, Fey, Roepstorff, & Larsen, 2001; Wuster & Babu, 2009).

Aromatic alcohols as potential QSMs of *S. cerevisiae* will be further discussed, as **I**) aromatic alcohols are the only molecules meeting the criteria for QSMs in fungi, **II**) QS in yeasts beyond *S. cerevisiae* were already reviewed by (Albuquerque & Casadevall, 2012; Barriuso et al., 2018; Nickerson et al., 2006), and most importantly **III**) *S. cerevisiae* serves as a model research organism and is regularly used by brewers for the production of top-fermented beers.

QUORUM SENSING IN *S. CEREVISIAE*

QS community-level behavior in *S. cerevisiae* has predominantly been associated with primary flocculation (Chen & Fink, 2006; Smukalla et al., 2009) and filamentation (Avbelj et al., 2015; Chen & Fink, 2006; González et al., 2018; Lenhart, Meeks, & Murphy, 2019; Lorenz, Cutler, & Heitman, 2000). However, as indicated recently by Winters and coworkers (2019), it is difficult to assess whether these shifts in morphology meet the criteria for a QS system. This hurdle represents a challenge as **I)** the exact point of differential gene expression has not been thoroughly examined in *S. cerevisiae* and **II)** populations undergoing morphological switches, such as filamentation, will inevitably contain both morphologies.

Previous studies conducted by Smukalla and associates (2018) investigated the physiological effect of the aromatic alcohols 2-phenylethanol, tryptophol, and tyrosol on the flocculence behavior in the diploid strain EM93 that typically does not exhibit flocculent behavior in nutrient-rich medium. The results of this investigation demonstrated that trytophol induced a significant difference in flocculation behavior at concentrations of 100 μM and 1000 μM, compared to the control (Smukalla et al., 2008). These authors suggested that mutual adhesion, leading to the formation of flocs, substantially impairs diffusion, therefore, providing the innermost cells physical protection from harmful compounds in the exterior environment. As flocculence requires a sizeable microbial population, and Flo adhesins expression is only functional at higher population densities, it stands to reason that this particular adaptive trait, flocculation behavior in *S. cerevisiae,* would be expressed when exposed to the QSM tryptophol.

Initial investigations by Chen and Fink (2006) have served as the foundational evidence for a QS mechanism in *S. cerevisiae* by demonstrating that the fungal QSMs 2-phenylethanol and tryptophol stimulated

pseudohyphal growth under nitrogen-limited conditions. Broadly, this investigation demonstrated the aforementioned aromatic alcohols were proportionally produced according to cell density, and that the secretion of these compounds was subject to positive feedback regulation in nitrogen-limited conditions starvation (Chen & Fink, 2006).

Avbelj and colleagues (2015) investigated the kinetics of 2-phenylethanol, tyrosol, and tryptophol in *S. cerevisiae* and confirmed some of the foundational work conducted by Chen and Fink (2006), by demonstrating the concentration of 2-phenylethanol, tyrosol and tryptophol were cell-density and growth-phase-dependent. In this investigation, aromatic alcohols 2-phenylethanol, tyrosol, and tryptophol were shown correlate well with the expression of *ARO8*, *ARO9,* and *ARO10* (Avbelj et al., 2015), encoding for aromatic aminotransferases I and II and an aromatic decarboxylase, respectively (Iraqui, Vissers, André, & Urrestarazu, 1999; Iraqui et al., 1998). Moreover, it was revealed that *S. cerevisiae* begins with the release of 2-phenylethanol in exponential phase, with peak synthesis occurring in the declining growth phase, followed later by tyrosol production, while the peak production of tryptophol corresponds with the stationary phase of cell growth (Avbelj et al., 2015).

However, more recent investigations conducted by Lenhart and associates (2019) revealed significant phenotypic variation among environmental isolates of *S. cerevisiae* collected from fermentations, clinical patients, soil, plant, and insects upon exposure to the 2-phenolethanol and tryptophol, suggesting the filamentous phenotypic response may be a strain-specific effect. Broadly, the results indicated that exogenous exposure to 2-phenolethanol and tryptophol exhibited a minimal effect on pseudohyphae formation, while in reality, a few strains even significantly decreased pseudohyphae formation in response aromatic alcohol exposure (Lenhart et al., 2019). Moreover, the conclusion of Lenhart and colleagues is to question whether the model laboratory strains of *S.cerevisae* are representative of the broader population of *Saccharomyces spp.* used in the commercial production of beer.

ELEMENTS INFLUENCING QUORUM SENSING MOLECULE SYNTHESIS

The synthesis of the aromatic alcohols in *S.cerevisiae* are regulated by local cellular density; therefore, when cells achieve quorum, the transcription of *ARO9* and *ARO10* genes is up-regulated rousing the production of aromatic alcohols (Avbelj et al., 2015; Chen & Fink, 2006). Moreover, aromatic alcohol production can likewise be autostimulated via the transcription factor Aro80p by tryptophol, which activates ARO9 and ARO10 transaminase and decarboxylase expression yielding a positive feedback loop (Iraqui et al., 1999, 1998). Consequently, *S. cerevisiae* within higher population density environments generates more 2-phenylethanol and tryptophol than cells at lower population densities (Avbelj et al., 2015; Avbelj, Zupan, & Raspor, 2016; Chen & Fink, 2006). Avbelj et al. (2015) expanded upon the initial research by Chen & Fink to further demonstrate that the production of 2-phenylethanol, tryptophol, and tyrosol in *S. cerevisiae* were all cell density dependant by comparing fermentations with low and high initial cell concentrations.

Aromatic alcohol production by *S. cerevisiae* was previously demonstrated to also be influenced by nitrogen availability in the local environment. Chen & Fink, (2006) reported that aromatic alcohols, like 2-phenylethanol, tyrosol, and tryptophol, were found in conditions with high concentrations of ammonium, as this represses the expression of transaminases, decarboxylases, and dehydrogenases that are required for the synthesis of aromatic alcohols (Avbelj et al., 2016). Previously, it was also demonstrated by Chen & Fink, (2006) that aromatic alcohol production occurs at their highest levels when the ammonium concentrations were less than or equal to 50 μM, and were significantly reduced at concentrations greater than 500 μM.

Furthermore, ethanol was also demonstrated to negatively influence the overall rate and commencement of 2-phenylethanol, tryptophol, and tyrosol synthesis (Avbelj et al., 2015). However, it remains unclear whether the reduction is linked to the suppression of aromatic alcohol synthesis or whether a quorate populationwas never achieved due to ethanolic inhibition. On the contrary, Avbelj and colleagues assert that *S. cerevisiae* produces higher concentrations of 2- phenylethanol, tryptophol, and tyrosol when grown in anaerobic conditions than aerobic conditions, however, the authors were unable to locate any literature to support this claim despite the phenomenon having been demonstrated in other fungal species, like *Candida albicans* (Avbelj et al., 2016; Ghosh, Kebaara, Atkin, & Nickerson, 2008). As ethanol and anaerobic conditions are environmental conditions frequently observed by brewer's yeast in a commerical brewing, it is probable that these two factors could have a significant impact on existing QS-induced behavior in brewer's yeast during fermentation.

Beyond external factors, previous studies have shown that phenotypic variation among nautral isolates exposed to aromatic alcohols indicate that QS-phenotypes, at least filamentation, may be strain specific and strongly influenced by allelic variation (Lenhart et al., 2019). However, it is currently unknown to the authors whether any further investigations have been conducted to assess the impact of natually occurring allelic variants on the QS-induced filamentous behavior in *S.cerevisiae*. As some isolates from the investigation by Lenhart (2019) were sources from industrial fermentations, this begs the questions whether commercial brewing strains are differently influenced by QS compounds then research-derived strains, such as Σ1278b.

FUTURE CONSIDERATIONS

QS molecules have already been demonstrated to be synthesized and secreted by a comprehensive collection of food-associated microbiota, including *S.cerevisiae*; however, limited knowledge exists on how QS may influence the brewing process and the quality and consistency of the finished product. Nevertheless, recent achievements over the past decade associated with the manipulation of microbial behavior through the use of QSMs or quorum quenching (QQ) (Grandclément, Tannières, Moréra, Dessaux, & Faure, 2015), has renewed interest regarding the impact of density-dependent communication on fermented food and beverages, including beer.

In brewing, further investigations into QS could reveal the genes associated with QS circuits in *S.cerevisiae* which could have a sizeable impact on; the rate of fermentation, efficiency of ethanol production, the formation of ester/higher alcohols, biomass generation in propagation, yeast physiology and vitality, and flocculation to only mention a select few. It is feasible that even the solution for significant industry concerns, such as premature yeast flocculation (PYF) in commercial fermentations, a phenomenon recognised by brewing scientists but where the mechanisms are still poorly understood. In PYF, yeast will sediment from fermenting wort earlier or more heavily before reaching full attenuation, resulting in higher residual extract, reduced alcohol and CO_2 production, and increased flavor anomalies which could be attributed to cell-to-cell interactions resulting from quorum sensing.

At the time of publication, the authors are unaware of any current or prior investigations specifically aimed at investigating the impact and application of quorum sensing on commercial brewing operations. However, considering the potential significance of quorum sensing to the brewing industry, future investigations could consider **I)** diving deeper into the effect of different QS associated gene knockouts (e.g. *ARO9*, *ARO10*) on brewing fermentations rate and yeast propagation compared to wild-type strains, **II)** examining the QS variation in behavioral response that may exist between commercially relevant brewing strains, **III)** determining the role of

QS in mixed cultured fermentation and its impact on increasing the consistency of sour beers, or **IV)** unraveling the role of QS in the biofilm formation of brewery spoilage organisms and how QQ could be employed as an alternative strategy to prevent the establishment of these detrimental microbial consortiums.

Therefore, even though research over the past decade has demonstrated the importance and impact of QS systems on a wide variety of fermented foods and beverages, little investigations thus far have been done on whether, and to what extent, QS influences commercial brewing and the quality of the finished product. Certainly, the further investigation into the QSS of *S.cerevisae* and its applications towards commercial brewing is clearly warranted.

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TABLE 1.		
Attribute	**Description**	**Citation**
Proportional to Cell-Density:	QSMs are created throughout the growth phase of the organism in a concentration proportional to cell density when cultured in a diffusion-restricted environment	(Albuquerque & Casadevall, 2012; Monds & O'Toole, 2014; Winzer et al., 2002)
Triggered Response:	The biological response, beyond the detoxification or metabolism of the QSM, is stimulated once a critical threshold concentration, the quorum, in the extracellular environment is reached	(Albuquerque & Casadevall, 2012; Monds & O'Toole, 2014; Winzer et al., 2002)
Extracellular & Identifiable:	QSMs are secreted and accumulate within the surrounding extracellular environment so that the cues are readily available to neighboring cells, and should be a recognized compound.	(Albuquerque & Casadevall, 2012; Monds & O'Toole, 2014; Winzer et al., 2002)
Defined Signaling Mechanisms:	Specific receptors and cascasde mechanisms exist to respond specifically to a QSM, and be identified. However, Monds and O'Tool (2008) deemed these criteria too restrictive, as some cells internalize QSMs and sense it at an intercellular concentration.	(Monds & O'Toole, 2014; Winzer et al., 2002)
Reproducible Physiological Response:	Application of a purified QSM reproduces the biological observable response at concentrations that are physiologically relevant	(Monds & O'Toole, 2014)
Community Adaptive:	The resultant biological response maximizes the fitness of the community involved in the signaling. Consideration of the evolutionary constraints implicit in ascribing biological function to the QSM required	(March & Bentley, 2004; Monds & O'Toole, 2014)
Catabolism Byproduct:	QSMs are not exclusively a chemical product derived from metabolic catabolism. Monds & O'Toole argue that metabolites are excellent candidates for cell-to-cell signaling, as these molecules are would play a duel-role and consequently be more time and energy-efficient, however in order to claim functionality as a QSM, these moleculares must elicit a community response dissimilar to its metabolic purpose.	(Monds & O'Toole, 2014)

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