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Research Article

RESEARCH ARTICLE

Exploring anti-quorum sensing and anti-virulence based strategies to fight *Candida albicans* infections: an *in silico* approach

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ABSTRACT

The complex virulence attributes of *Candida albicans* are an attractive target to exploit in the development of new antifungals and anti-virulence strategies to combat *C. albicans* infections. Particularly, quorum sensing (QS) has been reported as critical for virulence regulation in *C. albicans*. This work presents two knowledge networks with up-to-date information about QS regulation and experimentally tested anti-QS and anti-virulence agents for *C. albicans*. A semi-automatic bioinformatics workflow that combines literature mining and expert curation was used to retrieve otherwise scattered information from the scientific literature. The network representation offers an innovative and continuously updatable means for the *Candida* research community to query QS and virulence data systematically and in a user-friendly way. Notably, the reconstructed networks show the complexity of QS regulation and the impact that some molecules have on the inhibition of virulence mechanisms responsible for infection establishment (e.g. hyphal development) and perseverance (e.g. biofilm formation). In the future, the compiled knowledge may be used to build decision-making models that help infer new knowledge of practical significance. The knowledge networks are publicly available at http://pcquorum.org/. This Web platform enables the exploration of fungal virulence cues as well as reported inhibitors in a user-friendly fashion.

Keywords: *Candida albicans*; infection; virulence factors; quorum sensing; literature mining

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INTRODUCTION

The fungus *Candida albicans* is a common commensal microorganism, which asymptomatically colonises the gastrointestinal tract, the reproductive tract, the oral cavity and the skin of healthy individuals. However, it is also able to initiate invasive growth and develop health complications in compromised individuals. More specifically, this microorganism is a major cause of opportunistic infections, ranging from superficial infections (e.g. infections of skin and mucous membranes) to more life-threatening infections (e.g. airway and catheter-related bloodstream infections) that can result in serious disease and death (Mallick and Bennett 2013; Hirota *et al.* 2017). Notably, *C. albicans* is one of the few fungal species causing disease in humans (Nobile and Johnson 2015) and is the main pathogenic fungi causing nosocomial urinary tract infections (Behzadi, Behzadi and Ranjbar 2015). Remarkably, the *Candida* genus is present in approximately 80% of the nosocomial fungal infections, being the major cause of fungemia with associated high mortality rates (40%–60%) (Perlroth, Choi and Spellberg 2007; Doi *et al.* 2016).

The virulence mechanisms portrayed by *C. albicans* enable this microorganism to infect diverse host niches. *C. albicans* is a pleomorphic fungus, which means it exhibits polymorphic configuration (Fig. 1C) in response to a wide variety of conditions, namely growing as budding yeast or forming filaments, such as hyphae or pseudo-hyphae (Biswas, Van Dijck and Datta 2007; Sudbery 2011; Du and Huang 2016). The smaller yeast form is believed to be the form primarily involved in *C. albicans* dissemination within the host (Mayer, Wilson and Hube 2013), whereas the hyphal growth has an important role in the infection process, being responsible for tissue penetration and macrophage destruction (Sudbery 2011; Méar *et al.* 2013).

The infection process starts with the adherence to and invasion of host cells (Yang *et al.* 2014). After adhesion, a yeast-to-hypha transition and a directed growth occur towards weakened areas of the cell via another virulent trait called thigmotropism (Fig. 1A) (Mayer, Wilson and Hube 2013). Then, tissue invasion takes place with the help of invasins and hydrolases (Mayer, Wilson and Hube 2013; Yang *et al.* 2014).

Biofilm formation is another major virulence mechanism (Fig. 1B), as *C. albicans* is able to form biofilms on both abiotic (e.g. catheters, dentures) and biotic (e.g. host cells—skin, mucosa, etc.) surfaces, allowing fungal cell growth and dissemination in a variety of different conditions (Fanning and Mitchell 2012; Nobile and Johnson 2015). Similarly, phenotypic switching between white and opaque cells (Fig. 1D) contributes to a better adaptation of *C. albicans* to new host niches (Lan *et al.* 2002; Tsong *et al.* 2003; Tuch *et al.* 2010). While white cells are much more virulent than opaque cells, as shown in a case of intravenous infection (Kvaal, Srikantha and Soll 1997), opaque cells are better colonisers of the skin, as previously observed in a mouse model of disseminated candidiasis (Kvaal *et al.* 1999). Other *C. albicans* abilities include rapid adaptation to fluctuations in environmental pH, metabolic flexibility, powerful nutrient acquisition systems and robust stress responses (Fig. 1E) (Nicholls *et al.* 2011).

The regulation of these virulence traits is majorly coordinated through quorum sensing (QS), which is a cell-to-cell communication system that many microorganisms use to sense population density and regulate intra- and inter-species activities, as well as physiological processes, by releasing, sensing and responding to small QS molecules. When the concentration of QS molecules reaches a particular threshold, these signal molecules can bind and activate receptors with the ability to alter gene expression, and therefore promote behaviours considered beneficial under that particular condition (Avbelj, Zupan and Raspor 2016). In yeast, the major phenotypic process affected by QS is the transition between the filamentous and yeast forms (Wuster *et al.* 2008).

QS in eukaryotic organisms is a somewhat recent field of study with still unexplored potential regarding antimicrobial therapies. In 2001, the discovery of farnesol as a QS molecule in *C. albicans* triggered this exploration (Hornby *et al.* 2001). Besides farnesol, a number of other QS molecules have been identified for *C. albicans*, including farnesoic acid (Oh *et al.* 2001), tyrosol (Chen *et al.* 2004), tryptophol and phenylethanol (Lingappa *et al.* 1969; Chen and Fink 2006). As a whole, these molecules affect pivotal processes, such as morphogenesis, biofilm development, limitation of cell population density and control of nutrient competition, and control infection dissemination by regulating colony establishment at distal locations (Wongsuk, Pumeesat and Luplertlop 2016). QS molecules can also induce fungal apoptosis (Shirtliff *et al.* 2009) and can modify host immune cell behaviour (Abe *et al.* 2009).

Due to the essential involvement in virulence, QS mechanisms are now being targeted for the development of new antimicrobial strategies (Raina *et al.* 2009). The inhibition of virulence mechanisms and, in particular, the inhibition of the QS process are two main topics of study regarding the development of next-generation anti-candidal therapies.

In this work, a semi-automatic bioinformatics workflow combining text mining and expert curation is used to systematically retrieve and process experimentally validated information from the scientific literature and to reconstruct the first up-to-date and detailed knowledge network of QS regulation in *C. albicans* and, most importantly, a knowledge network of potential anti-QS and anti-virulence agents against this pathogen.

MATERIALS AND METHODS

Information retrieval

Text mining methods were used to automatically extract information on QS regulation and anti-virulence studies for *C. albicans* from the scientific literature. These textual findings were then integrated with data from public molecular databases in order to reconstruct comprehensive networks of experimental evidences. The curation pipeline used was adapted from previous reconstruction works of the authors (Jorge *et al.* 2016; Pérez-Pérez *et al.* 2017). The pipeline implemented in this work is depicted in Fig. 2.

The reconstruction of the QS regulation network for *C. albicans* focused on experimentally validated relations between genes and proteins associated with QS as well as QS molecules and virulence mechanisms. To this end, the scope of the PubMed queries was narrowed to those articles mentioning '*Candida albicans*', 'quorum sensing' and, at least, one term related to regulation (e.g. 'regulation', 'expression' or 'gene') and another related to virulence (e.g. 'farnesol', 'tryptophol' or 'virulence factor'). Similarly, for the reconstruction of the anti-virulence network, articles reporting antimicrobial, anti-virulence or anti-QS effects were screened. To this end, the corresponding PubMed query included the terms '*Candida albicans*', 'quorum sensing' and another term indicative of anti-candidal agents (e.g. 'antimicrobial' or 'antifungal').

For both network reconstructions, the retrieval of PubMed documents, the recognition of relevant named entities

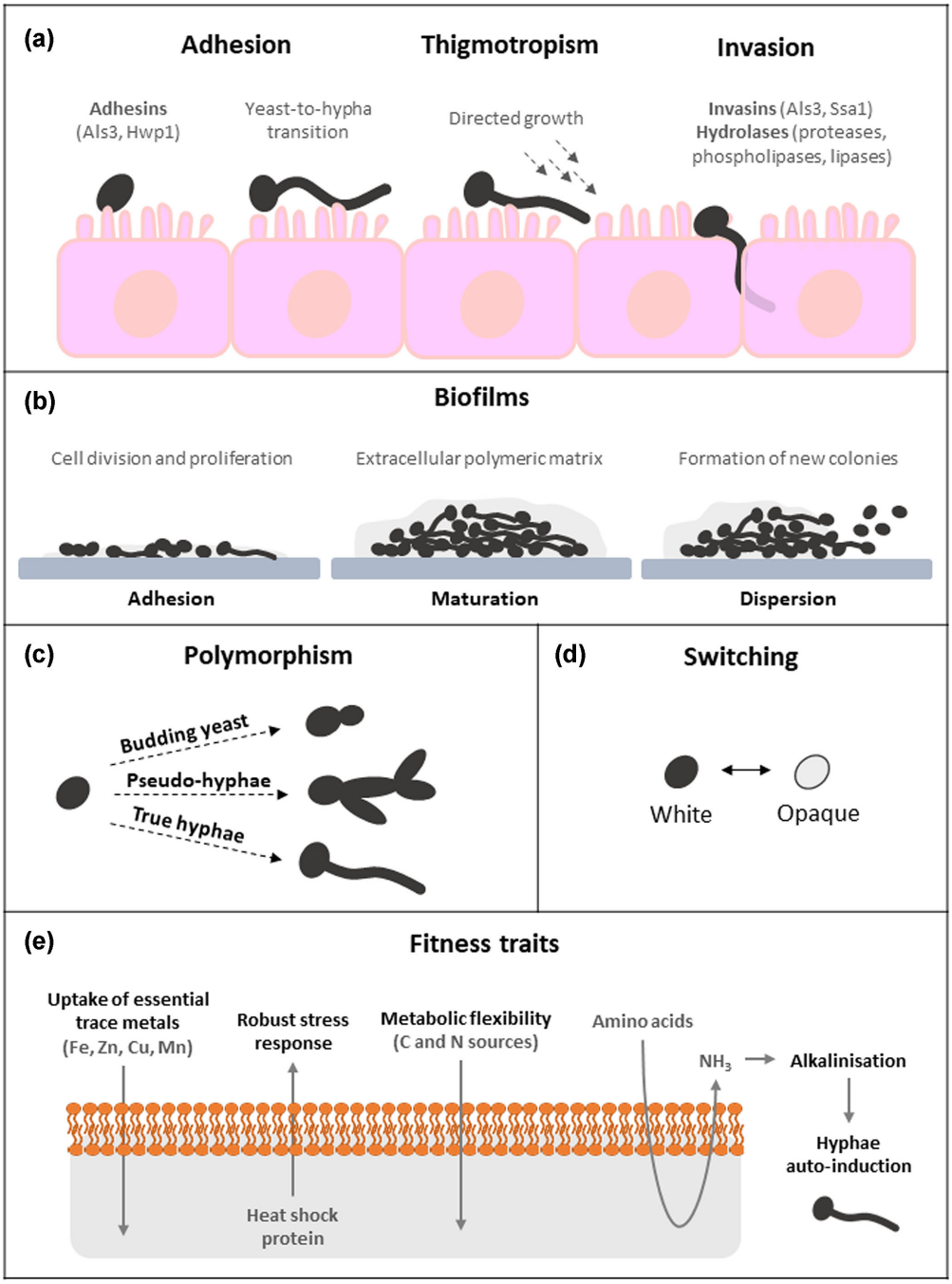

Figure 1. Schematic representation of *C. albicans* virulence mechanisms. (A) Main stages in adhesion and tissue penetration. Expression of adhesins and invasins on the cell surface mediates adherence and invasion to host cells. After initial adhesion, yeast-to-hypha transition and directed growth via thigmotropism occurs. Physical forces and fungal hydrolases facilitate invasion of the cell. (**B**) Development of biofilm. Attachment of yeasts to (a) biotic surfaces is followed by cell division and proliferation, thus establishing colonisation. Hyphal growth and extracellular polymeric matrix production leads to a mature biofilm architecture that subsequently disperses, leading to the formation of new colonies. (**C**) Polymorphism. Yeast daughter cells can form a germ-tube projection forming branches, which are divided by septa into separate fungal units that further extend outward into true hyphae. Pseudo-hyphae are formed by daughter cells budding from the original yeast cell, but which fail to detach. (**D**) Phenotypic plasticity (switching). Phenotypic switching between white and opaque can occur to better adapt *C. albicans* to new host niches. (**E**) Fitness traits. Fitness strategies influencing fungal pathogenicity.

(i.e. genes, proteins, drugs and virulence mechanisms) and the assessment of document relevance based on recognised contents were conducted automatically. The recognition of entities of interest was based on terminological resources of well-established and publicly available biological databases, namely DrugBank (Wishart *et al.* 2006), PubChem (Kim *et al.* 2016), ChEBI (Hastings *et al.* 2013), ChEMBL (Bento *et al.* 2014) and UniProt (Consortium 2015), and in-house dictionaries built for this effect. Moreover, expert curators performed the manual curation of the documents selected as potentially relevant, including the validation of the automatic document relevance assessment and the annotation of additional information (e.g. strains, mode

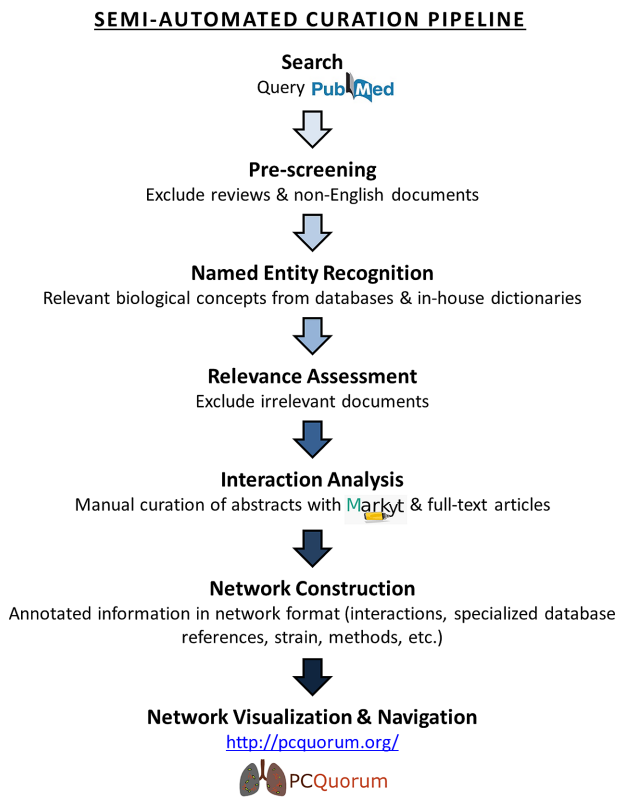

Figure 2. Step-by-step curation pipeline for the retrieval of relevant *C. albicans* QS regulation and anti-virulence/QS information from the scientific literature. The Markyt annotation tool (Pérez-Pérez *et al.* 2016) was used to assist in the manual curation step and the PCQuorum database (Pérez-Pérez *et al.* 2017) was used to provide public data access and allow network visualisation and navigation.

Table 1. Categories and examples of annotated entities.

Entity	Example
Gene	*CYR1*, *EFG1*, *orf19.3499*, *RAS1*
Protein	Adh3, Caspase, Cup9, Hog1
QS molecule	Dodecanol, Farnesol, Tyrosol
Virulence mechanism	Biofilm, Germ tubes, Hyphae
Drug	Pravastatin, Quercetin, 3-Hydroxyoctanoic acid
Environmental condition	Alkalinity, H_2O_2, Temperature
Other	Exopolysaccharide, Grapefruit EO, Bisbibenzyl

of growth and experimental methods), notably the category of the relation between the annotated entities (e.g. 'inhibition', 'upregulation' or 'stimulation'). Concomitantly, curators also revised the automatic annotations referenced from the databases and from the in-house dictionaries, which serve to update and increase upon the latter. Markyt annotation tool supported this manual curation of entities and relations (Pérez-Pérez *et al.* 2016).

Entity and relation annotation

Table 1 describes the entity categories considered relevant in the annotation of both networks. Given the variety of methodologies and techniques used in these studies to study gene expression, virulence regulation and antimicrobial effectiveness, a normalisation of relation categories was performed in order to guarantee the consistency of subsequent analyses. The list of relation categories is described in Table 2. The annotation of the relations was mainly based on the results section of each document, relying on the analysis of the figures, graphs and tables. Textual descriptions were used as means of confirmation of some inferences, as not all the results depicted were described or discussed in the main text (e.g. microarray data).

Network reconstruction

As a final step, all the compiled information was modelled as a knowledge graph or network, i.e. a representation that describes the entities of interest to the study of QS as nodes (Table 1) and the biological relations found among them as edges (Table 2). The reconstructed knowledge networks are publicly accessible at http://www.pcquorum.org. Web visualisation is supported by Cytoscape Web v.3.1.2 (Lopes *et al.* 2010) and advanced analyses are conducted in Cytoscape v.3.4.0 (Saito *et al.* 2012).

RESULTS AND DISCUSSION

Network visualisation and analysis

The PCQuorum website (http://www.pcquorum.org) provides public access to the curated information about QS/virulence regulation and anti-QS/virulence data for *C. albicans*. Users can specify search preferences by selecting nodes in one of the reconstructed networks (e.g. 'farnesoic acid' in the '*C. albicans* anti-QS' network) and, optionally, the relation category and relation type (e.g. expression level—downregulation) (Fig. 3A). The subnetwork meeting the search criteria is generated automatically (Fig. 3B). Its edges are coloured according to the category of the represented relations and different node shapes denote different entity categories (e.g. gene, protein, drug) (Fig. 3C). Users have access to additional data by clicking directly on the edges and nodes (Fig. 3D) and by consulting the data tables below the network visualiser, which describe network statistics, as well as produce comprehensive information about the effector (source) and target nodes, and the annotated relations (Fig. 3E).

QS regulatory network

In order to gain an in-depth understanding about QS and virulence regulation in *C. albicans*, the reconstruction of the respective regulatory network entailed the compilation and integration of experimental evidences scattered in the scientific literature. A total of 1187 regulatory relations described in 49 different documents (dating from 1997 till present) were considered. The noticeable discrepancy between the number of relations and documents is due to the fact that most of these studies focused on gene expression (something somewhat expected). These studies usually resorted to more (e.g. microarray) or less (e.g. PCR) high-throughput methodologies, which are known to output a significant amount of data. In fact, 74% of the annotated relations, corresponding to 60% of the total documents, included genes as the target entity and the most used methodologies in these studies were indeed PCR (including all categories—qRT-PCR, RT-PCR, etc.) (55%), northern blot (21%) and microarrays (12%).

Most of the annotated relations were 'gene–gene' (55%), followed by 'QS molecule–gene' (17%) and 'QS molecule–protein' (6.6%). The majority of the 'gene–gene' relations were derived from studies where a knockout mutant of the effector gene was

Table 2. Categories and descriptions of the normalised annotated relations.

Category	Relation	Description	Example
Expression level	Upregulation	Indicates any increase regarding the expression of a gene/protein.	'…appearance of stress response genes in a specific response to farnesol…' (Enjalbert and Whiteway 2005)
	Downregulation	Indicates any decrease regarding the expression of a gene/protein.	'…genes *CPH2*, *TEC1* […] *UME6* or *RDI1* […] were down-regulated in *eed1*Δ…' (Martin *et al.* 2011)
Protein level	Codification	Indicates the genes coding for a particular protein.[a]	N/A (information on protein coding extracted from public databases)
	Activation	Indicates when proteins are activated by another entity.	'…MDPs activate Cyr1p by directly binding to its LRR domain…' (Xu *et al.* 2008)
	Deactivation	Indicates when proteins are deactivated or degraded by another entity.	'…Cyr1p protein was directly inhibited by farnesol…' (Hall *et al.* 2011)
	Stabilisation	Indicates when proteins are stabilised or their degradation is impaired by another entity.	'…Hsp90 enables drug resistance by stabilising the catalytic subunit of the protein phosphatase calcineurin, Cna1…' (Shapiro *et al.* 2012)
	Interaction	Indicates a direct interaction between two proteins (with unreported relation effect).	'Sgt1 physically interacts with Cyr1…' (Shapiro *et al.* 2012)
Molecule level	Stimulation	Indicates any increase in the amount of QS or other molecules.	'Alkaline pH elevates aromatic alcohol yield.' (Ghosh *et al.* 2008)
	Inhibition	Indicates any decrease in the amount of QS or other molecules.	'Aromatic alcohol production was also five- to sevenfold lower when the cells were grown in medium with both l-proline and ammonium sulfate…' (Ghosh *et al.* 2008)
	Antagonism	Indicates any decrease in the action of a molecule caused by another.	'While […] cells grown with […] dodecanol […] were […] in the yeast morphology, the addition of db-cAMP […] restored true hyphae and pseudohyphae formation…' (Davis-Hanna *et al.* 2007)
Cell mechanism level	Stimulation	Indicates any increase in the appearance of virulence mechanisms.	'…hyphal extension […] was accelerated by tyrosol…' (Nagy *et al.* 2014)
	Inhibition	Indicates any decrease in the appearance of virulence mechanisms.	'…cells form […] filamentous colonies in the absence of farnesol and smooth colonies in the presence of farnesol…' (Langford *et al.* 2013)

[a]These relations were only included in the case where proteins were the entities annotated in the documents. This was not performed for the cases where only genes were annotated. N/A—not applicable.

used to assess its influence on the expression of the other genes. The regulatory information for the most common effector genes is presented in Table 3.

RAS1, *EFG1* and *CYR1* were the genes most evaluated as effectors (Table 3). The *RAS1* gene encodes the enzyme GTPase involved in the regulation of both the MAP kinase (MAPK) and the cAMP signalling pathways, which are crucial for controlling hyphal growth, and thus virulence (Feng *et al.* 1999; Leberer *et al.* 2001; Rocha *et al.* 2001). Accordingly, about 6% of the relations annotated for this gene relate to hyphae and metabolic pathway stimulation. In fact, *RAS1* was shown to cause the upregulation of genes related to hyphal development/adhesion (*HWP1*) and virulence/evasion of host immune response (*SAP4–6*), and the downregulation of genes/proteins involved in stress response (*CTA1*, *HSP12*, Hog1-P). In turn, *EFG1* encodes a transcription factor that influences filamentous growth (Bockmühl and Ernst 2001) and white-opaque phenotypic switching (Sonneborn, Tebarth and Ernst 1999), among other virulence mechanisms, upregulating genes that influence cell adhesion/filamentous growth (*HWP1*, *EED1*, *UME6*) and downregulating proteins that negatively influence these phenomena (Nrg1). Finally, *CYR1* encodes an adenylyl cyclase integrating the cAMP signalling pathway, upregulating filamentation (Ras1) and downregulating genes/proteins involved in stress response (Hsp12, Hog1-P) and filamentation repression (Nrg1, *NRG1*).

The case of farnesol in QS regulation

Farnesol is a sesquiterpene QS molecule, chemically related to another QS molecule—farnesoic acid—and it was the first QS molecule identified in an eukaryotic organism, namely in *C. albicans* (Hornby *et al.* 2001). Farnesol is well-known for its virulence regulation abilities as it inhibits the yeast-to-hypha transition (Hornby *et al.* 2001) and promotes reverse morphogenesis, i.e. the production of budding yeast from hyphae (Polke *et al.* 2017). Interestingly, farnesol has shown significant inhibitory effects on various fungi and bacteria (Allison *et al.* 2016), along with synergy with some types of antifungal products, which makes it a promising adjunctive therapeutic agent (Polke and Jacobsen 2017). On the other hand, farnesol is known to affect the mammalian host and may promote pathogenesis, possibly due to its detrimental effects on immune cells (Navarathna *et al.* 2007; Leonhardt *et al.* 2015).

However, the mechanisms underlying these relations and the complexity of the communication are still poorly understood. Furthermore, the molecular basis of farnesol signalling in *C. albicans* populations remains, despite intensive research in recent years, largely elusive (Polke and Jacobsen 2017). For this reason, we use farnesol to exemplify the query and exploration abilities available to look into the reconstructed QS regulation network (Fig. 4).

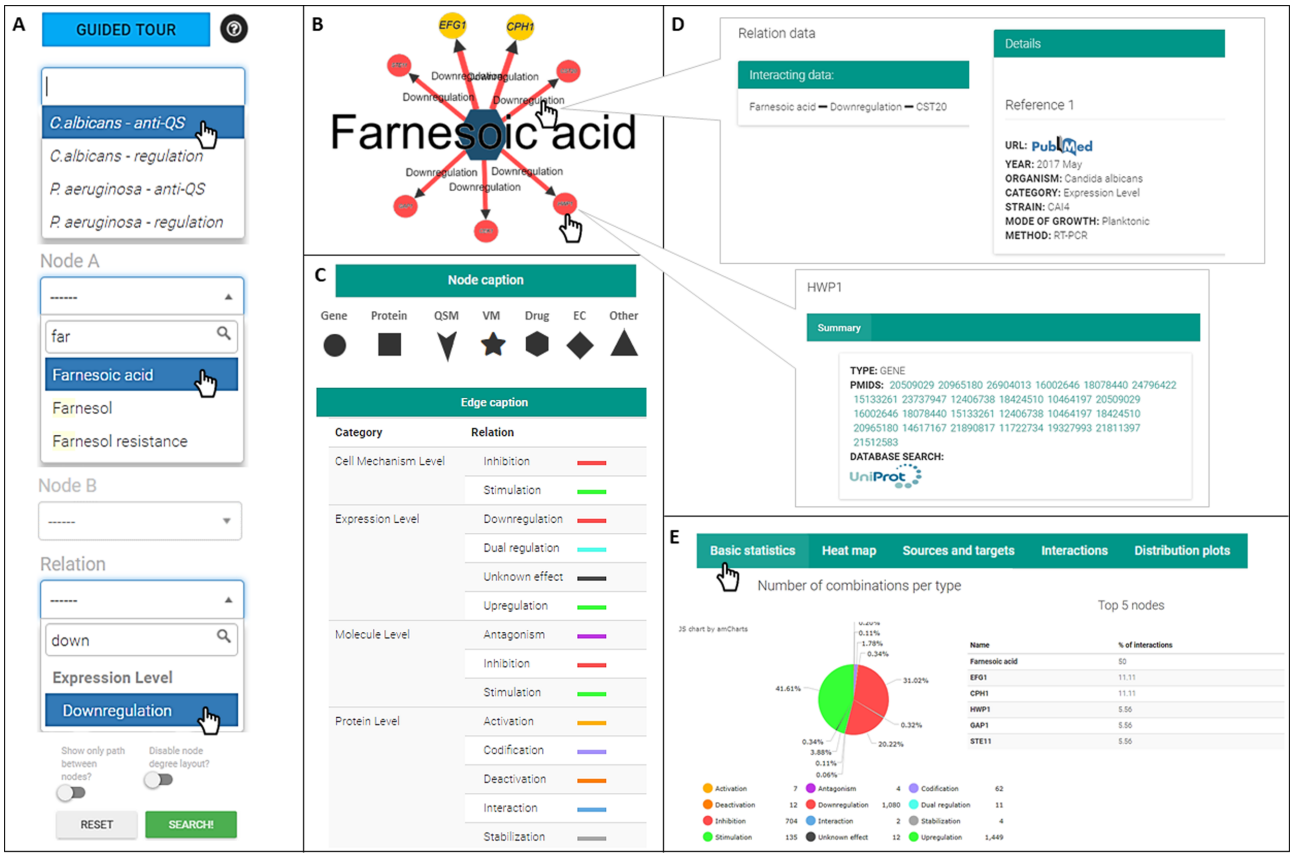

Figure 3. *Candida albicans* anti-QS/virulence and QS regulatory networks visualisation at PCQuorum. (**A**) Search options panel; (**B**) network rendering based on search criteria; (**C**) information mapping via edge colours and node shapes; (**D**) edge and node details; (**E**) additional information tables.

Table 3. Regulatory information for the three most tested effector genes.

Effector	Number of [a]		Top three targets[b]			
genes	Docs	Relations	Upregulation	Downregulation	Stimulation	Inhibition
RAS1	8 (16%)	17 (1.4%)	*HWP1* (20%) *SAP4–6* (6.7%) –	*CTA1*, *HSP12* (6.7%)	Hyphae (33%) cAMP-PKA Pathway, MAPK Pathway (6.7%)	Farnesol resistance (6.7%) –
EFG1	8 (16%)	13 (1.0%)	*ECE1*, *HWP1* (17%) Hsp12, *EED1*, *UME6* (8.3%)	Nrg1 (8.3%) –	Hyphae (17%) –	Hyphae (17%) –
CYR1	7 (14%)	9 (0.7%)	–	Hsp12, Nrg1, *NRG1* (17%)	Hyphae (17%) –	–

[a]% are relative to the total number of documents and relations, respectively;
[b]% respective to each mentioned target and relative to the total number of relations for each effector gene.

A total of 36 farnesol studies (73% of the total curated documents) were analysed and 255 relations (21% of all annotated relations) were annotated. Farnesol is mainly depicted as an effector node. Only three documents, encompassing one relation each, studied farnesol as a potential target (Fig. 4D). Specifically, these studies focused on farnesol production, namely in knockout mutants for the ZAP1 gene, which encodes a transcription factor that influences filamentous growth, and farnesol action, namely in the presence of other molecules that interfere with *C. albicans* morphogenesis, i.e. 3(R)-Hydroxy-tetradecaenoic acid and cAMP. Table 4 summarises the information gathered about farnesol as an effector node. Farnesol shows a complex regulation of a panoply of genes/proteins (Fig. 4C), namely the upregulation of those involved in filamentous growth inhibition (*TUP1*) and stress response (Hsp12, *MCA1*, *CDR1*, Cta1, Caspase). This correlates well with the stimulatory and inhibitory actions compiled from literature (Table 4) (Fig. 4B). On the other hand, farnesol downregulates genes/proteins necessary for hyphal development/adhesion (*HWP1*, *RBT1*, *HST7*, *GAP1*, *HSP90*).

Interestingly, as in the case of *RAS1*, where, conversely to farnesol, filamentation and stress response were respectively positively and negatively regulated (Table 3), filamentation and stress response appear to be usually counter-stimulated. This might indicate a mechanism through which *C. albicans*

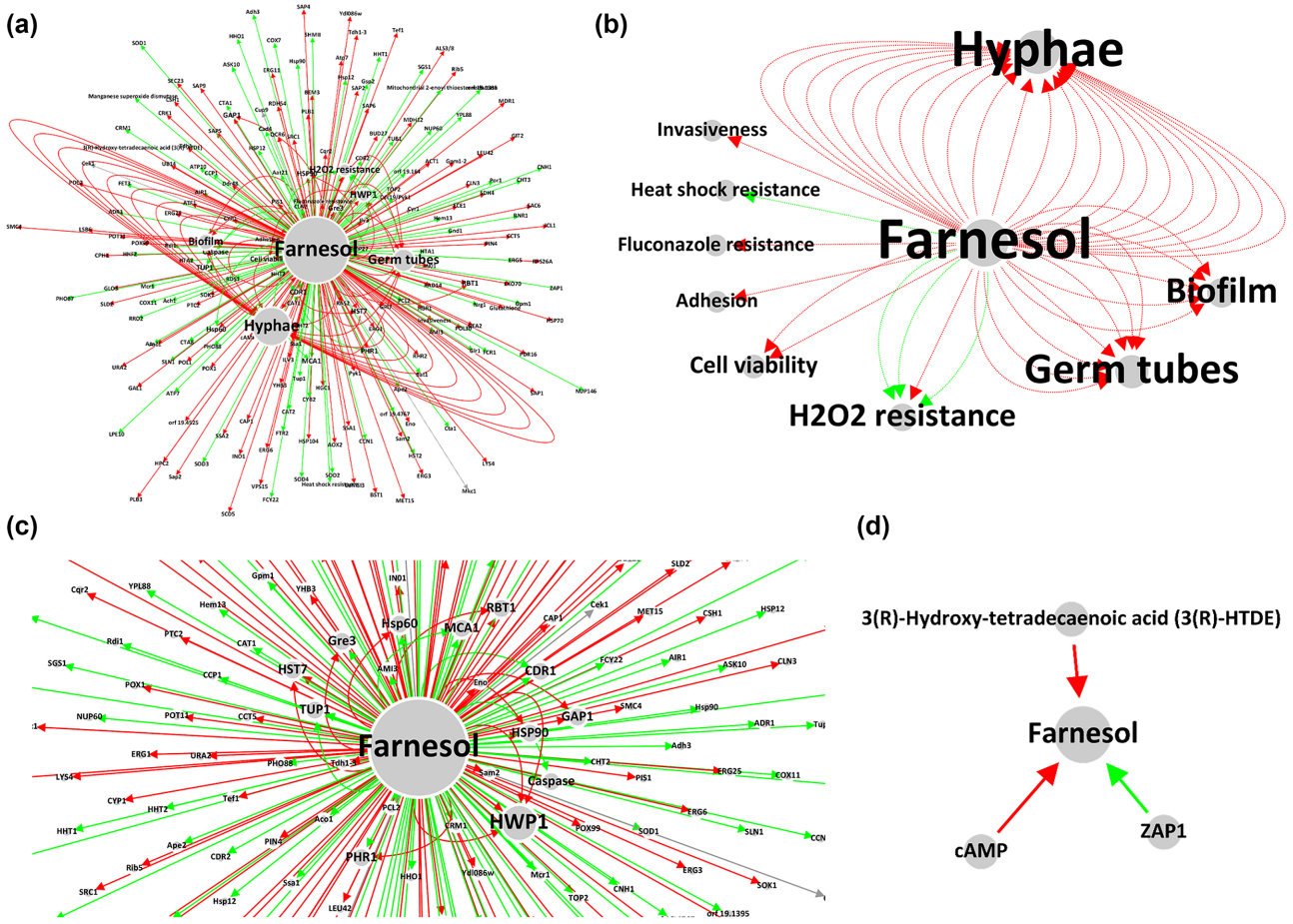

Figure 4. Network of farnesol QS regulation in *C. albicans*. (**A**) Global view of the curated QS regulatory network with farnesol as the central node; (**B**) farnesol regulation of virulence mechanisms; (**C**) farnesol regulation of genes and proteins; (**D**) farnesol as a target in QS regulation. Red and green coloured edges represent negative (e.g. inhibition and downregulation) and positive (e.g. stimulation and upregulation), respectively. Node size is positively correlated with the number of edge connections.

Table 4. Information about the QS targets regulated by farnesol.

Number of[a]		Top three targets[b]			
Docs	Relations	Upregulation	Downregulation	Stimulation	Inhibition
34 (69%)	252 (23%)	*TUP1* (3.3%) Hsp12, *MCA1*, *CDR1*, Cta1, Caspase (2.2%),	*HWP1* (3.6%) *RBT1*, *HST7*, *GAP1*, *SAP2*, *HSP90*, Ilv3 (1.8%)	H_2O_2 resistance (75%) Heat shock resistance (25%)	Hyphae (54%) Germ tubes (17%) Biofilm (12%)

[a]% are relative to the total number of documents and relations, respectively;
[b]respective to each mentioned target and relative to the total number of that particular relation category for farnesol.

prioritises and directs metabolic efforts towards the protection against environmental stresses (e.g. oxidants, temperature or antifungals) in detriment of growth as hyphae.

Anti-QS/Virulence Network

Presently, the reconstructed anti-QS/virulence network contains 62 unique agents (e.g. farnesol, thiazolidinedione-8 and quercetin) capable of influencing gene and protein expression as well as virulence mechanisms. These data are supported by 60 scientific documents, dated from July 2001 until May 2017. Effectors (or sources nodes) are mainly categorised into QS molecules and drugs, but also proteins and other molecules. All of these are separated into two sub-categories, natural and synthetic agents. The targets include QS molecules (e.g. farnesol), genes (e.g. *ALS1*, *TUP1* and *HWP1*), proteins (e.g. Sap2 and Hog1), virulence mechanisms (e.g. biofilm and hyphae) and other molecules (e.g. exopolysaccharides, glutathione). Figure 5 illustrates different relation categories annotated amongst the target categories. Noteworthy, the majority of the relations represent QS molecules targeting virulence mechanisms and genes, confirming that anti-QS and anti-virulence studies are mostly focused on the action of the naturally occurring compounds secreted by the targeted organism.

The in-depth curation of these studies led to the compilation of various categories of targets and relations (Fig. 6).

It is noticeable that the annotated agents were mainly tested for their influence on gene expression. Notably, one

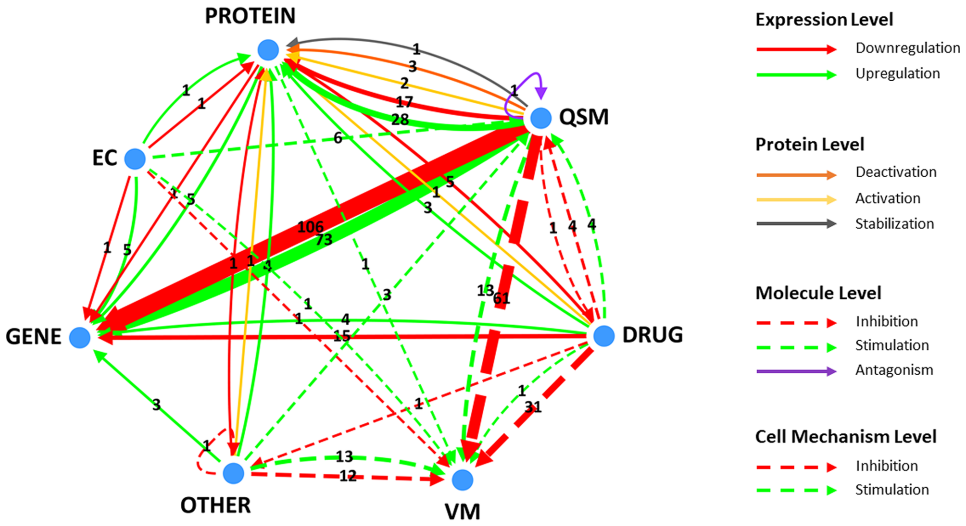

Figure 5. Overview of the relations reconstructed for the different node categories. Edge width is positively correlated with the number of relations. VM—virulence mechanism; QSM—quorum sensing molecule; EC—environmental condition.

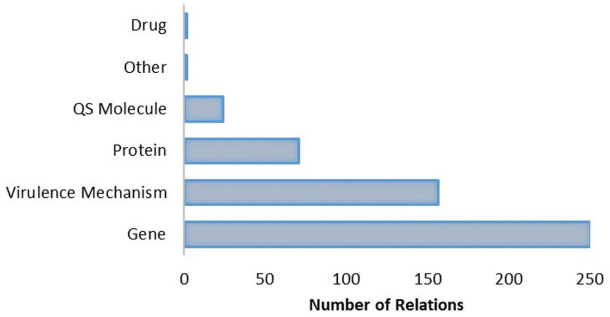

Figure 6. Total number of relations annotated for each target category.

of the most common experimental methods used was PCR—second most used technique (16%)—allowing a selective analysis of a small group of genes. Complementarily, the microarray results were also part of these studies (1.9%), illustrating changes in thousands of genes and aiding in the discovery of the pathways behind the mechanism that is affected by a given molecule or drug. Hence, the type of methodologies used account for the high number of annotated relations.

On the other hand, a substantial part of the documents (90%) focused in the resulting action of an agent directly towards a specific cellular physiology (e.g. virulence mechanisms). The most used agents and the most targeted virulence mechanisms are summarised in Table 5. Hyphae was the most studied virulence mechanism in the curated documents. The role of hyphae in the infection process and its contribution to the virulence of *C. albicans* makes the study of hyphal development and the screening of possible anti-hyphal agents critical (Mayer, Wilson and Hube 2013). In fact, about 73% of all curated effector agents were reported to affect hyphal development. Specifically, the main agent reported against this virulence mechanism was farnesol, with textual evidence of 19 relations with inhibitory effect. The second most studied virulence mechanism is biofilm formation, which was tested against 37% of all curated effector agents. The inherent robustness of biofilm development by *C. albicans* on several substrates and in different media (Nobile and Johnson 2015) makes this virulence mechanism another important focus of study. Lastly, the third most studied virulence mechanism was germ tubes, which has less relevance in terms of effector agents (9.7%) than the previous ones. The yeast and hyphal configurations represent the extremes of *C. albicans* morphology. The yeast cells can germinate and form germ tubes that can differentiate, grow and develop by mitosis to create somatic hyphae. Germ tubes can be considered the precursors of true hyphae (Brown, Argimón and Gow 2007), and hence tend to be less studied than the latter.

Looking into the effector agents, farnesol was the molecule most reported for being able to affect the production of hyphae, biofilm as well as germ tubes, emphasising its importance as a virulence regulator and possible adjuvant in anti-fungal therapies against *C. albicans*.

Most of the agents studied in the curated documents were classified as natural products (75%), while studies with synthetic products were less common, i.e. represent about 25% of the total agents tested (Fig. 7A). Included within the natural products category, there are QS molecules (60%), contributing farnesol with 44% of the evidences, drugs (14%) and, less commonly, proteins (1.2%) and other molecules (9.6%) (e.g. essential oils, plant extracts) (Fig. 7B). These percentages appear to indicate that the anti-QS and anti-virulence studies are mostly focused on the action of QS molecules, which are naturally involved in the communication and virulence regulation phenomena, as well as in the discovery of new natural therapies. Regarding synthetic products, all annotated agents were classified as drugs.

Regarding experimental methodologies, the curated documents describe a wide range of techniques. The three main experimental methods used were microscopy (36%)—mainly for visual inspection of hyphae/germ tube development—, PCR (16%)—for studying gene expression in different conditions,—and metabolic activity/cell viability measurements (10%)—, to access the effectiveness of the anti-fungal therapies tested. The most annotated relations were downregulation (33%), upregulation (29%) and inhibition (26%). Other relevant effects, annotated with less frequency, were stimulation (9.6%), activation (0.9%), deactivation (0.7%), and antagonism and stabilisation (0.2%). Null effects, i.e. no observed effect of an agent over a target, were

Table 5. Relation summary for the top three targeted virulence mechanisms.

Virulence mechanism	No. of docs[a]	No. of different effector agents[b]	Top three effector agents	No. of relations[c]	
				Stimulation	Inhibition
Hyphae	38 (63%)	45 (73%)	Farnesol	4 (5.5%)	19 (26%)
			Dodecanol	–	4 (5.5%)
			Farnesoic acid	1 (1.4%)	3 (4.1%)
Biofilm	21 (35%)	23 (37%)	Farnesol	–	7 (24%)
			Bisbibenzyls	–	3 (10%)
			Thiazolidine-8	–	3 (10%)
Germ tubes	11 (18%)	6 (9.7%)	Farnesol	–	8 (57%)
			Linoleic acid	2 (14%)	–
			2-Dodecanol, 3(R)-Hydroxy-tetradecaenoic acid (3(R)-HTDE), Propolis 5% ethanol extract (PEE), Tyrosol	2 (14%)	2 (14%)

[a]% are relative to the total number of different documents in the network;
[b]% are relative to the total number of different effector agents in the network;
[c]% are relative to the total number of relations for that specific virulence mechanism.

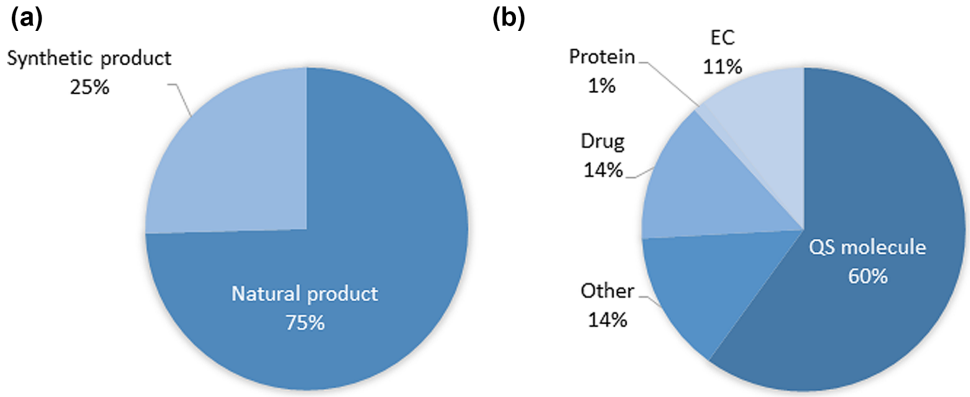

Figure 7. Distribution of agent classes. (**A**) Natural vs synthetic products; (**B**) categories of natural products. EC—environmental condition.

also annotated (supplementary material), but were not included in the network.

CONCLUSIONS

To know and to understand the molecular machineries underlying the expression of virulence mechanism by *C. albicans* is of upmost importance if researchers aim to prevent and/or control the development of infections. This work allowed for the creation of a valuable, new resource for researchers aiming to understand how *C. albicans* regulates the expression of virulence mechanisms and apply this knowledge to the design of novel anti-candidal therapies.

The PCQuorum database contains all currently available information on the scientific literature about *C. albicans* QS regulation and anti-QS/virulence topics. The network representation combined with the public web interface provides a novel, enhanced way to search, visualise and analyse information on this topic. A global analysis of the annotated data provided a portrait of the type of studies being carried out, including the most tested agents, genes, virulence mechanisms and experimental methods used.

As future work, authors anticipate the continuous update of the knowledgebase with additional information (including pharmacological and biochemical features). With a comprehensive knowledgebase, i.e. sufficient observations exist on different relations and effects, it will be possible to implement a data mining approach that may be of aid to infer new knowledge of practical significance.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSYR* online.

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