International Journal of Engineering & Technology, 7 (4.7) (2018) 1-4

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Application of Image Processing in Adaptation of Yeast to Environmental Conditions, Optimized by Autogegulatory Molecules and PSO

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Abstract

Unlike bacterial signal molecules, secreted yeast ones, which ensure their coordinated behavior as a single system, have been little studied. We used the image processing technique to evaluate the environmental conditions. Communication through quorum sensing molecules (QSM) is the dominant signaling in prokaryotic populations. In eukaryotic yeast cells, stress, caused primarily by the nutrient limit, causes a phenotypic manifestation of the mechanism of dimorphic switching, encompassing the repression of certain groups of genes and the activation of others, determining adhesion and virulence. Analysis of literature data and the results of the authors' own research emphasize the importance of signaling studies involving autoinducer molecules to elucidate the fundamental laws governing the regulation of yeast physiology, including growth parameters, morphogenesis and pathogenicity.

Keywords: *Image processing, quorum sensing, Medical engineering, yeast, particle swamp optimization*

1. Introduction

Adapter molecules that react to changes in environmental factors include secreted autoinducers found in bacteria and micromycetes. The regulating functions of the autoinducers are implemented through the signal systems necessary for the manifestation of various models of the behavior of microorganisms, including bioluminescence, the formation of biofilms, the aggregation of cells, the production of pathogenicity factors, antibiotics, exopolysaccharides and other secondary metabolites [1-3].

The factors of intercellular communication responsible for the density-dependent processes of eukaryotic microorganisms have been studied less than in prokaryotes [4-6]. In this review, the authors analyzed the literature data and their own experimental data obtained by studying the regulation of the physiological features of yeast growth in the presence of exogenous QSM.

2. Organization of Yeast Communities and the Regulatory Role of QSM

Survival and growth of microscopic fungi depend significantly on their ability to function as a single community. The determination of the regulatory role of signal molecules and contacts in yeast life processes revealed that structural changes in the community are due to the variability of expression and the functioning of adhesive proteins [7-8]. The induction of a "cohesive" state of yeast leads to dangerous consequences for human health. Thus, biofilms formed by pathogenic yeast on medical devices are the main cause of mortality from hospital-acquired mycoses [9, 10]. Significant harm is caused by communities of pathogenic fungi in the form of biofilms on food products [11].

The organization of yeast communities is determined by the transfer of intercellular signals that enhance the adhesion properties of cells, although such communities have a heterogeneous organization, since different genes are responsible for the morphological and physiological properties of a certain group of cells in different regions [9-11].

In a liquid medium, when shaken, the yeast grows as separate cells, and can also group into communities in the form of flakes, conglomerates, colonies, and biofilms. Taking into account the genotype, sources of nutrition and cultivation conditions, the yeast community is classified as: flakes - a cluster of cells in a suspended state in shaking liquid cultures; florces - thick layers of cells forming on the upper surface of liquid cultures; colonies - compact rounded structures on dense media; biofilms - a conglomerate of cells attached to each other in the extracellular matrix on a solid surface, mats - highly integrated multi-species communities on the surface of substrates [8].

Regulation of the organization of the yeast community is carried out by signal molecules that mediate the influence of environmental factors. Yeast has several types of signaling: pheromone, QS and ammonium. Pheromone is carried out in the process of pairing of two types (a- and α haploids) of *Saccharomyces cerevisiae* cells [12], while QS signals coordinate the behavior of a group of cells. QS signaling in yeast is involved in the regulation of virulence factor secretion, induction of cell competence and biofilm formation [13, 14]. The latter, like any form of community in yeast, is evolutionarily aimed at improving the survival of the microorganism: pseudohyphae and micellar structures are capable of invading the substrate in the process of actively searching for nutrients, and the formation of a matrix prevents their elution.

The existence of QS systems in microscopic fungi was revealed after the discovery in the dimorphic yeast of *Candida albicans* of the signal molecule of farnesol - terpenoid alcohol ($C_{15}H_{26}O$) regulating the transition: "plankton cells - filaments" and the formation of biofilms [15] (Fig.1). The presence of farnesol in the induction of the formation of the germinal tubes of yeast proline, N-acetylglucosamine or serum, prevented the transition to a mycelial growth [16]. The concentration of aromatic alcohol - tyrosol *C. albicans*, is proportional to the accumulation of yeast biomass both in plankton forms and in biofilm [4].

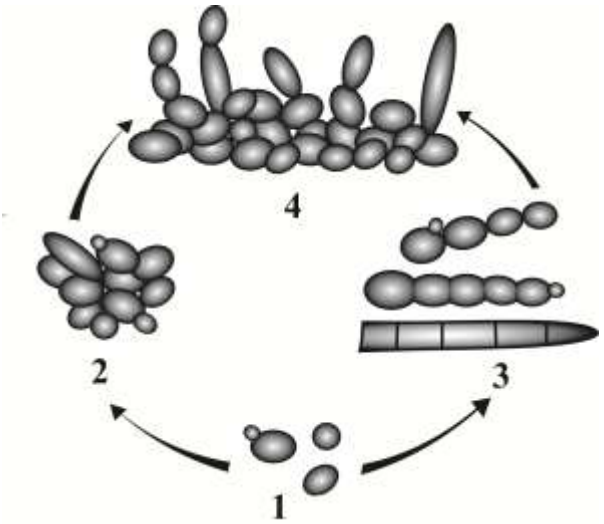

Fig. 1:. Autoregulator-induced yeast morphogenes. 1 - single plankton forms, 2 - conglomerate of colony-forming cells, 3 - filamentous forms (chain form, pseudohypha, hypha), 4 - biofilm

The role of QSM in yeast *S. cerevisiae* is performed by aromatic alcohols - phenylethanol and tryptophol, which control the morphogenesis of cells under nitrogen conditions in a medium that strictly regulates the biosynthesis of aromatic alcohols, which confirms the relationship between growth processes and QS inclusion conditions in *S. cerevisiae* [5]. Phenylethanol and tryptophol in yeast are subject to autoregulation by feedback type similar to QSM bacteria. Under minifermentation, when limiting the substrate and under high density of cells of wine yeast, not only 2-phenylethanol and tryptophol, but also tyrosol are secreted [17, 18]. The production of phenylated alcohols in non-traditional yeast strains of the genus *Pichia* in nitrogen deficiency is not associated with the formation of filaments, which indicates the presence of other molecules, presumably aromatic esters, which play the role of QSM in these yeasts [19].

2.1 Image processing for the detection in medical science

The main procedure of the image processing is illustrated in figure 2. PSO is utilized for detecting global optimization of image process to find the most optimal condition. For this purpose we utilize the PSO integrated with image processing to find the most suitable environmental conditions as follows:

$$J_m = \sum_{i=1}^{N} \sum_{j=1}^{C} u_{ji}^{m} \left\| x_i - c_j \right\|^2$$

where N is the data items number, c is cluster number, cj is the centroids of the jth cluster,xi is the ith data, uij is the membership DOF of xi in the jth sector.

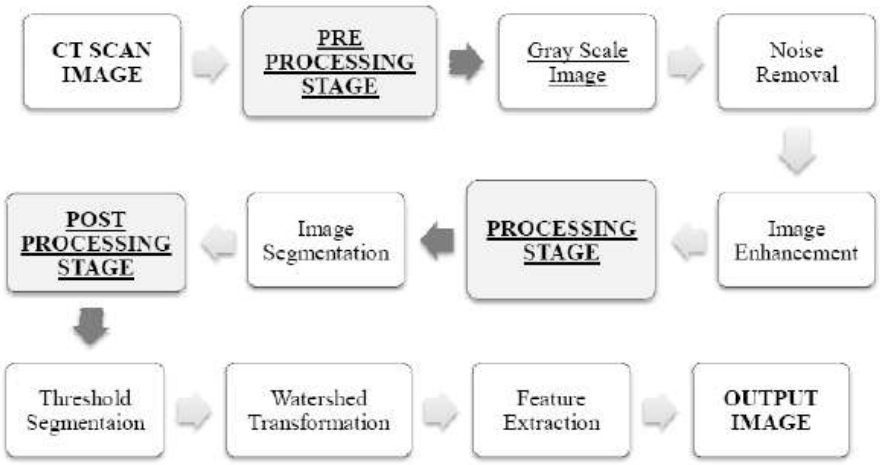

Fig. 2:. The process of image processing in the medical issues

A significant role in the life of *S. cerevisiae* is assigned to such factor of intercellular signaling as ammonium [20]. Ammonium diffuses on an agar plate from one colony to another and stimulates the effect of synchronization of the alkaline phase in neighboring colonies. The mutant *S. cerevisiae*, which does not produce ammonium, is defective in the ability of the colonial division into apoptotic and vegetative zones [21].

3. The Role of Adhesive Proteins in the Formation of Communities and the Morphogenesis of Yeast

The processes of intercellular contacts and cell adhesion in the formation of communities in *S. cerevisiae* are carried out with the help of a family of adhesive proteins - floculins. Floculins mediate intercellular attachment by binding to oligosaccharides on the surface of other cells [22]. When anchored in the cell wall with the C-terminus near glycosylphosphatidylinositol, the flocculins are recruited by the N-terminal A domain containing the lectin to bind to the oligosaccharides of neighboring cells. The homologues of Flo11, Flo1, Flo5, Flo9 and Flo10 consist of three domains (A, B, and C), and the N-terminal A domain provides adhesive properties [23].

Adhesins in *Candida spp.* and *Saccharomyces spp.*, in addition to the functions of attachment to mammalian tissues, hard surfaces and other yeast cells, play a key role in the cellular response to environmental changes by participating in switching strategies of yeast morphogenesis [24]. Some strains of *S. cerevisiae* described the allele of the Flo11 gene (Flo11F), which has high expression and gives them a number of additional properties - the ability to form compact fluffy colonies and floras in the production of sherry. The Flo11 mutant *Saccharomyces* strain is defective in diploid (pseudohyphalic) and haploid (invasive) growth, and this defect is

not suppressed by the addition of phenylethanol and tryptophol [25].

The formation of *S. cerevisiae* filaments involves the Ras2/cAMP-PKA signaling pathway, where cAMP induces the activity of homologous protein kinases of the Tpk group. Tpk2 mutations that inhibit filamentation lead to elimination of the stimulating effects of aromatic alcohols due to inhibition of the expression of the Tpk2 gene, the transcription factor Flo8, and the surface protein of the Flo11 adherent regulated by it [26]. The addition of phenylethanol or tryptophanol to the medium induces the expression of Flo11 and suppresses the defect of filament formation in the mutant strain of the aro8 and aro9 genes [26].

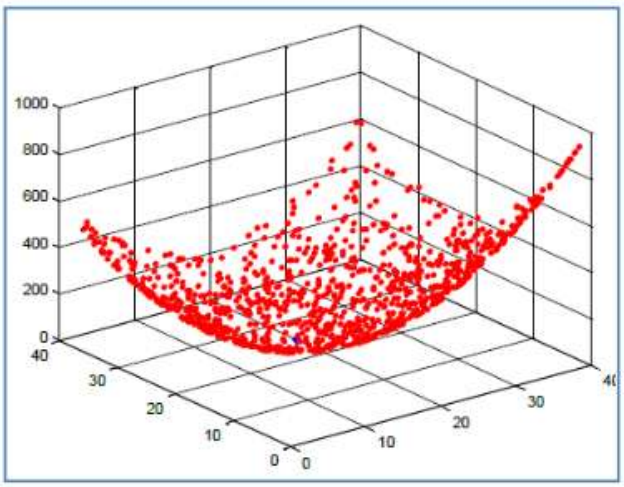

Fig. 3:. Optimization results of image processing based on the environment conditions

4. Exogenous Autoinducers in the Regulation of Yeast Growth

Natural antagonists of signal QSM - furanones, which are capable of enhancing the formation of biofilms in some bacteria, are of interest in subinhibitory concentrations. We investigated the effect of furanone-1 on the growth of yeast *C. tropicalis* and *S. cerevisiae*. It was found that the addition of furanone at the beginning of the lag phase stimulated the entry of culture into the exponential phase due to the reduction of the G-1 period of the cell cycle [27].

The study of the effect of bacterial QSM-acylated homoserine lactone (HSL) derivatives on the growth of yeast *S. cerevisiae* revealed the opposite effect. The addition of N-hexanoyl homoserine lactone and N-octanoyl-DL-homoserine lactone to the medium simultaneously with the inoculum in the microdoses increased the lag period, shortened the exponential phase, and accelerated the transition of the culture to the stationary growth phase. The inhibitory effect of exogenous HSL on yeast at the beginning of the lag phase is due to the low level of cells and, correspondingly, to the minimum content of endogenous HSL. In the exponential phase, HSL abruptly suppresses yeast budding. In the phase of growth retardation, exogenous HSL had no effect in connection with an increase in endogenous HSL to the threshold level [28]. Thus, the regulation of the population size of *S. cerevisia* by bacterial QSM depends on the concentration of the autoinducers, the physiological age of the culture and the competence of the cells to these compounds.

The overarching obstacle to understanding the mechanisms of physiological functions of autoinducer molecules in yeast is the lack of consensus in the regulation of intercellular communication processes. In the opinion of the authors [29, 30], the signal molecules of micromycetes, like bacterial molecules, should accumulate in proportion to the increase in the number of cells in a growing population, with consequences that limit the growth of culture at a particular stage of its development. It is important that after reaching the threshold level in yeast culture, QSM should induce a coordinated growth of the entire cell population, rather than induce the processes of assimilation or detoxification of the autoinducer molecule. According to [5], phenylethanol and tryptophol synthesized by yeast cells functionally similar to QSM of gram-negative bacteria [31] induce such phenotypic manifestations as the ability to regulate the morphogenesis of *S. cerevisiae* under conditions of nitrogen deficiency.

The study of the effect of exogenous auto-inductor molecules *S. cerevisiae* of phenylethanol (PEL) and its isoforms showed that among all studied isoforms of PEL, its R-isoform maximally inhibited the development of the culture when introduced into the medium together with the inoculum in microdoses (10 μM). The addition of PEL at the same concentration to the culture of the exponential phase completely suppressed the budding of yeast, and the introduction of PEL at the beginning of the deceleration phase and the stationary phase did not cause changes in growth, but induced the cell morphogenesis associated with the transition of unicellular forms of yeast into chain ones [32]. Elimination of the effect of exogenous PEL in the onset of the stationary phase is due to the accumulation of autoregulators in the culture synthesized by the cells themselves, which agrees with the data [5] on the correlation of the processes of increase in the number of *S. cerevisiae* cells in the population with an increase in the level of extracellular PEL by 20-50 times. Achieving a common pool of exogenous and endogenous signaling molecules to a critical level that blocks proliferation processes means that exogenous PEL, like the endogenous PEL, is perceived by the proliferating culture as a signal of a stressful situation in which the stopping of fission and the development of filamentous forms are preferred.

Thus, under the influence of various external factors on cells, the phenomenon of QSM-mediated phenotypic switching covers repression of some groups of genes and activation of others, determining adhesion, penetration and virulence. The genetic mechanism, called dimorphic switching, allows different forms of yeast to adapt to a changing environment [33]. It can prove to be an attractive target for the development of antimycotic drugs in the strategy of prevention and treatment of fungal infections

5. Conclusion

Summarizing the literature data and the results of our own research, we can conclude:

Coordination of the behavior of yeast in the population depends on the number of cells and is associated with the transformation of the external signal into intracellular, inducing a change in the expression of genes after the action of QSM autoinducers.

The role of QSM in the intercellular communication of yeast *C. albicans* is played by farnesol, in *Saccharomyces* - phenylethanol and tryptophil. Synthesis of aromatic alcohols, induced by the nitrogen limit in the medium, has a regulatory effect on the population size by feedback type.

Phenylethanol and tryptophol induce the expression of adhesin proteins - flocculins, the key role of which is to switch the strategies of yeast morphogenesis. Exogenous phenylethanol, furanone and homoserine lactones are perceived by yeast cells as a stress signal that induces an adaptive response leading to the stopping of cell division and the formation of filamentous yeast forms.

The directed regulation of the level of QSM in fungi, and, possibly, direct use of signaling molecules at the onset of the infection process will allow suppressing the development of pathogens such as *C. albicans, Magnaporthe grisea, Ustilago maydis*, capable of causing systemic mycoses.

Acknowledgements

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

References

[1] C.N. LaRock, J. Yu, A.R. Horswill et al. "Transcriptome analysis of acetyl-homoserine lactone-based quorum sensing regulation in *Yersinia pestis*", *PloS One*, 2013, vol. 8, № 4, pp. e62337.

[2] G.I. El-Registan, A.L. Mulyukin, Yu.A. Nikolaev et al. Adaptogenic functions of extracellular autoregulators of microorganisms. *Microbiology (Mikrobiologiya)*, 2006, vol. 75, № 4, pp. 380-389.

[3] G.F. Sprague, S.C. Winans "Eukaryotes learn how to count: quorum sensing by yeast", *Genes & development*, 2006, vol. 20, № 9, pp. 1045-1049.

[4] H. Chen, M. Fujita, Q. Feng et al. "Tyrosol is a quorum-sensing molecule in *C. albicans*", *PNAS*, 2004, vol. 101, № 14, pp. 5048-5052.

[5] H. Chen, G.R. Fink "Feedback control of morphogenesis in fungi by aromatic alcohols", *Genes & development*, 2006, vol. 20, № 9, pp. 1150-1161.

[6] M. Kruppa "Quorum sensing and *C. albicans*", *Mycoses*, 2009, vol. 52, № 1, pp. 1-10.

[7] P. Albuquerque, A. Casadevall "Quorum sensing in fungi–a review", *Medical mycology*, 2012, vol. 50, № 4, pp. 337-345.

[8] S.M. Honigberg "Cell signals, cell contacts, and the organization of yeast communities", *Eukaryotic cell*, 2011, vol. 10, № 4, pp. 466-473.

[9] J. Perlroth, B. Choi, B. Spellberg "Nosocomial fungal infections: epidemiology, diagnosis, and treatment", *Medical mycology*, 2007, vol. 45, № 4, pp. 321-346.

[10] M.A. Pfaller, D.J. Diekema "Epidemiology of invasive candidiasis: a persistent public health problem", *Clinical microbiology reviews*, 2007, vol. 20, №. 1, pp. 133-163.

[11] M. Stratford "Food and beverage spoilage yeasts. Yeasts in food and beverages", Berlin, 2006, pp. 335-379.

[12] J. Kurjan "The pheromone response pathway in *S. cerevisiae*", *Annual review of genetics*, 1993, vol. 27, № 1, pp. 147-179.

[13] W.L. Ng, B.L. Bassler "Bacterial quorum-sensing network architectures", *Annual review of genetics*, 2009, vol. 43, pp. 197-222.

[14] J.J. Walker, N.R. Pace "Endolithic microbial ecosystems", Annu. Revol. Microbiol, 2007, vol.61, pp. 331-347.

[15] D.A. Hogan "Talking to themselves: autoregulation and quorum sensing in fungi", *Eukaryotic cell*, 2006, vol. 5, № 4, pp. 613-619.

[16] J.M. Hornby, E.C. Jensen, A.D. Lisec et al. "Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol", *Applied and environmental microbiology*, 2001, vol. 67, № 7, pp. 2982-2992.

[17] J. Zupan, M. Avbelj, B. Butinar et al. "Monitoring of quorum-sensing molecules during minifermentation studies in wine yeast", *J of agricultural and food chemistry*, 2013, vol. 61, № 10, pp. 2496-2505.

[18] M. Avbelj, J. Zupan, L. Kranjc, P. Raspor "Quorum-sensing kinetics in *S. cerevisiae*: a symphony of ARO genes and aromatic alcohols", *J of agricultural and food chemistry*, 2015, vol. 63, № 38, pp. 8544-8550.

[19] I.M.H. Rijswijck, J. Dijksterhuis, J.C.M. Wolkers-Rooijackers et al. "Nutrient limitation leads to penetrative growth into agar and affects aroma formation in Pichia fabianii, P. kudriavzevii and *S. cerevisiae*", *Yeast*, 2015, vol. 32, № 1, pp. 89-101.

[20] Z Palkova, L. Vachova "Ammonia signaling in yeast colony formation", *International review of cytology*, 2003, vol. 225, pp. 229-272.

[21] L. Vachova, Z. Palková "Physiological regulation of yeast cell death in multicellular colonies is triggered by ammonia", *The Journal of cell biology*, 2005, vol. 169, № 5, pp. 711-717.

[22] K.J. Verstrepen, G.R. Fink "Genetic and epigenetic mechanisms underlying cell-surface variability in protozoa and fungi", *Annual review of genetics*, 2009, vol. 43, pp. 1-24.

[23] R.K. Bojsen, K.S. Andersen, B. Regenberg "*S. cerevisiae* – a model to uncover molecular mechanisms for yeast biofilm biology", *FEMS Immunology & Medical Microbiology*, 2012, vol. 65, № 2, pp. 169-182.

[24] R.R. Barrales, J. Jimenez, J.I. Ibeas "Identification of novel activation mechanisms for FLO11 regulation in *S. cerevisiae*", *Genetics*, 2008, vol. 178, № 1, pp. 145-156.

[25] M. Fidalgo, R.R. Barrales, J.I. Ibeas, J. Jimenez "Adaptive evolution by mutations in the FLO11 gene", *Proceedings of the National Academy of Sciences*, 2006, vol. 103, № 30, pp. 11228-11233.

[26] X Pan, J. Heitman "Protein kinase A operates a molecular switch that governs yeast pseudohyphal differentiation", *Molecular and cellular biology*, 2002, vol. 22, № 12, pp. 3981-3993.

[27] A.B. Margulis, E.T. Gilyazova, F.G. Kuprianova-Ashina. Growth peculiarities of the *C. tropicalis* and *S. cerevisiae* culture in the presence of furanone. *Advances in Medical Mycology*, 2013, vol. 11, № 9, pp. 326-329. (In Russian).

[28] E.T. Kalimullina, A.B. Margulis, A.I. Kolpakov, F.G. Kuprianova-Ashina Growth peculiarities of the *S. cerevisiae* culture in the presence of exogenous autoregulators of the cell population density. *Uchenye Zapiski Kazanskogo Universiteta. Seriya Estestvennye Nauki*, 2015, vol. 157, № 4, pp. 27-38. (In Russian).

[29] K. Winzer, K.R. Hardie, P. Williams "Bacterial cell-to-cell communication: sorry, can't talk now - gone to lunch!", *Current opinion in microbiology*, 2002, vol. 5, № 2, pp. 216-222.

[30] S. Raina D. De Vizio, M. Odell "Microbial quorum sensing: a tool or a target for antimicrobial therapy", *Biotechnology and applied biochemistry*, 2009, vol. 54, № 2, pp. 65-84.

[31] K.L. Visick, C. Fuqua "Decoding microbial chatter: cell-cell communication in bacteria", *J of bacteriology*, 2005, vol. 187, № 16, pp. 5507-5519.

[32] E.T. Kalimullina, A.A. Bayazitova, I.S. Nizamov, A.I. Kolpakov, F.G. Kupriyanova-Ashina, O.N. Ilyinskaya "Impact of exogenous autoregulators of intercellular communication in yeast on the growth of *S. cerevisiae*", *International J of Pharmacy & Technology*, 2016, vol. 8, № 4, pp. 24496-24507.

[33] K.J. Boyce, A. Andrianopoulos "Fungal dimorphism: the switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host", *FEMS microbiology reviews*, 2015, vol. 39, № 6, pp. 797-811.