The second approach is based on the separate detection of electron-dense markers at the top and the bottom side of ultrathin sections [3]. Labels at the top side of ultrathin section are detected in backscattered electron imaging using primary electrons with the energy around 1keV. Primary electrons with the energy between 15 and 30 keV are used for image recording of the same area in the STEM mode, when transmitted electrons are detected. In this case we can visualize labels on both sides of the ultrathin section together with its cell ultrastructure. Using of both sides of sections for immunolabeling means doubling of the number of detected epitopes compared to commonly one-sided immunolabeling methods.

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EVALUATION OF DIMORPHIC YARROWIA LIPOLYTICA STRAINS GROWTH IN DIFFERENT CULTURE MEDIA BY QUANTITATIVE IMAGE ANALYSIS

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Yarrowia lipolytica is a dimorphic microorganism capable of growing as a mixture of yeasts and short mycelial cells. However, under controlled conditions, it may grow as a quasi-homogeneous population of either yeast-like cells or hyphae [1]. The role of different factors in dimorphic transition of *Y. lipolytica* has been previously described, namely carbon and nitrogen sources [1], blood serum [2], citrate and medium pH [3]. This yeast has the ability to use hydrophobic substrates (n-alkanes, oils, fats) in production of lipases and esterases, single cell oil and in the treatment of wastewater, as well as non-hydrophobic subtracts (sugars, alcohols and organic acids) [4].

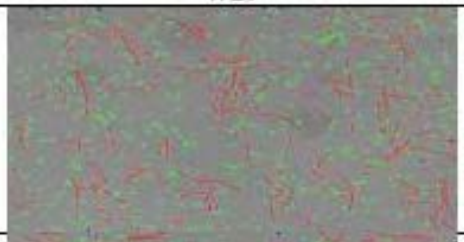
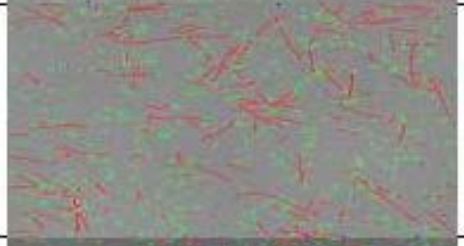
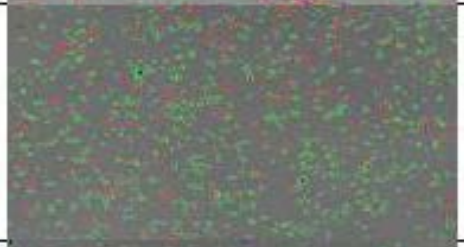
Cell morphology has a strong impact in several activities of industrial interest revealed by this yeast. This wide morphology greatly affects fermentation performance, since it in-

duces rheological changes and consequently leads to mass and heat transfer alterations in the bioreactor. Hence, understanding cell morphology became an important key to enhance and optimize the productivity of the process.

Quantitative image analysis (QIA) procedures can provide valuable information about the biological processes, and allow for a monitoring tool to decide if a given action should be carried out in the system. However, to the authors' knowledge, QIA has never been applied for characterizing dimorphic growth of *Y. lipolytica* in different carbon sources.

During the monitoring period, samples were collected for morphological characterization using QIA in bright-field microscopy. The yeasts-to-hyphae dimorphic transition in *Y. lipolytica* W29 and MTLY40-2P strains, were determined over time. The growth of *Y. lipolytica* strains showed a differential dimorphic behaviour in response to the presence of hydrophobic (olive oil and castor oil) or hydrophilic (N-Acetyl glucosamine (Glc) and glucose) carbon sources. Images at the end of each growth test are presented in Table 1. It was verified by QIA that, for both strains, the presence of olive oil (Ol), Glc, and glucose in the culture media induced the transition from yeast to hypha form. Furthermore, when castor oil (Co) was used as carbon source no morphological changes were observed.

Table 1. Images obtained at the end of each test with the four carbon sources and for each strain studied, after QIA (hyphae in red, yeasts in green)

Carbon source	Strain	
	W29	MTLY40-2P
Glc		
Glucose		
Ol		
Co		

In conclusion, it could be found that QIA can be used as a simple and accurate methodology to assess and characterize yeasts-to-hyphae dimorphic transition in near real time.

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