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Simulated gastric digestion survivability and bioprocess compatibility of a novel *Pichia kudriavzevii* FOL-27

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Abstract: The objective of this study was to investigate *Pichia kudriavzevii* FOL-27's: i) survivability in simulated gastric juice (SGJ) and simulated bile juice (SBJ), ii) growth kinetics under batch bioreactor trials (BT) and fed-batch bioreactor trials (FBT). Viability of FOL-27 as determined by calculating relative cell density ratio (RCDR) under SGJ and SBJ conditions was conducted at pH=3, pH=2, pH=1.5, and control and ox-bile levels of 0.2%, 1%, 2%, and control (0%), respectively. Microbial growth kinetics was obtained by measuring absorbances at OD_{600} in BT or in FBT where pH, dissolved oxygen (DO) and temperature were controlled at 5.5, 25%, and 30°C, respectively. In addition, effect of DO at 12.5% or 25% were evaluated to determine the growth and performance of FOL-27 in FBT by utilizing exponential feed. The doubling-time, maximum specific growth rate, and final cell densities achieved for BT were 101.8 min, 8.202 h^{-1} and 28.7, respectively. FBT at 25% O_2 or 12.5% O_2 level yielded a doubling-time, maximum specific growth rate, and final cell density of 90.18 min, 3.95 h^{-1}, 22.51 and 88.8 min, 2.83 h^{-1}, 26.6, respectively. RCDRs achieved were similar for pH=3 and control vs both were significantly higher ($p<0.05$) than pH=1.5 and pH=2 with the latter two pH-levels were significantly different ($p<0.05$) from each other. RCDRs were not significantly different across control, 0.2%, 1%, and 2% ox-bile levels ($p>0.05$). *P. kudriavzevii* FOL-27 exerts probiotic characteristics via tolerating SGJ and SBJ conditions similar to that of human gastrointestinal conditions. An observable elevation in biomass when grown under FBT conditions reveals that *P. kudriavzevii* FOL-27 is compatible to bioprocess development.

Keywords: *P. kudriavzevii* FOL-27, probiotics, fed-batch, bioprocess, dissolved-oxygen.

Yeni bir *Pichia kudriavzevii* FOL-27'nin simüle edilmiş mide sindiriminde hayatta kalma yeteneği ve biyoproses uyumluluğu

Özet: Bu çalışmanın amacı, *Pichia kudriavzevii* FOL-27'nin: i) simüle edilmiş mide suyu (SGJ) ve simüle edilmiş safra suyunda (SBJ) hayatta kalma kabiliyetini, ii) kesikli biyoreaktör denemeleri (BT) ve beslemeli kesikli biyoreaktör (FBT) denemeleri altında büyüme kinetiğini araştırmaktır. SGJ ve SBJ koşulları altında bağıl hücre yoğunluğu oranının (RCDR) hesaplanmasıyla belirlenen FOL-27'nin canlılığı, pH=3, pH=2, pH=1,5'te ve %0,2, %1, %2 ve kontrol (%0) sığır-safrası seviyelerinde gerçekleştirildi. Mikrobiyal büyüme kinetiği, pH'nın çözünmüş oksijenin (DO) ve sıcaklığın sırasıyla 5,5, %25 ve 30°C'de kontrol edildiği BT veya FBT'de OD_{600}'de absorbansların ölçülmesiyle elde edildi. Ek olarak, logaritmik besleme kullanılarak FOL-27'nin FBT'deki büyümesini ve performansını belirlemek için DO'nun %12,5 veya %25'teki etkisi değerlendirildi. BT için elde edilen iki katına çıkma süresi, maksimum spesifik büyüme oranı ve nihai hücre yoğunlukları sırasıyla 101,8 dakika, 8,202 h^{-1} ve 28,7 olarak tespit edildi. %25 O_2 veya %12,5 O_2 seviyesindeki FBT, sırasıyla 90,18 dakika, 3,95 h^{-1}, 22,51 ve 88,8 dakika, 2,83 h^{-1}, 26,6'lık bir ikiye katlama süresi, maksimum spesifik büyüme oranı ve nihai hücre yoğunluğu sağladı. Elde edilen RCDR'ler pH=3 için benzer ve kontrole karşı her ikisi de pH=1.5 ve pH=2'den önemli ölçüde daha yüksek olarak bulundu ($p<0.05$). Son iki pH seviyesi ise birbirinden önemli ölçüde farklı RCDR ile sonuçlandı ($p<0.05$). RCDR değerleri kontrole karşı %0,2, %1 ve %2 sığır-safra seviyeleri arasında önemli ölçüde farklı olarak bulunmadı ($p>0,05$). *P. kudriavzevii* FOL-27, insan gastrointestinal durumlarına benzer SGJ ve SBJ koşullarını tolere ederek probiyotik özellikler sergileyebilmektedir. FBT koşulları altında büyütüldüğünde biyokütlede gözlemlenebilir bir artış, P. kudriavzevii FOL-27'nin biyoproses geliştirme konusunda uyumlu olduğunu ortaya koymaktadır.

Anahtar Kelimeler: *P. kudriavzevii* FOL-27, probiyotikler, kesikli besleme, biyoproses, çözünmüş oksijen.

Research article

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1. Introduction

Fermentation technique is one of the oldest technics used to preserve food and enhances the aroma and flavour of the food systems. In addition, products such as enzymes, vitamins or antimicrobials can be produced by fermentation. Therefore, fermentation technology is still being considered as one of the critical applications in fermented food industry. The most common microorganisms used for production of fermented beverages and foods are bacteria and yeast (Alakeji and Oloke, 2020). Yeasts are being classified under eucaryotic organisms and are unicellular. The history of the yeasts goes back to ancient times, and it is still one of the most important organisms used by human beings (Liti, 2015). For example, yeasts have been used to produce bread, wine, and beer for thousands of years. When selecting a yeast organism for manufacturing an alcoholic beverage, the major criteria would be ethanol production rate during fermentation and its contribution to the sensorial profile of the resulting product (Walker, G.M, 2016). Yeasts can exist in variety of different ecological niches such as plant-based or even animal-derived systems which they could impact the organoleptic properties of foods (Chelliah et al., 2016).

Yeasts could rapidly form colonies when grown in nutrient-rich environments and co-exist with other microorganisms such as probiotic bacteria (Yetiman et.al 2022). Yeasts are extremely influential in the realms of biotechnology, food, everyday chemicals, and pharmaceuticals, and they have played a crucial role in human development throughout history. They were domesticated to power industrial fermentations and have been genetically altered for the manufacturing of pharmaceuticals and industrial chemicals. Normally, they predominate in spontaneously fermented foods, adding to desired tastes. Of these, *Saccharomyces cerevisiae* is thought to be a prominent workforce for bioprocesses because of its well-characterized physiology, ease of engineering, and reputation as "generally regarded as safe." (Nielsen, 2019). However, *S. cerevisiae* biotechnological processing is becoming more difficult, in part because of its innate traits, which include low stress tolerance and limited carbon sources (Thorwall et al., 2020). Furthermore, it is challenging to satisfy the wide range of consumer demands when *S. cerevisiae* is the only strain used in controlled fermentation operations, which limits the sensory qualities of products (Steensels & Verstrepen, 2014). Because of these disadvantages, non-conventional yeasts have become popular biotechnological hosts because of their beneficial phenotypes, which include inherent stress tolerance, the capability to metabolize a variety of carbohydrates, and the release of distinct tastes. Most of the probiotic research in the literature has been conducted bacterial organisms. For example, lactic acid bacteria from the species Lactobacillus and Bifidobacterium are commonly studied and produced in probiotic dietary supplements (Saavedra, 2001). *Bifidobacterium bifidum* and *Streptococcus thermophilus* effectively reduce acute diarrheal and rotavirus transmission (Vlasova et al., 2016; Martinez. et al., 2015; Sharma et al., 2014). The positive effects seen in the intestine may be due in part to the induction of protective antimicrobials (Power et al., 2014) which could possess antagonistic properties (Cintas et al., 2001). Probiotic yeasts have gained popularity in recent years, not just for animal nutrition preparations as well as for clinical applications. Currently, the yeast *Saccharomyces boulardii* is one of the most common yeast-based probiotic dietary supplements especially prescribed to those showing diarrhea-type diarrheal intestinal symptoms. It has been reported that *S. boulardii* was discovered by experimental methods, so this yeast species offers antitoxin properties for a variety of gastrointestinal diseases (Vandenplas et al., 2009; Buts, 2009). As a result, it is regarded as a non-bacterial probiotic product. Many mechanisms have been reported to explain the vast range of health-supporting effects of food-grade yeast ingestion (Czerucka et al., 2007). Antibiotic-associated and infectious diarrhea including recurrent *Clostridium difficile*; irritable bowel syndrome; and inflammatory bowel disease are some of the diseases that probiotic yeasts are shown effective in clinical trials (Foligné et al., 2010).

This species was formerly known as *Issatchenkia orientalis* and was later renamed *Pichia kudriavzevii* by Kurtzman et al. (2008). This species is distinguished by the production of spherical ascospores.

Initially, it was suggested that *Candida krusei*, which is frequently used to refer to clinical isolates, was *P. kudriavzevii*'s asexual form (anamorph), as they shared the same sequences in the D1/D2 sections of the 26S ribosomal DNA (Kurtzman et al., 2008). The non-conventional yeast *Pichia kudriavzevii* is found in traditionally fermented foods in the world and is widely distributed in natural habitats. Chelliah (2016) reported that several experimental evaluations on *Pichia kudriavzevii* resulted in technological advantages for sustainable bioenergy production of this yeast species. It has also been isolated as an indigenous microbiota in olive fermentation and pickled wax gourd (Golomb et al., 2013, Wu et al., 2016). When nutritional constraints are induced, *P. kudriavzevii* exhibits dimorphic transitions marked by the establishment of pseudohyphae and the creation of biofilms (Van Rijswijck et al., 2014), (Gómez-Gaviria & Mora-Montes, 2020). According to Chu et al. (2023), extremely low pH stress also causes pseudohyphae to grow and multicellular clusters to gradually evolve; these processes are incompatible with mass transfer bioprocess development. This phenotype differs from *C. albicans* (Villa et al., 2020). Because the creation of biofilm is thought to be linked to invasive growth and food spoiling, it has gained interest in both medical studies and the industry of food. *P. kudriavzevii* is found in many naturally fermented foods across the world, which may be partially attributed to its strong resilience to environmental challenges. This makes the species a promising strong framework for chemical biosynthesis. According to Chelliah (2016), no reports has been found on probiotic evaluation of *P. kudriavzevii.* Moreover, apart from Gumustop and Ortakci (2022) no reports have been found on evaluating the bioreactor processability and biomass optimization of probiotic candidate *P. kudriavzevii* strains. We isolated a novel *P. kudrivzevii* FOL-27 strain from fermented plant material called “Shalgam” and performed in vitro gastrointestinal survival experiments to determine fundamental probiotic characteristics along with bioreactor trials to optimize biomass development of this novel strain. To our knowledge, no reports have been published on this novel isolate with regards to its simulated gastric survival and bioprocess development capacity.

2. Materials and Methods

2.1. DNA isolation and PCR Fingerprinting

P. kudriavzevii FOL-27 culture was medium in YPS or yeast extract peptone sucrose, is a complete medium for yeast growth and incubated aerobically at 30 ℃ for 24h with 225 rpm. DNA extraction was performed according to DNA extraction kit manufacturer’s protocol. The DNA of *P. kudriavzevii* FOL-27 was kept at 4°C upon isolation. The conserved region of 5.8S ITS rRNA was amplified using ITS1 and ITS4 primers. D1/D2 domains of the 26S rRNA region was amplified using NL1 and

NL4 primers. The nucleotide sequence of primers used in the PCR runs were shown in Table 1. The individual components of PCR mix were purchased from Transgen Biotech. PCR reagents and final concentrations are shown in Table 2. PCR cycling started with thermal cycling and the first denaturation of samples at 95°C for 5 min. The denaturation phase started at 94°C for 30 seconds followed by annealing which occurred at following temperatures of 55°C (ITS1/4), 52.5°C (NL1/4). For extension phase, specimens were extended at 78°C for 2 minutes. All three phases were repeated 36 times. Samples were incubated at 72°C for 10 min for the last extension step. Then, samples were stored at 4°C. After PCR amplification, specimens were run on 1% agarose gel with 140 voltage. NL primer amplified DNA product was further processed for sanger sequencing application. Sanger sequencing reads were evaluated by using the NCBI's BLAST tool.

Table 1. Primer names and their sequences.
Tablo 1. Primer adları ve bunların sekansları.

Primer Name	Sequence
ITS1	TCCGTAGGTGAACCTGCGG
ITS4	TCCTCCGCTTATTGATATGC
NL1	CGCATATCAATAAGCGGAGGAAAAG
NL4	GGTCCGTGTTTCAAGACGG

Table 2. Components of PCR reactions.
Tablo 2. PCR reaksiyonlarının bileşenleri.

Component	Volume (µL)	Final Concentration
Template DNA	2	-
Forward primer (10 µM)	1	0.2 µM
Reverse primer (10 µM)	1	0.2 µM
10X EasyTaq® buffer	5	1X
2.5 mM dNTPs	4	0.2 µM
EasyTaq® DNA polymerase	1	2.5-5 units
Nuclease-free water	36	-
Total volume	50	-

2.2. Inoculum preparation

YPS was prepared with the following w/w inclusions of 1% yeast extract, 2% peptone, and 2% sucrose in a shake flask. First, a cryovial of *P. kudriavzevii* FOL-27 stored at -80°C was subculture in a 10 mL YPS broth at 30°C for 24h. After pre-inoculation, a 1% v/v of sub-cultured FOL-27 was inoculated into a 50 mL of YPS media placed in Erlenmeyer flask followed by incubation at 30°C for 24-h with 225 rpm shaking. At the end of incubation optical density of samples was measured at 600 nm by using Shimadzu UVmini-1240 spectrophotometer.

2.3. Cultivation in batch bioreactor

For batch fermentation, bioreactor was filled with 650 mL of YPS media which was inoculated with a 50 mL of pre-cultivated *P. kudriavzevii* FOL-27. Since batch fermentation doesn't require any fresh media to feed in and cultures to harvest while running the process neither fresh media was added nor grown cells were removed from the bioreactor. The temperature, pH and dissolved oxygen (DO) levels were controlled at 30°C, 5.5, and 25% during the entire batch fermentation process, respectively. The samples were taken every 2h until the first 6-h of the fermentation, and the fifth sampling was carried out at 22h for OD_{600} measurements to determine biomass development of FOL-27. Upon cease of fermentation, another 50 mL of fermentative were taken, and the samples were centrifuged to discard the supernatant after which they were put into the incubator until they dry out to determine dry weight of *P. kudriavzevii* FOL-27.

2.4. Cultivation in fed-batch bioreactors

Fed-batch bioreactor trials were carried out to evaluate the performance boost in terms of biomass yield and to test the effect of two different DO levels on growth and performance of *P. kudriavzevii* FOL-27 strain. Fed-Batch bioreactor fermentations started off with 700 mL of YPS media. A 50 mL of pre-cultivated *P. kudriavzevii* FOL-27 strain was inoculated into bioreactors. The temperature and pH of the bioreactors were maintained at 30°C, and 5.5 respectively, while dissolved oxygen levels were adjusted to 25% (control) or 12.5% (treatment) through the entire fermentation process. A sterile 33.3% sucrose solution was prepared for the fed-batch phase and feed pumps were kicked in from 6h onset of fermentation. Fed-batch fermentations were allowed to continue for 20h in which samples were taken every hour until 12h after which samples were taken every two hours for OD measurements at 600 nm. Fed-Batch bioreactor trials were performed in duplicates.

2.5. Simulated gastric and bile juice

To evaluate the survival of FOL-27 under simulated gastric juice and simulated bile juice, modified methods from Sun & Griffiths (2000), Yetiman et al. (2022), Klaenhammer & Kleeman (1981), Song et. al. (2003) were applied. To prepare SGJ, YPS media was adjusted to pH conditions of control (no acid supplementation), pH = 1.5, pH = 2, and pH = 3 by adding HCl solution. To prepare SBJ, YPS broth was supplemented with control (no bile), 0.2%, 1%, and 2% (w/v) ox bile extract (Sigma, Germany). Later, each treatment condition was inoculated with *P. kudriavzevii* FOL-27 fresh culture followed by incubation at 30°C x 200 rpm shaking conditions for 48 h. The final optical cell densities were achieved by using Shimadzu UVmini-1240 spectrophotometer at a wavelength of 600 nm. The trials were conducted in 3 reps and results were shown as relative cell density ratio (OD_{600} t_{final}/OD_{600} t_0). Statistical analysis was conducted using the analysis of variance (ANOVA) and Tukey tests in excel.

3. Results and Discussion

3.1. PCR and sanger sequencing

The DNA samples amplified with NL primers are shown in Figure 1. However, there was nothing observed on the sample that was amplified with ITS primers. For this reason, the PCR product that was amplified with NL primer was sent for sequencing. The BLAST tool (Altschul et al., 1990) was used to examine the Sanger sequencing results. Examining genetic links among *Pichia* species has been made possible by phylogenetic analysis of gene sequences. The PCR fragment of the NL primer was found on the CK5 large subunit ribosomal RNA gene of *Pichia kudriavzevii* strain with 99.47% similarity and a 0.0 E-value score, according to BLAST data. Furthermore, when we checked the BLAST result, the close species was found *Pichia occidentalis* have 99.27% homology.

Nuclear DNA reassociation was used to identify *Issatchenkia occidentalis* as a unique species among strains of *P. kudriavzevii* and the taxonomy database presently lists *Issatchenkia occidentalis* as a synonym for *P. occidentalis* (Kurtzman et al., 2008).

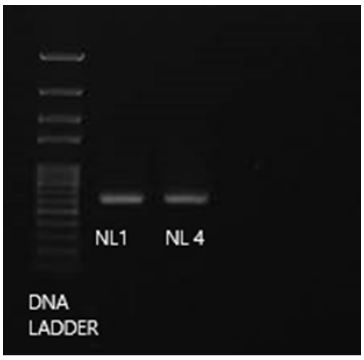

Figure 1. Gel picture of NL primers amplified DNA fragments.
Şekil 1. NL primerleri ile amplifiye edilmiş DNA parçalarının jel resmi.

3.2. Biomass in batch cultivation

The biomass evolution of *P. kudriavzevii* FOL-27 over time is shown in Fig 2. The samples were taken for the first 6 hours, after last sample was taken at around 22h after which stationary phase has been approached. Results indicated the lag and log phases of *P. kudriavzevii* FOL-27 was achieved at around 3 and 7 h, respectively (Figure 2).

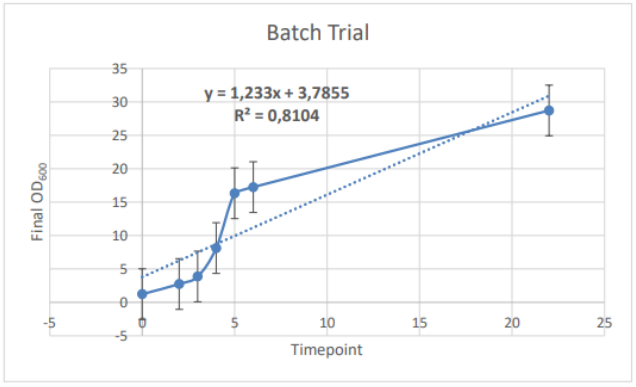

Figure 2. Evolution of biomass over time for batch fermentations.
Şekil 2. Batch fermantasyonlar için biyokütlenin zaman içindeki gelişimi.

Throughout the batch trial pO_2 and pH were controlled. The pH decline started at 8th hour perhaps due to acid production in the environment and base pump dosed NaOH until pH level reaches pH=5.5. The stirrer maintained a constant mixing to avoid sedimentation and uniformity across the medium (Figure 4). During the batch trials, the doubling-time, maximum specific growth rate, and final cell densities achieved were 101.8 min, 8.202 h^{-1} and 28.7 respectively.

Figure 3. The evolution of pO_2, pH, agitation, temperature, base pump duration over time. The graph shows the pH level (blue), stirrer (pink), base pump duration (dark blue), total flow(grey).
Şekil 3. pO_2, pH, çalkalama, sıcaklık, temel pompa süresinin zaman içindeki gelişimi. Grafik pH seviyesini (mavi), karıştırıcıyı (pembe), temel pompa süresini (koyu mavi), toplam akışı (gri) gösterir.

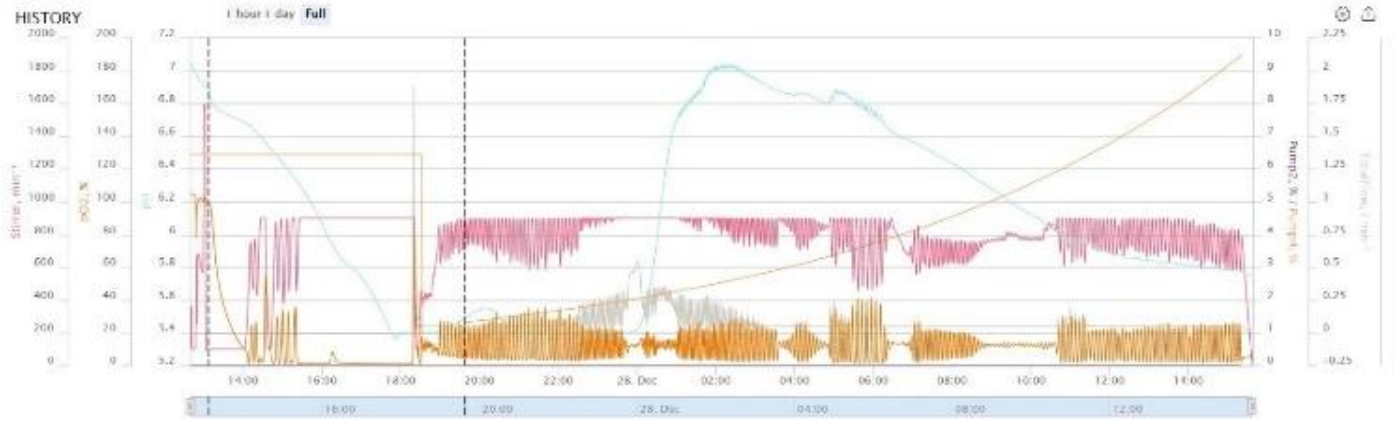

Figure 4. The evolution of pH (light blue) and base pump duration (orange line), dissolved oxygen (pO_2) (orange), airflow rate (grey), and stirrer rate (pink) against time during fed-batch cultivation. pO_2 set to 12.5%.
Şekil 4. Beslemeli kesikli ekim sırasında pH'ın (açık mavi) ve baz pompa süresinin (turuncu çizgi), çözünmüş oksijenin (pO_2) (turuncu), hava akış hızının (gri) ve karıştırıcı hızının (pembe) zamana karşı gelişimi. pO_2 %12.5'e ayarlanmıştır.

3.3. Biomass in fed-batch cultivation

Fed-batch trials (FBT) at 25% pO_2 or 12.5% pO_2 yielded doubling-time, maximum specific growth rate, and final cell densities of 90.18 min, 3.95 h^{-1}, 22.51 and 88.8 min, 2.83 h^{-1}, 26.6, respectively (Figure 5 and 6). Similar biomass yields were achieved in the first 12 hours of bioreactor cultivation. Later, biomass yield was increased perhaps due to feeding with 33.3% sucrose. The stirrer and airflow intake through the cascade system were used to modify pre-set levels of dissolved oxygen throughout the fed-batch operation.

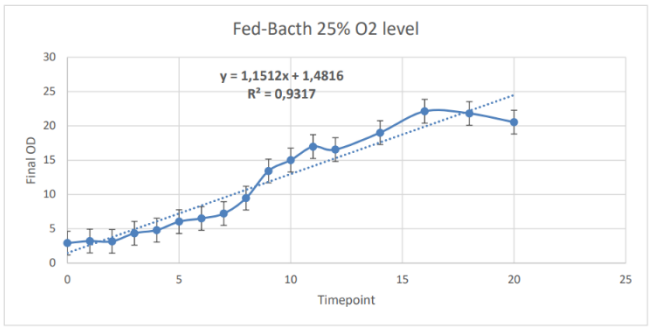

Figure 5. Evolution of biomass over time in fed-batch 25% pO_2 level bioreactor.
Şekil 5. Beslemeli kesikli %25 pO_2 seviyeli biyoreaktörde biyokütlenin zaman içindeki gelişimi.

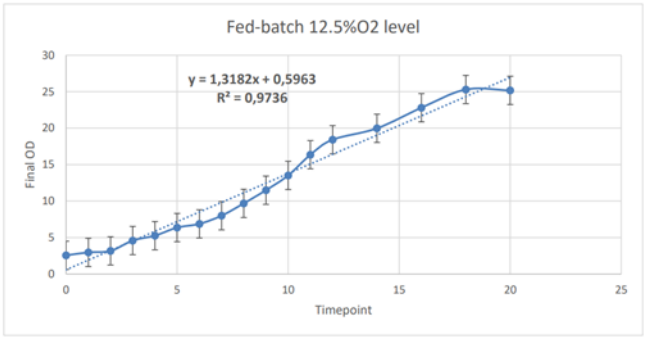

Figure 6. Evolution of biomass over time in fed-batch at 12.5% pO_2 level bioreactor.
Şekil 6. Beslemeli kesikli %12.5 pO_2 seviyeli biyoreaktörde biyokütlenin zaman içindeki gelişimi.

The pH was oscillating at 5.4 at the onset of the batch operation, as shown in Figures 4 and 7. At that point, the base pump starts in and raises the pH to 5.5. When the pH rises to above 5.5, the base pump shuts down. At around 10h, the base pump resumes to adjust the pH of the fermentate in the vessel.

At around 6h after the inoculation, a pH bump was seen (Figure 7) perhaps because of *P. kudriavzevii* FOL-27 producing more alkaline characteristic metabolite than acidic. According to ANOVA results, no significant difference seen between means of biomass in FBT at 25% pO_2 or 12.5% pO_2 level (p=0.83).

Figure 7. The evolution of pH (light blue) and base pump duration (orange line), dissolved oxygen (pO_2) (orange), airflow rate (grey), and stirrer rate (pink) against time during fed-batch cultivation. pO_2 set to 25%.
Şekil 7. Beslemeli kesikli ekim sırasında pH'ın (açık mavi) ve baz pompa süresinin (turuncu çizgi), çözünmüş oksijenin (pO_2) (turuncu), hava akış hızının (gri) ve karıştırıcı hızının (pembe) zamana karşı gelişimi. pO_2 %25'e ayarlanmıştır.

3.4. Survival against SGJ and SBJ

The SGJ survivability trials indicated that *P. kudriavzevii* FOL-27 can proliferate in pH=3 at a similar rate to control condition of pH=6.5 ($p > 0.05$). When the pH was below 2; however, the viability of *P. kudriavzevii* FOL-27 significantly declined ($p<0.05$). According to ANOVA, viability of *P. kudriavzevii* FOL-27 between pH=2 vs pH=1.5 was significantly different ($p< 0.05$). (Table 3).

Table 3. Survival of *P. kudriavzevii* FOL-27 against SGJ.
Tablo 3. P. kudriavzevii FOL-27'nin yapay mide ortamında hayatta kalması.

pH	Relative Cell Density Ratio (OD_{600} t_{final}/OD_{600} t_0)	Standard Deviation
1.5	2.03^c	±0.5
2	13.8^b	±2.0
3	25.2^a	±4.2
6.5 (control)	24.9^a	±5.7

P. kudriavzevii FOL-27 can grow in YPS media with a bile salt concentration of 0.2 percent. Furthermore, when the bile concentration is greater than 1%, *P. kudriavzevii* FOL-27 is still alive. Moreover, when the bile salt concentration is between 1% and 2%, no significant difference in survival of *P. kudriavzevii* FOL-27 was achieved (p=0.86) (Table 4).

Table 4. Survival of *P. kudriavzevii* FOL-27 under SBJ.
Tablo 4. P. kudriavzevii FOL-27'nin yapay safra ortamında hayatta kalması.

Bile Salt Concentration (%)	Relative Cell Density Ratio (OD_{600} t_{final}/ OD_{600} t_0)	Standard Deviation
0 (control)	19.2^a	±0.77
0.2	18.1^a	±0.61
1	18.3^a	±1.26
2	19.0^a	±2.63

P. kudriavzevii has been identified as a potential candidate for ethanol production (Díaz-Nava et al., 2017). *P. kudriavzevii* is an unusual yeast that can withstand a variety of stresses, including low pH, elevated temperature, and high salt concentrations. In the manufacture of xylonic acid, lactic acid, and succinic acid, the potential for genetic engineering of *P. kudriavzevii* for organic acid synthesis has been established (Ndubuisi et al., 2020). *P. kudriavzevii* can use glucose, fructose, and glycerol as carbon sources. On the other hand, *P. kudriavzevii* ITV-S42, does not use sucrose or xylose sugars and ferments ethanol when sugar concentrations are high (Díaz-Nava et al., 2017). Ndubuisi et al. (2020) demonstrated that the first 7.5 hours of *P. kudriavzevii* LC375240 growth weas practically identical across 30°C and 37°C. In the present study, the biomass development of *P. kudriavzevii* FOL-27 was evaluated at 30°C and a stationary phase was reached at 10 h. The results of SGJ and SBJ tests suggested that *P. kudriavzevii* FOL-27 could be a probiotic yeast strain owing to the resilience and survivability seen in acid and bile conditions mimicking human gastric digestion system. A previous study on probiotic characterization of isolated yeasts from Iranian traditional dairies showed that *Pichia fermentans* and *Pichia kudriavzevii* yeast strains showed probiotic potentials because of exhibiting antibacterial and antifungal properties (Saber et al., 2019). *P. kudriavzevii* FOL-27 is resistant to bile salts at concentrations comparable to other *P. kudriavzevii* strains examined, for example M31, M30, M29, M28, M26, O6, G6, G5 (Greppi et al., 2017). Similar to FOL-27, those strains survived satisfactorily at pH=2. Previous research has shown that the carbon source has a significant impact on *P. kudriavzevii* kinetic characteristics (Díaz Nava et al., 2017). Conditions of oxygen limitation encourage ethanol production but not biomass development in some yeast species. As a result, two-step fermentation methods have been designed: an aerobic stage to produce vast amounts of biomass, followed by a low-oxygen stage to increase ethanol production and the kinetic study was carried out on *P. kudriavzevii* 4A in an oxygen-limited environment. (Galafassi et al., 2010). *P. kudriavzevii* FOL-27 exhibited tolerance to 12.5% oxygen level. Chen et al. (2010) reported that raw milk isolates of *P. kudriavzevii* strains of BY15 and BY10 demonstrated probiotic capacity with regards to their survivability in SGJ and SBJ. The probiotic properties of the *P. kudriavzevii* FOL-27 strain used have not been tested before. Therefore, acid-bile tolerance tests were carried out on the strains.

4. Conclusion

Probiotics research has been rapidly evolving in recent years, such as the use of probiotic yeast strains, which has been underutilized so far but is becoming increasingly attractive. The most recent research demonstrates the immense potential of probiotic yeast in the food market, as well as the application of their special characteristics that are not present in probiotic bacteria. Many of the properties of the most well-known probiotic yeast, *S. cerevisiae* var. *boulardii,* have been studied, including positive effects on human health and negative or positive impacts on food products. In this study, the potential probiotic activity of a newly isolated *P. kudriavzevii* FOL-27 strain was investigated under simulated gastric juice and simulated bile juice conditions. We found that FOL-27 is can tolerate high ox-bile salt and low pH conditions mimicking the human gastrointestinal conditions. *P. kudriavzevii* FOL-27 was also subjected to batch reactor and fed-batch reactor conditions to characterize its growth kinetics. Fed-batch fermentation trials using an exponential feeding regimen with 33.3% sucrose supplementation yielded significantly more final biomass than batch trial, as determined by optical density at 600 nm wavelength. Our study demonstrates the preliminary data for probiotic potential of *P. kudriavzevii* FOL-27 and provides tools to improve the biomass development and fermentation growth kinetics of this newly isolated yeast strain. *P. kudriavzevii* FOL-27 carries potential durableness in harsh conditions of SGJ and SBJ, and possesses bioprocess compatibility, leading further probiotic characterizations via in vitro and in vivo experiments. *P. kudriavzevii* is a food fermentation starter culture that can be used to increase fermentation efficiency and achieve controlled food processing. It is commonly found in and dominates spontaneously fermented foods, which lead to distinctive flavors and demonstrate its probiotic potential.

5. Acknowledgment

This work was a capstone project for bachelor of degree.

6. Conflict of Interest

Authors declare no conflict of interest.

7. References

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