Mechanism of Electric Field Assisted Screening for Candida Tropicalis

Chaozheng Zhang (zhangchaozheng@tust.edu.cn)
Tianjin University of Science and Technology
Tianshuang Zhang
Tianjin University of Science and Technology
Peng Tan
Tianjin University of Science and Technology
Xueqing Zhang
Tianjin University of Science and Technology
Hua Zhao
Tianjin University of Science and Technology

Research Article

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Posted Date: July 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-734522/v1

Mechanism of electric field assisted screening for *Candida tropicalis*

Chaozheng Zhang[1, *], Tianshuang Zhang[1], Peng Tan[1], Xueqing Zhang[1], Hua Zhao[1, *,]

[1]Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, Tianjin Key Laboratory of Industrial Microbiology, College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, China

*Corresponding author: zhaohua@tust.edu.cn (H. Zhao), zhangchaozheng@tust.edu.cn (C. Zhang); Tel：0086-22-60602067, Fax：0086-22-60602298

Abstract:

Electric field assisted technology has been widely used in many fields. *Candida tropicalis* degrading thiophene has been successfully screened by electric field assisted screening (EFAS). However, the mechanism of EFAS is yet unclear. In this study, the cell concentration of *C. tropicalis* at different locations in the electric field was studied. In the electric field, *C. tropicalis* cells migrate towards the cathode plate. When the loading electric field intensity was 0.6 V /cm, the concentration of cells near the cathode plate reached the maximum and it was nearly stable at 15 minutes of loading time. Analysis of the cell morphology at different locations showed that only the budding cells could migrate in the electric field. The charged characteristic of the budding cells in the electric field explained the screening mechanism of thiophene-degrading *C. tropicalis* by EFAS. The results can provide a theoretical foundation for EFAS in screening other biodegradable microorganisms.

Keywords: *Candida tropicalis*; Electric field; Migration; Budding cell; mechanism

1. Introduction

Electric and magnetic fields can convert to each other, and they are closely related to the growth and metabolism of living things[1]. Almost all living creature has some changes in properties and physiological activities under a certain electric field(EF)[2]. Therefore, in recent years, electric-field assisted (EFA) technology has been gradually applied in various researches[3,4]. Through application of EFA technology, the efficiency of soil remediation was improved [5,6], the growth and trace metal content of aquatic plants are affected [2], and the harmful contaminants, which strictly limited the land application of the sludge, were reduced from sludge [7]. The EFA technology was also being used in drug delivery research[8]. In order to improve the activity and degradation efficiency of microorganisms, the EFA technology has been applied more and more in biodegradation[9,3,6].

In our previous studies[10], the EFA technology was used to screen functional microorganisms, and a *Candida tropicalis* biodegrading thiophene was obtained. The EFA screening (EFAS) method is simple, timesaving, and can also improve the activity of *C. tropicalis* at the same time. The screened strains showed excellent biodegradation efficiency in study of thiophene biodegradation. However, the mechanism that EFAS can screen for the microbes remains unclear. Single molecule electrophoresis under the EF has been proved to be a feasible method for DNA sequencing[11]. At low voltages (1.5-3.0V), the EF could provide electron donors and receptors for microbial degradation of PCBs[12]. These researches explained the microbial aggregation and migration in the EF.

Therefore, the objective of this study was to investigate the migratory and aggregate behavior of *C. tropicalis* in the EF by examining the distribution of *C. tropicalis* cells under different EF. The

mechanism of EFAS was explored by the migratory behavior of the cells under different EF.

2. Materials and methods

2.1 Strain and medium

The strain used in the experiment was *Candida tropicalis* TCCC30004, which was stored in the Tianjin University of Science and Technology's center of culture collection (TCCC).

The yeast extract peptone dextrose (YEPD) medium was prepared under the following conditions: yeast extract 10 g/L, peptone 20 g/L, glucose 20 g/L, pH 7.0, at 115°C for 20 min.

2.2 Yeast counting

C. tropicalis cultured on the inclined surface was transferred to 5 mL YEPD medium in a 30°C shaker at 180 rpm for 12h, and then 5% (v/v) of broth was inoculated into 50 mL YEPD medium. After shaking culture at 30°C and 180 rpm for12 h, the yeast were collected by centrifugation at 4°C and 5000 r/min. They were suspended in 50mL YEPD medium. YEPD was used to dilute the suspension into samples with different concentrations of yeast, and OD_{600} value of each sample was determined. Yeast concentrations in samples were counted by hemocytometer. Taking OD_{600} as the abscissa and yeast concentration as the ordinate, a curve was drawn to fit the relation curve between OD_{600} and yeast concentration. When counting yeast, 200 μL yeast suspension was placed in a 96-well plate and OD_{600} value was measured by a microplate reader. The results were repeated for three times and the mean value was obtained.

2.3 Experimental device

An EFA experiment device was set up (Fig. 1). A cube with a length of 25cm, a width of 8cm and a height of 6cm was used as the culture tank, with a cover on it. There were eight sampling holes on the cover with an interval of 3cm between the holes. During the experiment, a sampling

needle could be used to sample through the sampling hole. The anode plate and the cathode plate were two platinum plates located at the ends of the culture tank and connected to a direct current power supply with adjustable voltage and current.

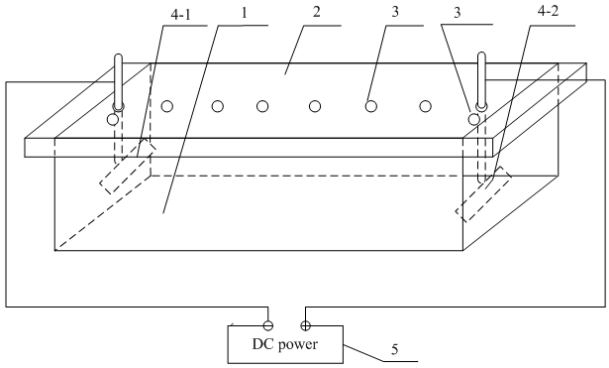


Fig. 1 The EF experimental device (1, culture tank; 2, lid; 3, Sampling hole; 4-1, cathode plate; 4-2, anode plate; 5, direct current power)

2.4 Experiment of aggregation or/and migration in the EF

The experimental device was placed in the clean workbench, the inside and electrode of the device were cleaned with alcohol, and then ultraviolet sterilization was conducted for 30min. Afterwards, 1000mL of yeast suspension of a certain concentration was added to the EFA culture tank. Then at 0.3 V/cm of EF strength, power was applied for 5min, and 1mL *C. tropicalis* suspensions at different sampling holes(0, 3, 6, 9, 12, 15, 18, and 21cm distance from the anode plate) were quickly collected into the sampling tube with a pipette. The OD_{600} value of 200 μL *C. tropicalis* suspension was measured by a microplate reader in a 96-well plate. The OD_{600} value was repeated three times and the mean value was obtained. The above operations were repeated when the loading EF strength was changed to 0.4, 0.5, 0.6 and 0.7 V/cm, respectively. The suspension must be shaken well before the loading voltage was changed each time. According to

the measured OD_{600} value, the cell concentrations at different location of the culture tank under different loading voltages were calculated. The loading EF that were most conducive to *C. tropicalis* migration or/and aggregation were obtained.

After the loading EF was determined, the loading time was explored under the condition of the optimum EF. The selected loading time was 5, 10, 15, 20, 25 and 30min, respectively. The cell concentration at different location of the culture tank under different loading times was obtained according to the above mentioned method. The loading time to facilitate *C. tropicalis* migration or/and aggregation was determined.

3. Results and discussion

3.1 Verification of counting method

In this study, the number of samples was large, and the count of *C. tropicalis* cell needed to be fast and accurate, so the hemocytometer method could not meet the requirements. The microplate reader that could simultaneously determine 96 samples at a high speed was selected for counting *C. tropicalis* cell in solution. The amount of cell in the solution corresponding to different OD_{600} was measured using hemocytometer. The results were shown in Fig.2. The mathematical equation of the corresponding curve was $Y = 0.9235\ X + 5.5193$. Y was the cell concentration. X stood for OD_{600}. The linear correlation coefficient was greater than 0.98.

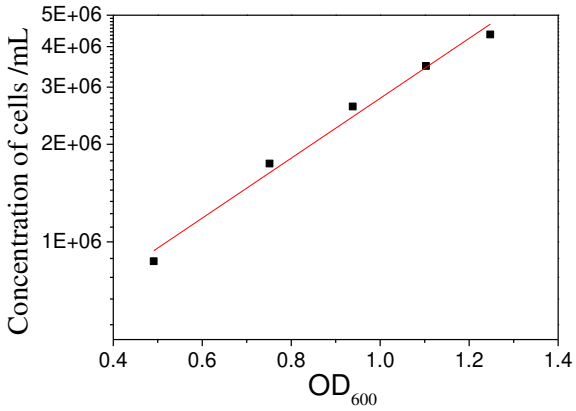


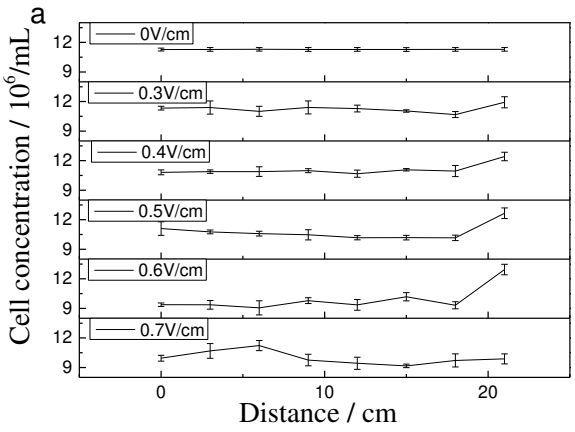
Fig. 2 The counting curve of *C. tropicalis*

3.2The behavior under different EF

The loading voltage at both ends of the culture tank was changed, and the concentration of *C. tropicalis* at different locations was sampled and analyzed. The results were shown in Fig. 3a. Under a certain loading voltages(in the test range), the cell concentration of *C. tropicalis* fluctuated slightly with the distance (distance from anode plate) in the culture tank. However, at the loading EF of 0.3-0.6 V/cm, the concentration of *C. tropicalis* at 21cm distance(near the cathode plate) was significantly higher than that at other distance. And the concentration of *C. tropicalis* at 21 cm distance increased with the increase of the loading EF. When the loading EF reached 0.6 V/cm, the cell concentration at 21 cm distance reached its maximum value. When the loading EF reached 0.7 V/cm, the cell concentration at 21 cm distance decreased again. The overloading EF, whose strenght was 0.7 V/cm, might cause damage to the *C. tropicalis* cells. Therefore, the loading EF strength of 0.6 V/cm was the most suitable for the migration of the cells to cathode plate. The physiological activities of cells are different in different living environments[14]. Subsequently, the above experiments were carried out with saline instead of YEPD medium (Fig. 3b). The results showed that the movement trend of *C. tropicalis* cells in saline was consistent with that in YEPD medium. However, the cell concentration of *C. tropicalis*

120 at 21cm distance(near the cathode plate) in saline was lower than that in the YEPD under the same
121 condition.

122 Stimulated by an EF, cell growth, metabolism, enzyme activity, membrane permeability and
123 other aspects were affected accordingly[13]. Previous studies have shown that the biomass in
124 fermentation broth increased after direct current stimulation[10]. However, *C. tropicalis* did not
125 reproduce for less than five minutes. It just proved that *C. tropicalis* could migration and
126 aggregation to cathode plate in a certain EF. Samples taken at different distances were examined
127 by microscope. Cells in the samples taken from 21cm distance(near of cathode plate) were
128 predominantly budding cells, whereas cells in the sample taken from other distances were not so.
129 The external energy can also acted in some form as the metabolic regulator of cells[15]. The cell
130 activity of *C. tropicalis* was enhanced under the EF. The migration and aggregation of *C.*
131 *tropicalis* cells to the cathode plate may be related to the physiological activity of the cells. Under
132 a certain EF, the centers of negative charge and positive charge in the budding cell were shifted.
133 This caused the cells to become positively charged and move towards the cathode plate.
134 Therefore, the following conclusions were drawn: in the budding stage, *C. tropicalis* cells were
135 positively charged and move towards the negative pole (cathode plate) in the EF, whereas cells at
136 other growth stages do not have this property.



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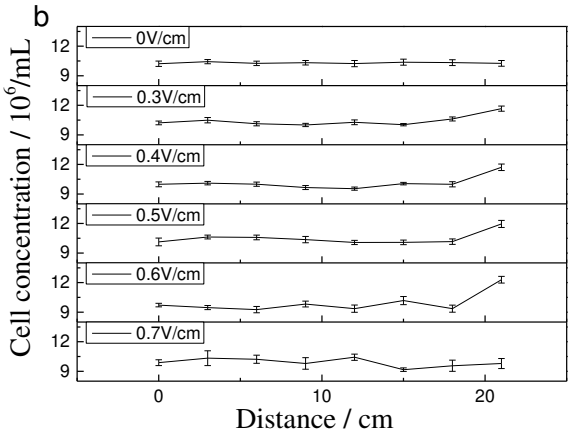

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139 **Fig. 3 Effect of loading EF on cell migration (Distance, the distance from the anode plate;**

140 **5min of loading time; a-YEPD, b-Saline)**

141 **3.3 The behavior under different loading time**

142 Under the loading EF of 0.6 V/cm, the cell concentrations at different locations were analyzed

143 by changing the loading time. The results were shown in Fig. 4. The *C. tropicalis* cells became

144 active in an EF. After loading the EF, the cell concentration began to decrease at almost all

145 locations, except the cell concentration near the cathode plate(21cm of distance) was

146 increased(Fig. 4a). The cell concentration near the anode plate(0cm of distance) was the lowest

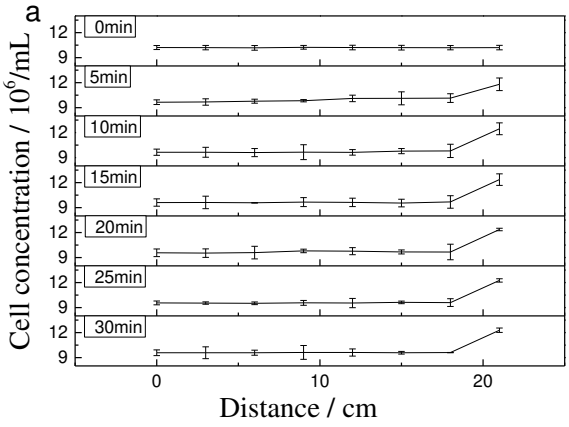
147 after 5min of EF loading. As the cells migrated, the concentration of cells near the anode plate did

148 not change. And then at a distance of 12cm, the concentration of the cells didn't change when the

149 EF was applied for 10min(Fig.4b). After loading the EF for 15min, the cell concentration at all

positions did not change. Except near the cathode plate(21cm of distance), the cell concentration at all positions was almost the same and lower than the initial value. This was the concentration of non-budding cells in the solution. At this time, almost all the budding cells gathered near the cathode plate. And the cell concentration at a distance of 21cm was significantly higher than that at other distance. With the extension of the loading time, the cell concentration near the cathode plate became higher and higher, however the increasing trend slowed down (Fig.4 b). It might be due to a decrease in the number of budding cells in the solution other than near the cathode plate.

After loading the EF for about 20min, the plaque that proved the attachment of bacteria to the cathode plate began to appear. Therefore, in the experiment to investigate the migration behavior of *C. tropicalis* in the EF, the loading time of EF should be decreased as much as possible to reduce the influence of cell aggregation in the cathode plate on experimental results. The *C. tropicalis* capable of biodegrading thiophene was screened by EFAS method[10] because only the budding cells could aggregate in the cathode plate. The aggregation behavior of *C. tropicalis* cells on the cathode plate is the key to the successful screening of it. Only the cells that can degrade thiophene could grow and germinate in the corresponding enrichment medium and the stronger the capacity to degrade thiophene, the stronger the capacity to grow and germinate.



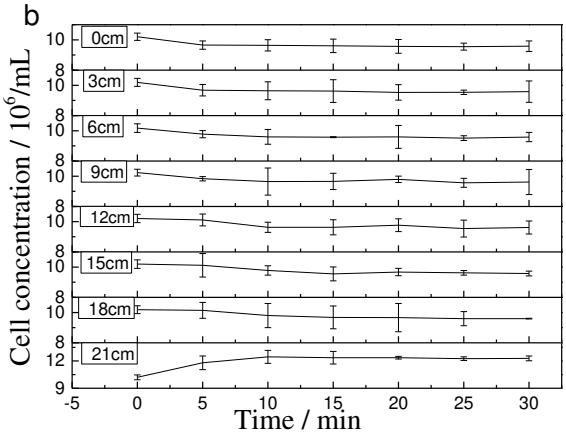


Fig.4 Effect of loading time on cell migration (a- Different distances at the same time, b-Same distance at different times)

4. Conclusions

In this study, the migration of *C. tropicalis* cells in the direct current field were observed. Under different EF, the migration rate of cells was different. Budding *C. tropicalis* cells with a positive charge were more likely to move towards the cathode plate in the EF. Plaque attachment to the cathode plate can be observed in a short period of time (about 20 minutes). The mechanism by which thiophene-degrading *C. tropicalis* was screened by an EFAS method was explained by the characteristic of budding cells being positively charged in EF.

Acknowledgments

This work was supported by the Foundation (No.2020KF005, 2020KF009) of Key Laboratory of

Industrial Fermentation Microbiology of Ministry of Education and Tianjin Key Lab of Industrial Microbiology (Tianjin University of Science &Technology) and the Undergraduate Laboratory Innovation Fund of Tianjin University of Science and Technology(No.1904A204).

Compliance with ethical standards

Conflict of interest

All authors declare that there are no conflict of interests.

Ethical guidelines

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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