The Potential of Thermotolerant Yeast *Pichia kudriavzevii* 1P4 as an Ethanol Fermentation Agent

Darojatul Ulya (G351180061)

Supervised by:

Dr. Rika Indri Astuti M.Si, Prof. Dr. Anja Meryandini, MS

SEKOLAH PASCASARJANA
INSTITUT PERTANIAN BOGOR
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Introduction

Progressive consumption of fossil fuel

Global warming effect

Renewable and environmental-friendly energy alternatives

Bioethanol

High-Temperature Ethanol Fermentation

Advantages:

- Reduced cooling cost
- Higher fermentative activity and saccharification
- Lower risk contamination

(Banat *et al*. 1998)

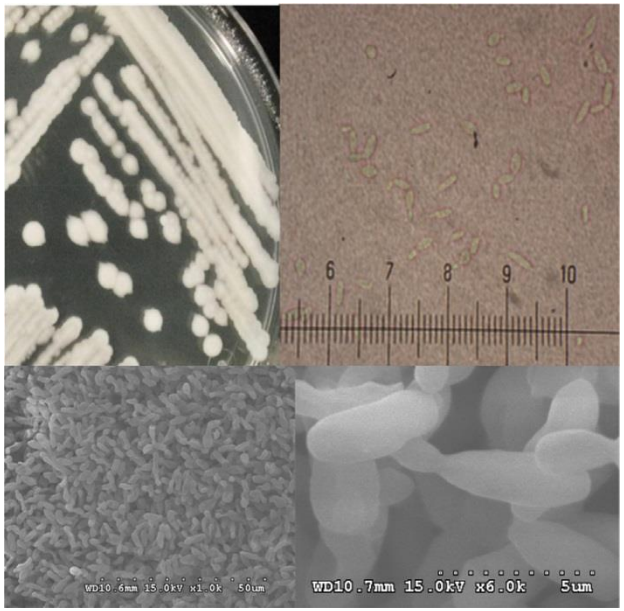
Problems

Fermentation related-stresses, including:

- High temperatures
- High sugar content
- High ethanol content

Introduction

Pichia kudriavzevii



- Ethanologenic yeast which is capable to use hexose and pentose sugars as a substrate for ethanol fermentation

  (Mussatto *et al*. 2012)
- Yeast *P. kudriavzevii* 1P4 can grow at YPD (Yeast Pepton Dextrose) media at 40 °C.

  (Inderiani 2017)
- Ethanol production and fermentative activity of *P. kudriavzevii* 1P4 still unidentified

Objectives

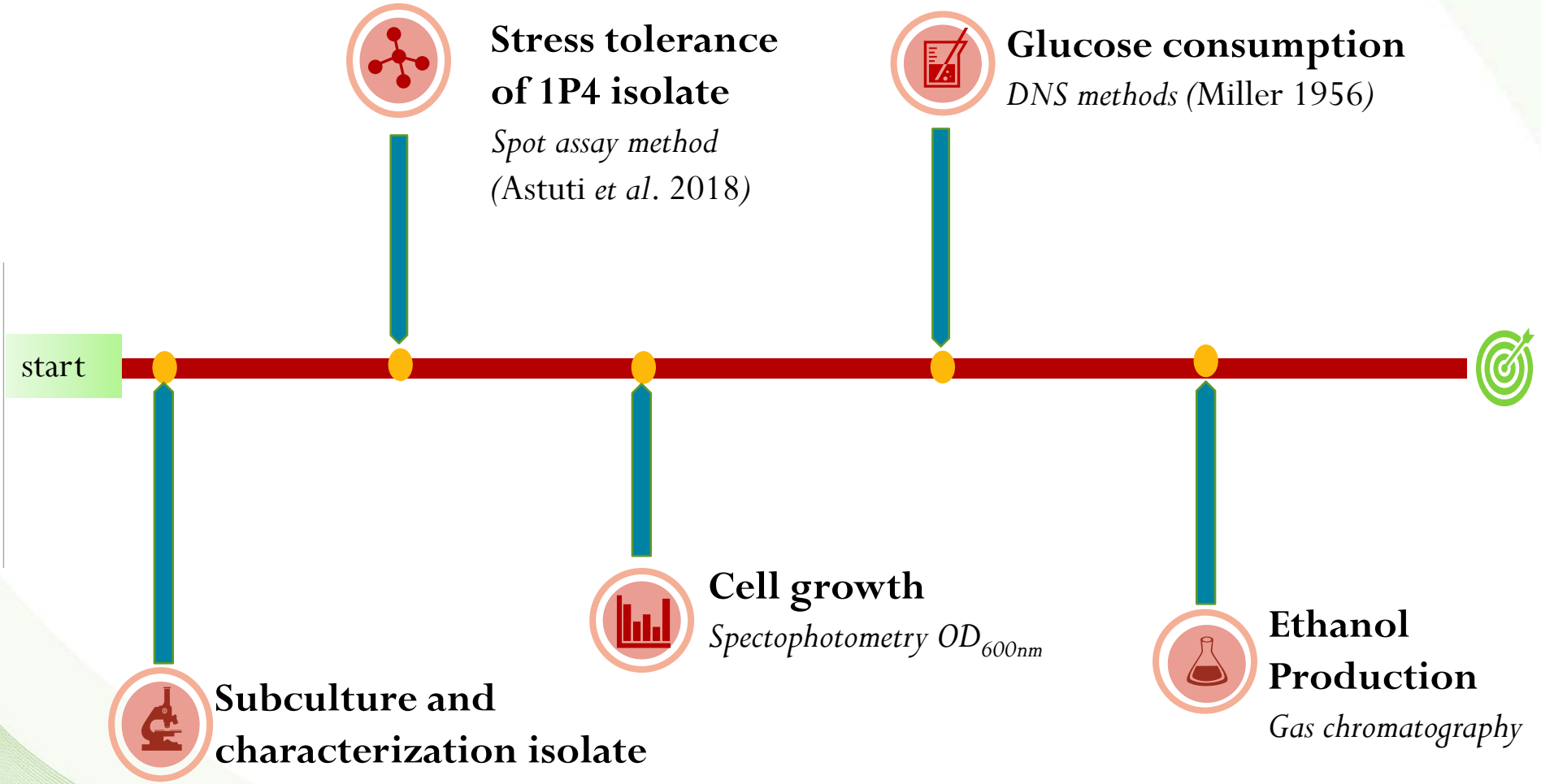
- Evaluating stress tolerance phenotype of isolate *P. kudriavzevii* 1P4 in dealing with fermentation related-stresses at YPD media including high temperatures, high sugar content and ethanol content.
- Determining fermentative activity of isolate *P. kudriavzevii* 1P4 at YPD media

Time and place

Juli 2019 until Juli 2020

Laboratorium Penelitian Biologi, FMIPA IPB and Laboratorium Mikrobiologi PPSHB IPB

Procedures


Stress tolerance of 1P4 isolate
Spot assay method (Astuti et al. 2018)
Glucose consumption
DNS methods (Miller 1956)
start
Subculture and characterization isolate
Cell growth
Spectophotometry OD_{600nm}
Ethanol Production
Gas chromatography

Procedures:
Subculture and characterization isolate

Subculture

YPD liquid media

Incubation 24 hours, 27 °C

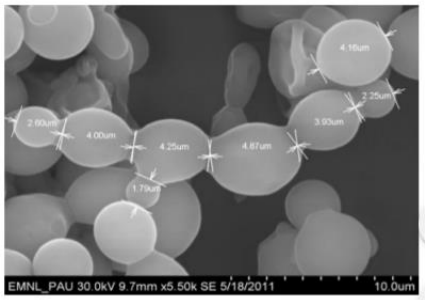
streak

YPD solid media

Characterization

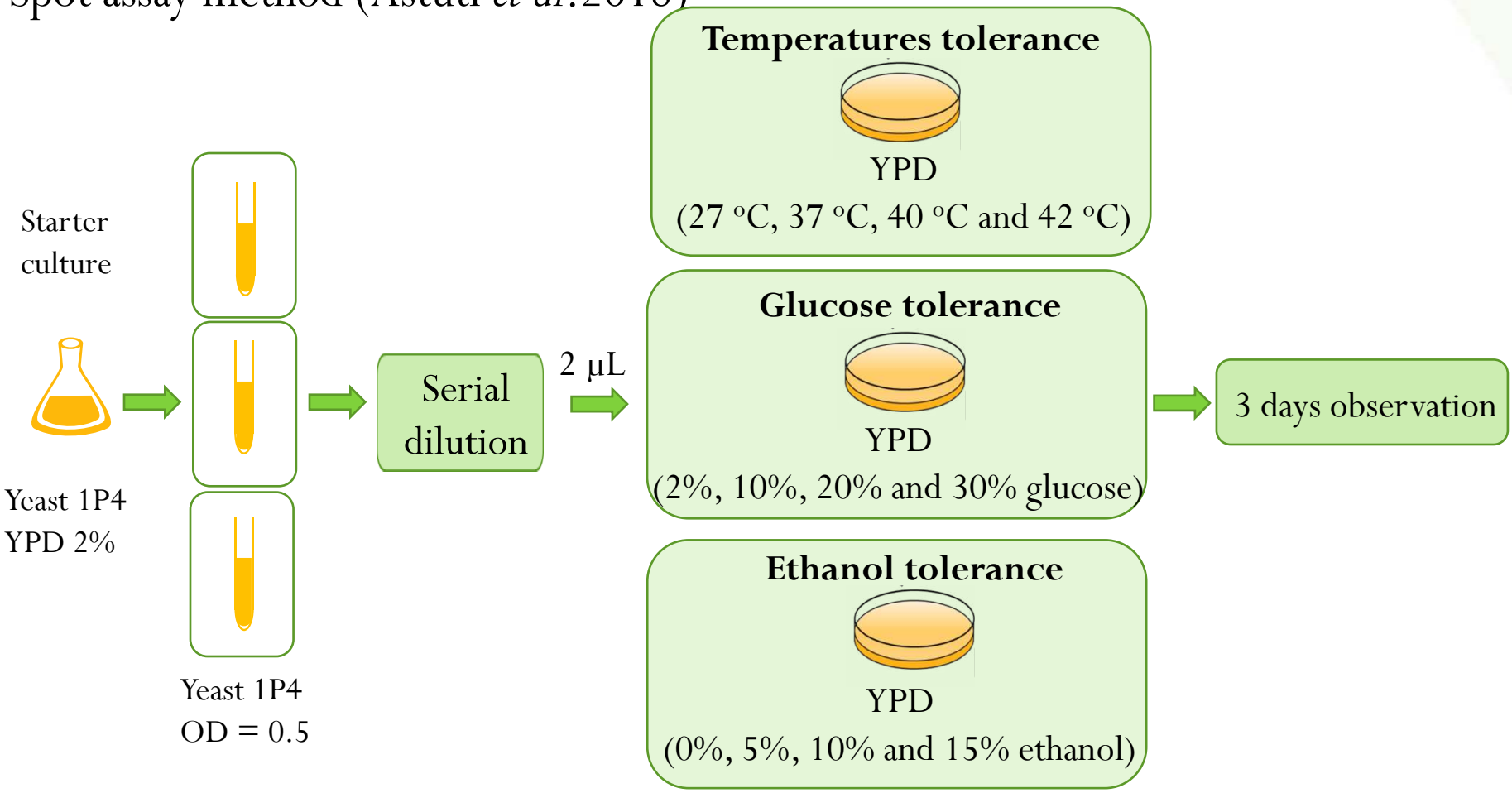
Using light microscope to identify:

- Cell shape
- Cell size
- Budding cell



Procedures:
Stress tolerance test

- *Saccharomyces cerevisiae* BY4741 as a comparison isolate
- Spot assay method (Astuti *et al*.2018)



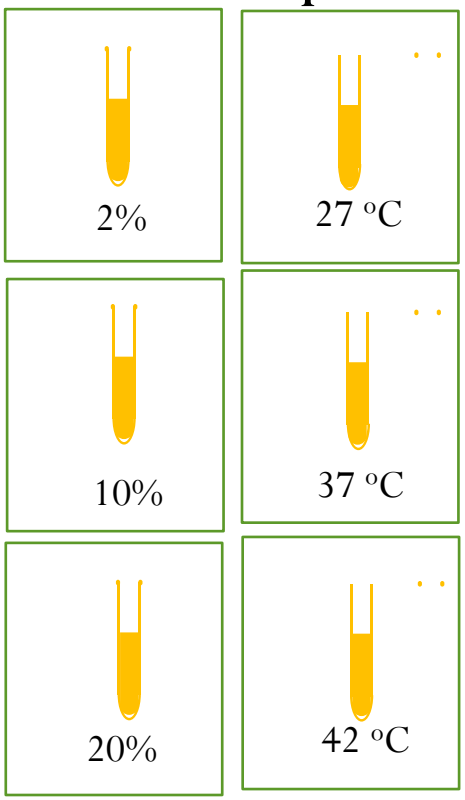
Procedures:
Glucose consumption, cell growth and ethanol production

1 lup

1P4 isolate
16 – 24 hours

OD_{600} 0.8 – 1
Liquid YPD

Glucose Temperatures



Glucose consumption

Measuring OD550nm per 8 hours during 48 hours experiment

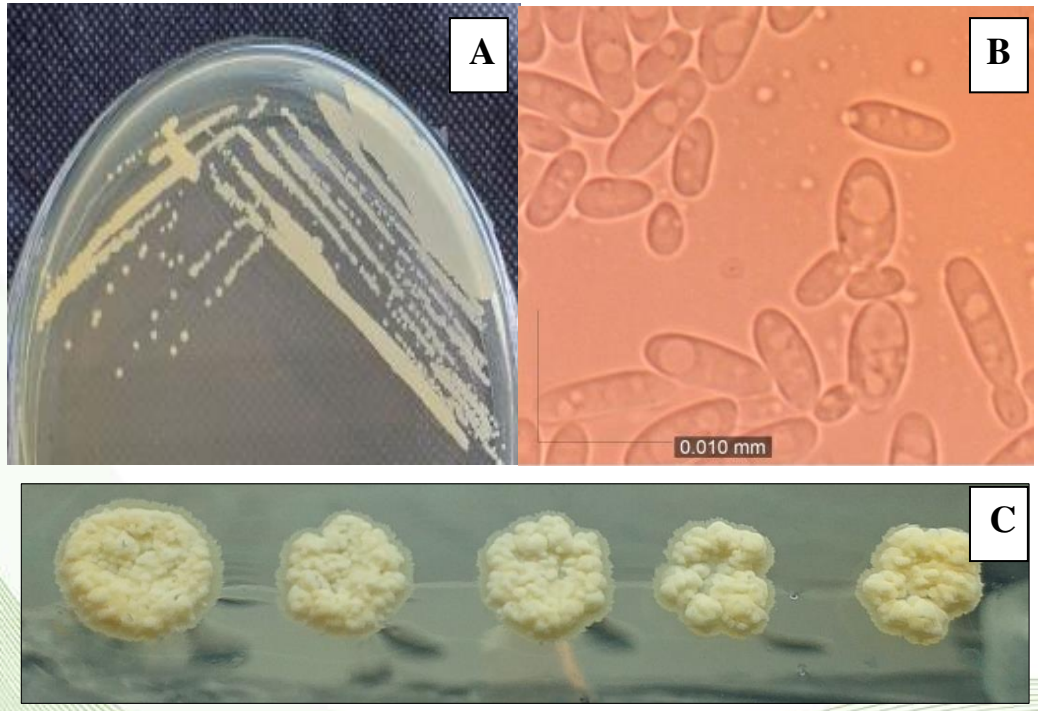
Cell growth

Measuring OD600nm per 3 hours during 48 hours experiment

Ethanol production

Measuring ethanol concentration at 48 hours experiment using gas chromatography

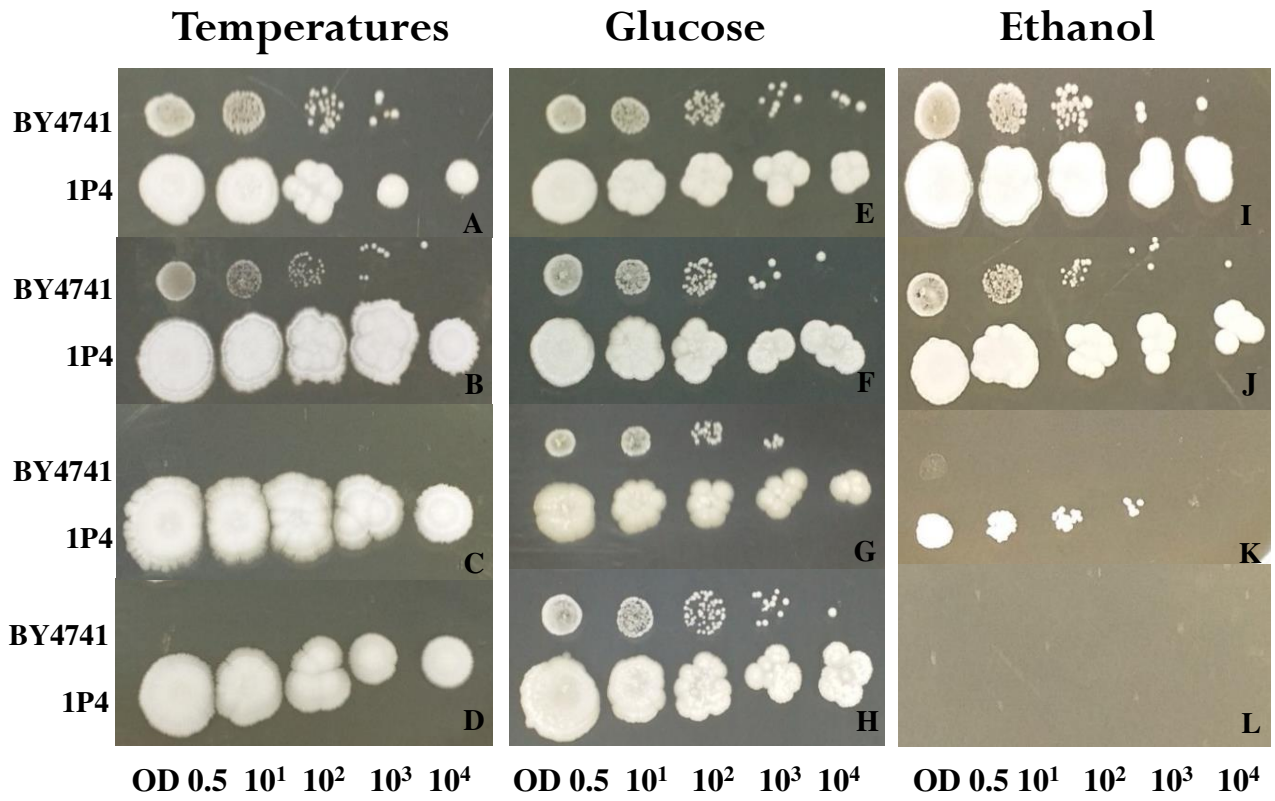
Result and discussion:
Morphology characterization of *P. kudriavzevii* 1P4



- Colony isolate round and white-cream
- Single cell ovoid
- Size 4-10 µm
- Monopolar budding cell type
- Pseudohyphae was not found

Morphology characterization of *P. kudriavzevii* 1P4. **A**. Colony yeast 1P4 at 2% YPD media. **B.** Single cells of 1P4 isolate under light microscope (1000X). **C.** Colony 1P4 isolate at YPD media with 20% initial glucose.

Result and discussion:
Stress tolerance of *P. kudriavzevii* 1P4



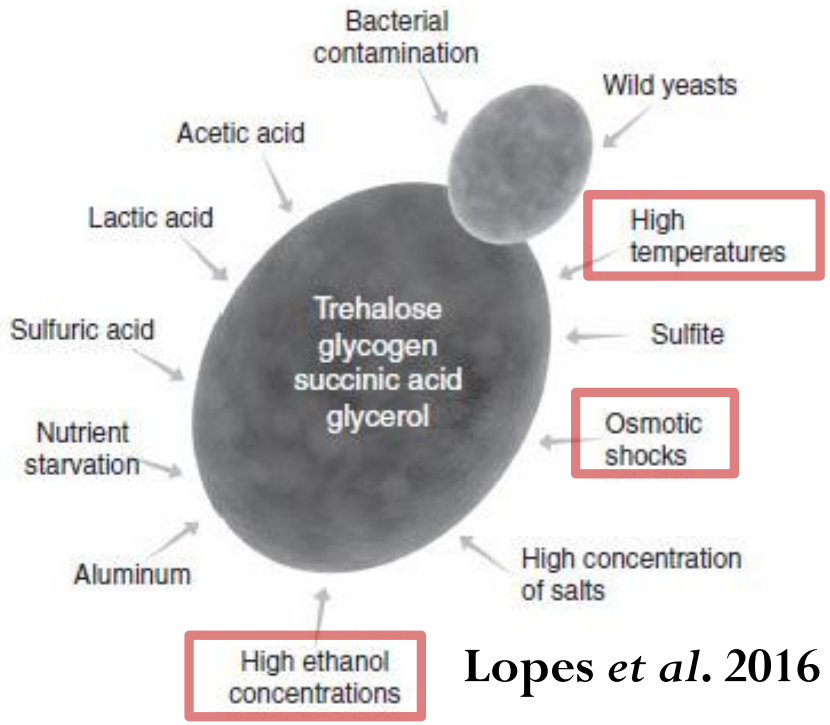
Temperatures: **A**. 27 °C, **B**. 37 °C, **C**. 40 °C, **D**. 42 °C.
Glucose: **E**. 2%, **F**. 10%, **G**. 20%, **H**. 30%.
Ethanol: **I**. 0%, **J**. 5%, **K**. 10%, **L**. 15%.
BY4741: Isolate *S. cerevsiae* BY4741, **1P4**: Isolate *P. kudriavzevii* 1P4

References:

- *P. kudriavzevii* S26 can grow at YPD media with 6% ethanol (Ndubuisi *et al*. 2018)
- *P. kudriavzevii* ITV-S42 survive at semi-sintetic media with 20% glucose (Diaz-Nava *et al*. 2017)
- *P. kudriavzevii* RZ8-1 was capable to produce ethanol at YPD media at 45°C (Chamnipa *et al*. 2018)

Result and discussion:
Stress tolerance of *P. kudriavzevii* 1P4

Response yeast cells against stress-related fermentation



Lopes *et al.* 2016

- High level of trehalose strongly correlates with the induction of stress tolerance factors, making it an important marker for potential stress resistance in yeast.
- The thermal stress response in yeast is the induction of Hsps (Heat shock proteins). Hsps function as to protect thermally damaged protein.

(Saini *et al.* 2018)

- Stress tolerance of yeast was depends on yeast strain, composition of the fermentation medium, intracellular ethanol accumulation, incubation temperature and osmotic pressure.

(Banat *et al.* 1998)

Result and discussion:
Glucose consumption of *P. kudriavzevii* 1P4

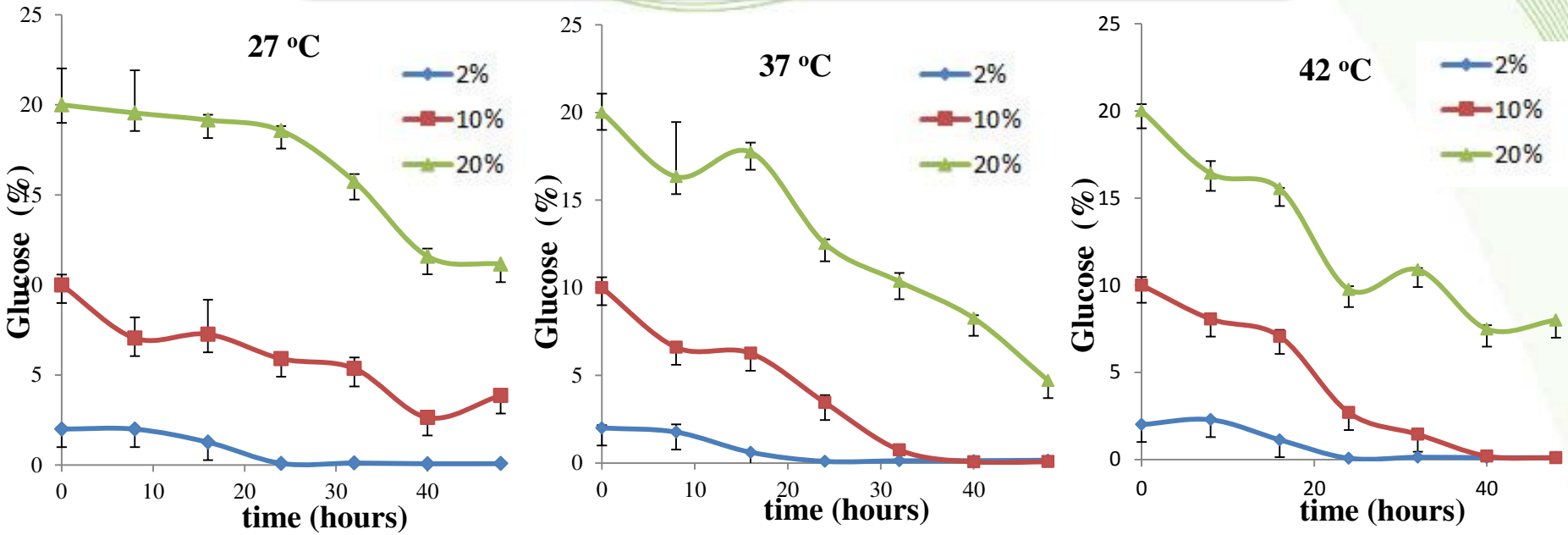


Fig.3. Changes in reducing sugar content at YPD media with initial glucose 2%, 10% and 20% at temperatures (27 °C, 37 °C and 42 °C) during 48 h fermentation 2% 2% initial glucose, 10% 0% initial glucose , 20% 20% initial glucose.

- 1P4 consumed the highest glucose at 20% initial glucose at 37 °C (15.28%).
- Glucose consumption rate was faster at high temperature ethanol fermentation

Result and discussion:
Cell growth of *P. kudriavzevii* 1P4

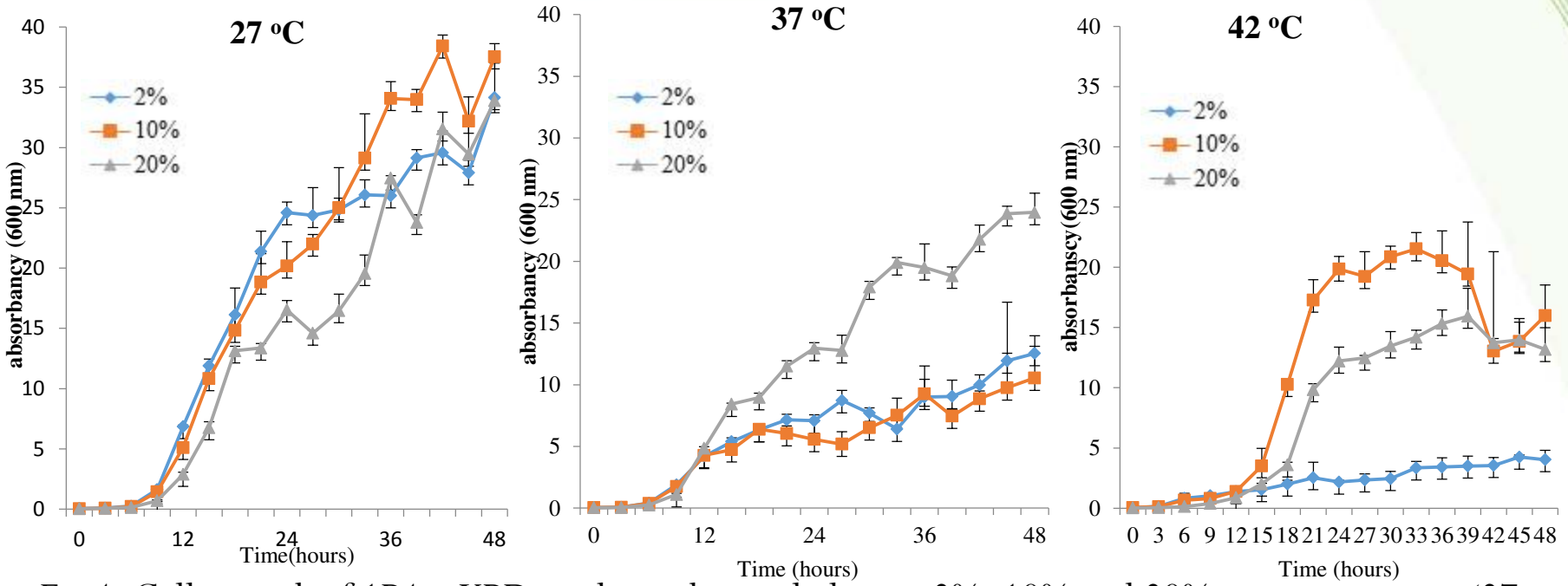
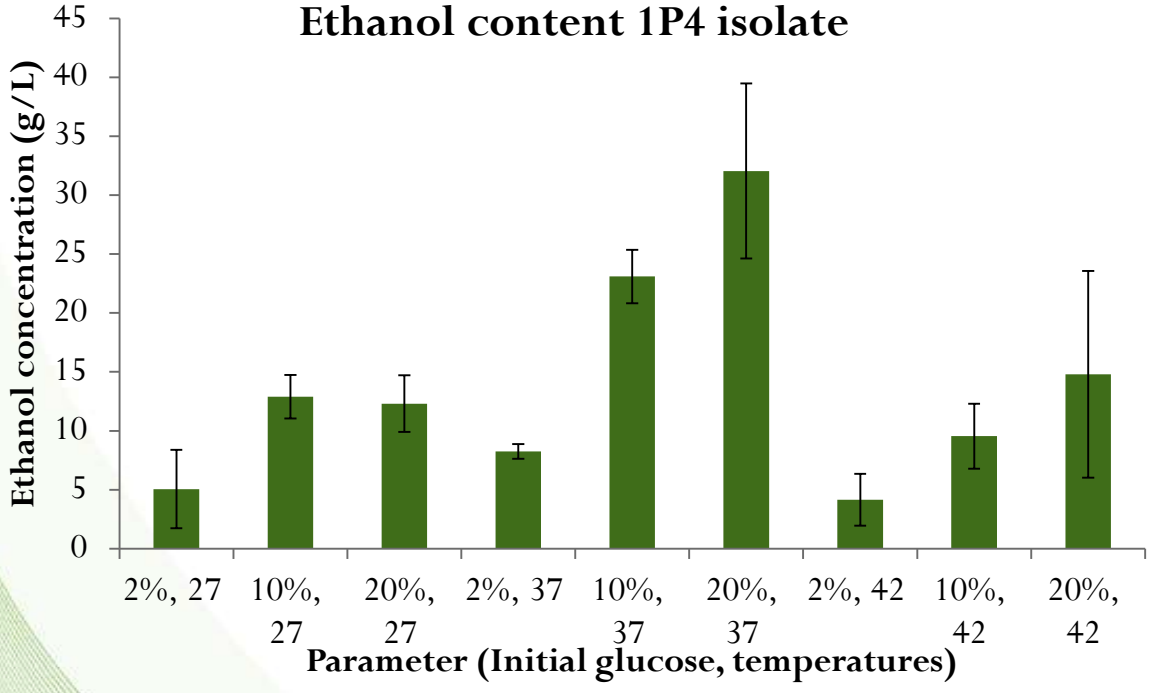


Fig.4. Cell growth of 1P4 at YPD media with initial glucose 2%, 10% and 20% at temperatures (27 °C, 37 °C and 42 °C) during 48 h fermentation

- Lag phase of 1P4 isolate was shorter at high-temperatures ethanol fermentation.
- Growth of 1P4 isolate increased with higher initial glucose condition yet decreased as temperature during fermentation was raised

Result and discussion:
Ethanol production of *P. kudriavzevii* 1P4



References:

- *P. kudriavzevii* RZ8-1 produced 35.14 g/L ethanol at 37 °C (Chamnipa *et al*. 2018)

- Ethanol production of 1P4 was higher than control at high-temperature ethanol fermentation

Several factors which influence the production of bioethanol including *strain*, temperature, sugar concentration, pH, fermentation time, agitation rate and inoculum size (Zabed *et al*. 2014, Lopes *et al*. 2016)

Result and discussion:
Ethanol production of *P. kudriavzevii* 1P4

Parameter	Actual Ethanol (g/L)	Theoretical ethanol (g/L)	Fermentation time (hours)	% yield	Volume production (g/L/h)
2%, 27 °C	5.05	9.81	48	51.46	0.10
10%, 27 °C	12.90	31.37	48	41.11	0.26
20%, 27 °C	12.30	45.22	48	27.19	0.25
2%, 37 °C	8.25	9.41	48	87.61	0.17
10%, 37 °C	23.10	50.77	48	45.49	0.48
20%, 37 °C	32.05	78.24	48	40.95	0.66
2%, 42 °C	4.15	9.68	48	42.84	0.08
10%, 42 °C	9.55	50.70	48	18.83	0.19
20%, 42 °C	14.80	61.40	48	24.10	0.30

Conclusion

- *P. kudriavzevii* 1P4 performed higher fermentative activity at HTEF.
- 1P4 consumed the highest concentration of glucose of 20% at 37°C (15.29%), simultaneously with the highest concentration of ethanol (32.05 gl^{-1} ethanol and 0.67 g l^{-1} h volumetric ethanol productivity).
- 1P4 also showed high tolerance against sugar and temperature stresses.
- Cell growth analysis exhibited short lag phase of 1P4 as temperature of fermentation was raised.

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Thank you ☺