Evaluating the bioethanol production potential of *Spathaspora passalidarum* and *Kluyveromyces marxianus* using spent mushroom substrate

Pathamaporn Prasongsup* and Nadchanok Rodrussamee
Department of biology, Faculty of science, Chiang Mai University, Chiang Mai 50200, Thailand
Email address: aomp40858@gmail.com

OBJECTIVE

To evaluate the potential of *Spathaspora passalidarum* and *Kluyveromyces marxianus* for ethanol production from spent mushroom substrate

METHODOLOGY

Sample preparation

SMS → Air-dry for 7 days → Dry using hot air oven at 60°C for 24 h → Dried SMS → Grind → Sieve through 710-micron mesh → Sieved SMS → Compositional analysis

Pretreatment
2% (w/v) NaOH

Sieved SMS → Add SMS into 2% NaOH with ratio of SMS: NaOH (1 g: 20 ml) → 2% (w/v) NaOH → Incubate in a water bath at 55°C for 1 h → Filter through muslin cloth

- Liquid → NaOH hydrolysate → Analyse
  - -Reducing sugars (DNS method)
  - -Hydrolysate tolerance (Spot test)
- Solid → Add Solid into 0.1% H_2SO_4 with ratio of Solid: H_2SO_4 (1 g: 5 ml) → 0.1% (v/v) H_2SO_4 → 121°C for 15 minutes → Filter through muslin cloth

Hydrolysis
0.1% (v/v) H_2SO_4

Liquid → H_2SO_4 hydrolysate → Analyse
- -Reducing sugars (DNS method)
- -Hydrolysate tolerance (Spot test)

Fermentation

Yeast culture in YPD broth for 24 hours. → X 3 → Inoculate in 100 mL of
- YPD broth
- YPD+NaOH hydrolysate
- YPD+H_2SO_4 hydrolysate

Monoculture
- *S. passalidarum* CMUWF1-2
- *K. marxianus* DMKU3-1042
- *K. marxianus* CMUMK40-1

Co-culture
- CMUWF1-2 + DMKU3-1042
- CMUWF1-2 + CMUMK40-1
- DMKU3-1042 + CMUMK40-1

ABSTRACT

Spent mushroom substrate (SMS) is a byproduct of the mushroom cultivation industry and poses a significant disposal challenge. This study aimed to evaluate the potential of xylose-fermenting yeasts, *Spathaspora passalidarum* and *Kluyveromyces marxianus*, for bioethanol production from SMS. The proximate composition of SMS was analyzed, revealing cellulose, hemicellulose, and lignin contents of 37.29% ± 0.21, 20.07% ± 0.72, and 27.12% ± 0.87, respectively. SMS pretreated with 2% (w/v) NaOH and further hydrolyzed with 0.1% (v/v) H_2SO_4 yielded total reducing sugar concentrations of 19.95 ± 0.25 g/L and 38.80 ± 0.76 g/L, respectively. To assess yeast tolerance, hydrolysates obtained from NaOH pretreatment and NaOH followed by H_2SO_4 hydrolysis were tested at concentrations of 25%, 50%, 75%, and 100% (v/v) using a spot test. The results showed that *S. passalidarum* and *K. marxianus* could thrive at 25% (v/v) and 100% (v/v) hydrolysate concentrations after NaOH pretreatment and NaOH followed by H_2SO_4 hydrolysis, respectively. To further evaluate their ethanol production potential, fermentation of the SMS hydrolysate at each selected concentration is currently in progress.

INTRODUCTION

Spent Mushroom Substrate (SMS) → Lignocellulosic biomass (Hemicellulose, Cellulose, Lignin) → Bioethanol → *Saccharomyces cerevisiae* (C5 ✗, C6 ✓) → Normally, *S. cerevisiae* can't ferment C5-,C6-sugars in SMS

Use xylose-fermenting yeast
- *Spathaspora passalidarum*
- *Kluyveromyces marxianus*

RESULTS

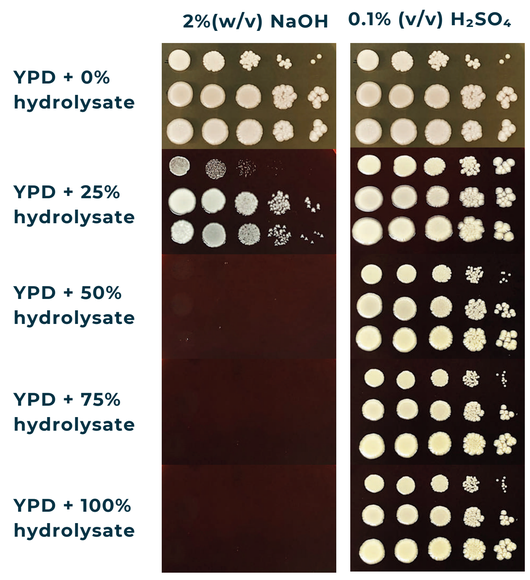
Table. 1 Compositional analysis of SMS.

Composition	Quantity (%)
Total solids (TS)	92.49 ± 0.02
Volatile solids (VS)	82.88 ± 0.51
Cellulose	37.29 ± 0.21
Hemicellulose	20.07 ± 0.72
Lignin	27.12 ± 0.87
Ash	17.12 ± 0.51

Table. 2 Reducing sugars in NaOH and H_2SO_4 hydrolysates.

Samples	Reducing sugars (g/l)
NaOH hydrolysate	19.95 ± 0.24
H_2SO_4 hydrolysate	38.80 ± 0.71

Reducing sugars in H_2SO_4 hydrolysate > NaOH hydrolysate



S. passalidarum and both strains of *K. marxianus* could tolerate 25%(v/v) NaOH hydrolysate and 100% (v/v) H_2SO_4 hydrolysate

Fig 1. Spot test for hydrolysate tolerance of *S. passalidarum* CMUF1-2, *K. marxianus* DMKU3-1042 and *K. marxianus* CMUMK40-1 in NaOH and H_2SO_4 hydrolysates at concentration of 25%, 50%, 75% and 100% (v/v).

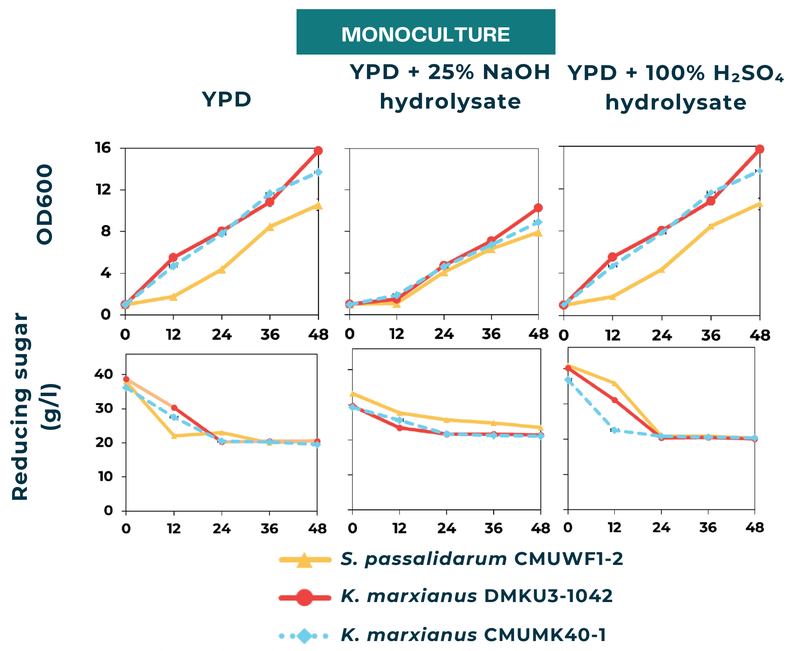


Fig 2. Growth and reducing sugars utilization in YPD, YPD + 25% NaOH hydrolysate, YPD + 100% H_2SO_4 hydrolysate using monoculture of *S. passalidarum* CMUF1-2, *K. marxianus* DMKU3-1042 and *K. marxianus* CMUMK40-1.

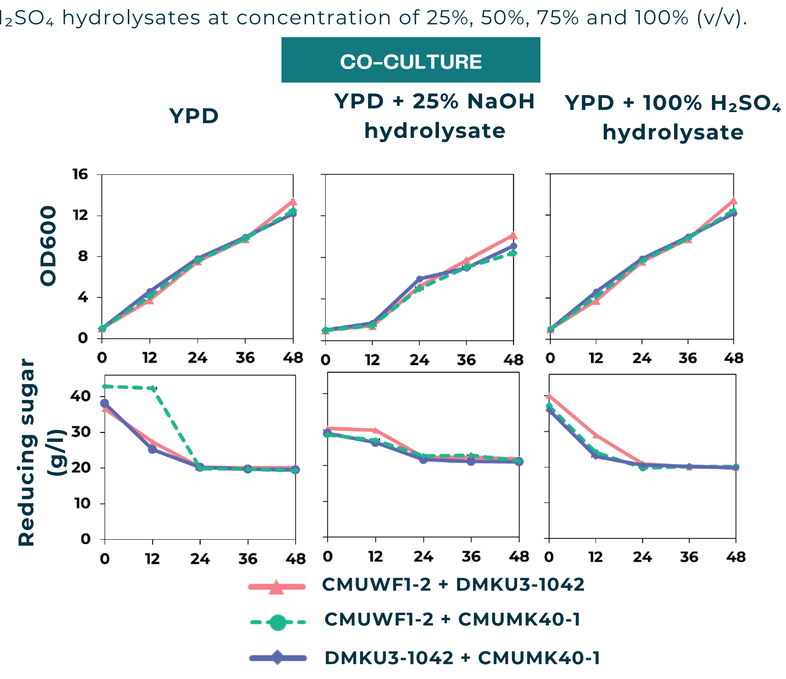


Fig 3. Growth and reducing sugars utilization in YPD, YPD + 25% NaOH hydrolysate, YPD + 100% H_2SO_4 hydrolysate using co-culture of CMUF1-2 + DMKU3-1042, CMUF1-2 + CMUMK40-1 and DMKU3-1042 + CMUMK40-1.

Growth
***K. marxianus* DMKU3-1042** > *K. marxianus* CMUMK40-1 > *S. passalidarum* CMUWF1-2
CMUWF1-2 + DMKU3-1042 > CMUWF1-2 + CMUMK40-1 > DMKU3-1042+ CMUMK40-1

Reducing sugars utilization
***K. marxianus* CMUMK40-1** > *K. marxianus* DMKU3-1042 > *S. passalidarum* CMUWF1-2
CMUWF1-2 + CMUMK40-1 > CMUWF1-2 + DMKU3-1042 > DMKU3-1042+ CMUMK40-1

CONCLUSION

- SMS contained cellulose, hemicellulose, and lignin in concentrations of 37.29% ± 0.21, 20.07% ± 0.72, and 27.12% ± 0.87, respectively.
- The reducing sugar concentration in the H_2SO_4 hydrolysate (38.80 ± 0.71 g/L) was higher than that in the NaOH hydrolysate (19.95 ± 0.24 g/L).
- *S. passalidarum* and both strains of *K. marxianus* were able to tolerate 25% (v/v) NaOH hydrolysate and 100% (v/v) H_2SO_4 hydrolysate.
- Growth and reducing sugar utilization in monocultures grown in YPD, YPD + 25% NaOH hydrolysate, and YPD + 100% H_2SO_4 hydrolysate showed that both strains of
- *K. marxianus* had higher growth rates and sugar utilization compared to S. passalidarum CMUWF1-2.
- However, co-culturing *S. passalidarum* with *K. marxianus* enhanced the growth and reducing sugar utilization of *S. passalidarum*.
- The DNS method may not be sufficient, as it contains errors and cannot detect all sugars or ethanol concentrations in hydrolysates, making HPLC analysis necessary.