

Biotechnology for Biofuels

Additional file 1:

From mannan to bioethanol: cell surface co-display of β -mannanase and β -mannosidase on yeast *Saccharomyces cerevisiae*

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From mannan to bioethanol: cell surface co-displays of β -mannanase and β -mannosidase on yeast *Saccharomyces cerevisiae*

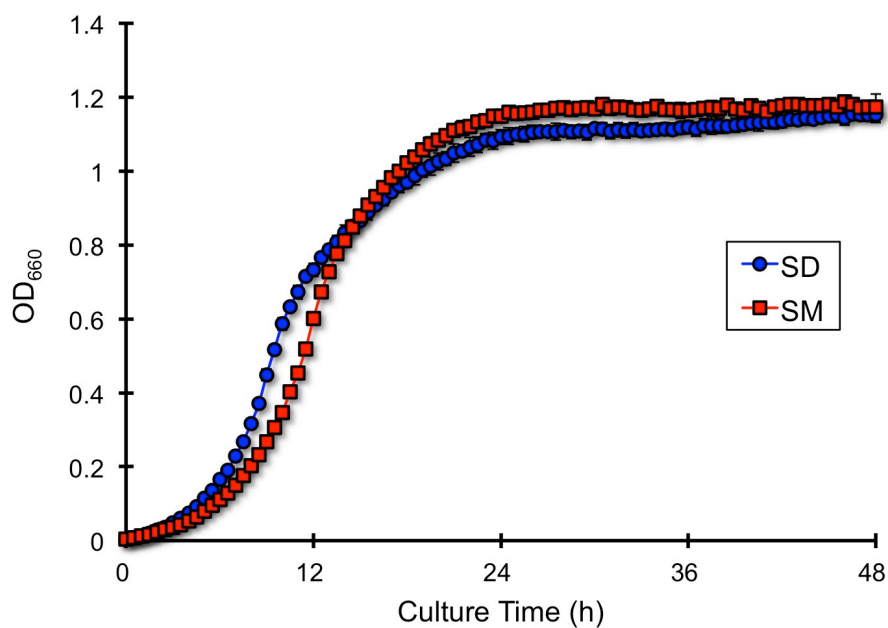


Figure S1. Growth curves of YPH499 yeast strain in SD and SM media. YPH499 yeast cells were inoculated at an OD₆₆₀ of 0.05 and cultured in SD and SM minimal media containing 2 g/L of glucose and mannose, respectively. The cell growth was determined by monitoring OD₆₆₀ every 30 min using a TVS062CA biophotorecorder. Data are presented as the mean \pm standard deviation of separate cultivations ($n = 3$ each).

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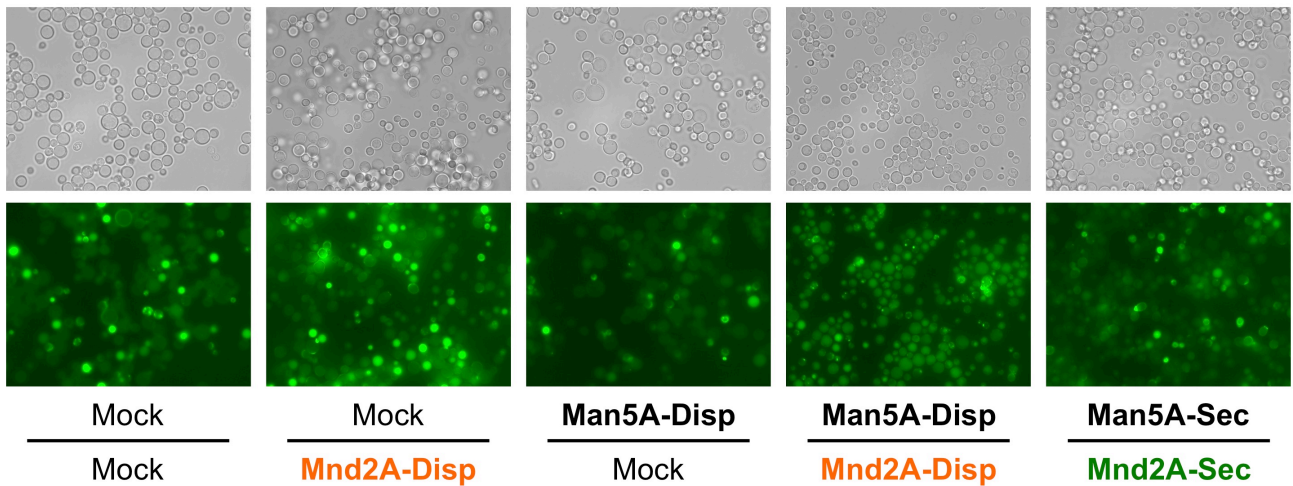


Figure S2. Fluorescence images of immunostained Mnd2A-displaying and -secreting yeast cells. Yeast cells immunostained with Alexa Fluor 488-labeled anti-HA antibody. Upper panels show visible light images and lower panels show green fluorescence images. Images correspond to high magnification ($\times 1,000$).

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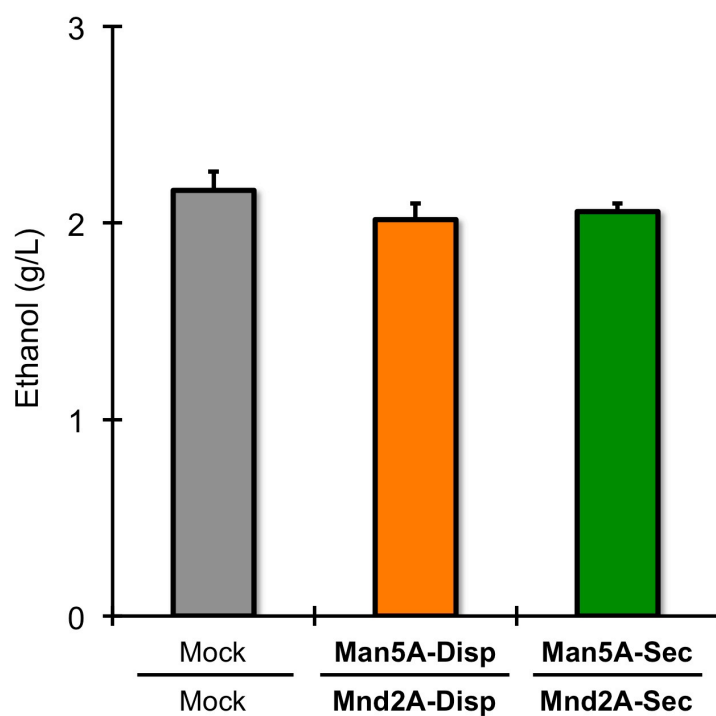


Figure S3. Ethanol fermentations by yeast cells using mannose as a carbon source. Fermentations were performed in YPA medium (pH 5.0) containing 5 g/L mannose as a sole carbon source. Fermentations were started with an $OD_{600}=20$ of yeast cells. The ethanol titers show the values at 18 h of fermentations. Data are presented as the mean \pm standard deviation of separate cultivations ($n = 3$ each).

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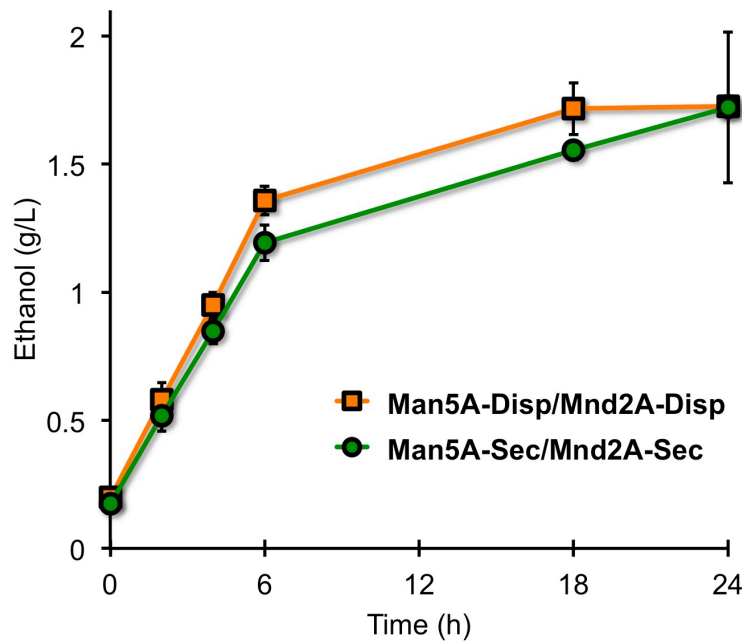


Figure S4. Ethanol fermentation by Man5A- and Mnd2A-displaying and -secreting yeast cells in 1,4- β -D-mannan-containing medium supplemented with purified enzymes. Fermentation was performed in YPA medium (pH 5.0) containing 5 g/L 1,4- β -D-mannan as a carbon source, and 500 U β -mannanase and 8 U β -mannosidase as purified enzymes. Cultures were initiated with an $OD_{600}=20$ of yeast cells. Data are presented as the mean \pm standard deviation of separate cultivations ($n = 3$ each).

Table S1. List of primers

Construction of pFGK424

Amplification of the coding sequences for secretion signal sequence of α -factor and FLO428 anchor

5'–GGGGGCTAGCATGAGATTTCTTCAATTTT

5'–GGGGGGATCCTTAAATAATTGCCAGCAAT

Construction of pFGK426-AaMan5A

Amplification of man5A-FLAG (w/o TAA) gene

5'–TAAAAGAGACGTCGACCTTCCCCGGACGCCGAACCACAAC

5'–TTAAGCATGCGTCGACCTTGTCATCGTCATCCTTGTCGCTTCGACTGCG
CATTGATGGCCGCCAC

Construction of pFGK426-AaMan5A-TAA

Amplification of man5A-FLAG (w/ TAA) gene

5'–TAAAAGAGACGTCGACCTTCCCCGGACGCCGAACCACAAC

5'–TTAAGCATGCGTCGACTTACTTGTCATCGTCATCCTTGTCGCTTCGACT
GCGCATTGATGGCCGCCAC

Construction of pFGK424-AaMnd2A

Amplification of mnd2A-HA (w/o TAA) gene

5'–TAAAAGAGACGTCGACCAATATGTTTCGTGACCTGGGTACTGAAC

5'–TTAAGCATGCGTCGACAGCGTAATCTGGAACATCGTATGGGTATTCTCCCT
CATTCAGATCCCACAG

Construction of pFGK424-AaMnd2A-TAA

Amplification of mnd2A-HA (w/ TAA) gene

5'–TAAAAGAGACGTCGACCAATATGTTTCGTGACCTGGGTACTGAAC

5'–TTAAGCATGCGTCGACTTAAGCGTAATCTGGAACATCGTATGGGTATTCTC
CCTCATTCAGATCCCACAG

*Underlining indicates restriction enzyme sites.

**Undulating lines indicate the coding sequences for FLAG tag or HA tag.