

Pectinases Secretion by *Saccharomyces cerevisiae*: Optimization in Solid-State Fermentation and Identification by a Shotgun Proteomics Approach

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Table S1. ANOVA for the influence of the tested variables on the secretion of total pectinolytic activity (U mL⁻¹), by *S. cerevisiae* strain, according to the factorial design.

| | square sum | degrees of freedom | quadratic mean | F |
|-------------|------------|--------------------|----------------|-------------|
| regression | 7.21 | 7 | 1.03 | 28.47 |
| residue | 0.43 | 12 | 0.04 | |
| lack of fit | 0.39 | 9 | | tabulated F |
| pure error | 0.05 | 3 | | 5% = 2.91 |
| total | 7.65 | 19 | | |

$r^2 = 0.94$.

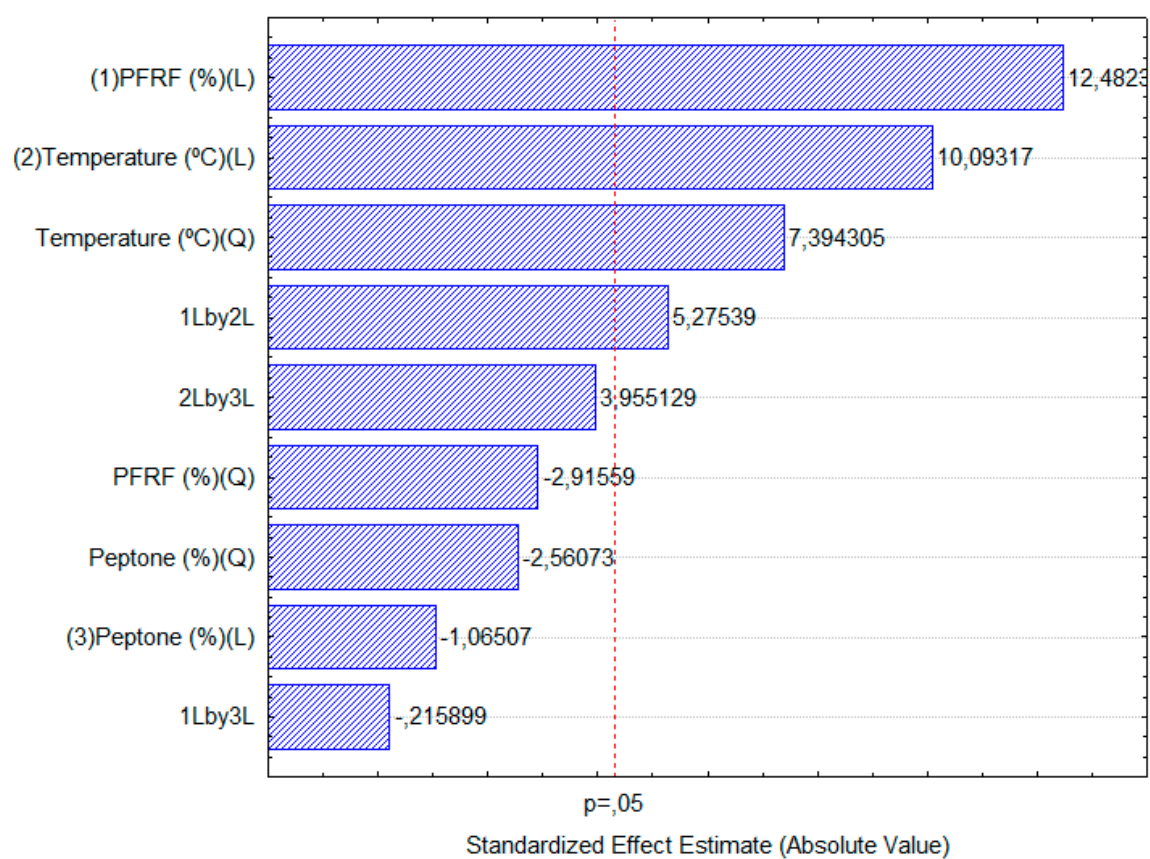


Figure S1. Pareto chart for the influence of the tested variables on the secretion of total pectinolytic activity (U mL^{-1}) by *S. cerevisiae* strain, according to CCRD - 1.

Table S2. ANOVA for the influence of the tested variables on the secretion of total pectinolytic activity (U mL⁻¹) by *S. cerevisiae* strain, according to CCRD - 1.

| | square sum | degrees of freedom | quadratic mean | F |
|-------------|-------------------|---------------------------|-----------------------|-------------|
| regression | 36.38 | 4 | 9.09 | 5.73 |
| residue | 19.03 | 12 | 1.59 | |
| lack of fit | 18.83 | 10 | | tabulated F |
| pure error | 0.20 | 2 | | 5% = 3.26 |
| total | 55.41 | 16 | | |

$r^2 = 0.71$

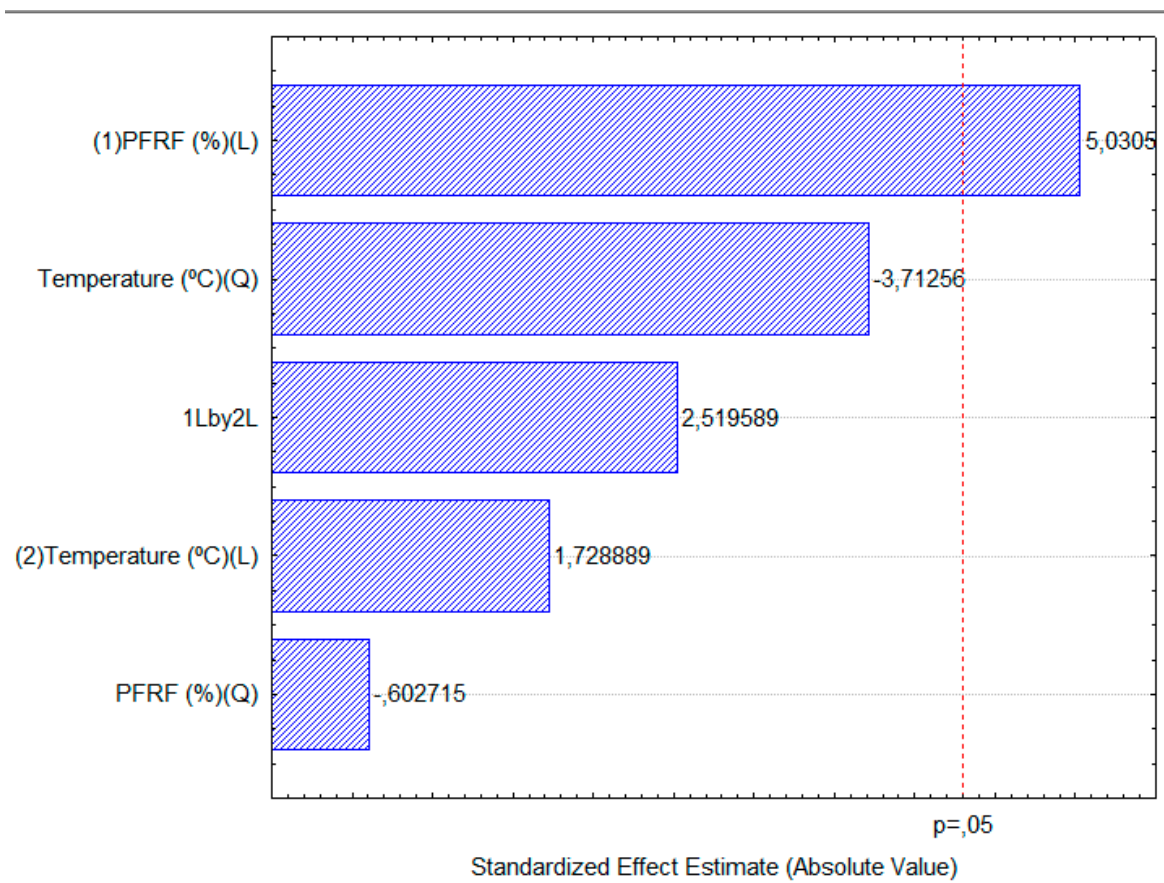


Figure S2. Pareto chart for the influence of the tested variables on the secretion of total pectinolytic activity (U mL^{-1}) by *S. cerevisiae* strain, according to CCRD - 2.

Table S3. ANOVA for the influence of the tested variables on the secretion of total pectinolytic activity (U mL⁻¹) by *S. cerevisiae* strain, according to CCRD - 2.

| | square sum | degrees of freedom | quadratic mean | F |
|-------------|-------------------|---------------------------|-----------------------|-------------|
| regression | 38.14 | 5 | 7.63 | 6.80 |
| residue | 5.61 | 5 | 1.12 | |
| lack of fit | 4.04 | 3 | | tabulated F |
| pure error | 1.57 | 2 | | 5% = 5,05 |
| total | 43.75 | 10 | | |

$r^2 = 0.87$

Table S4. Matrix for CCRD - 2, total pectinolytic activity response and expected results by the model descriptive equation, after 24 h of fermentation by *S. cerevisiae* strain.

| Assay | PFRM (%) | Temperature (°C) | U mL ⁻¹ (Observed) | U mL ⁻¹ (Expected) |
|-------|----------|------------------|-------------------------------|-------------------------------|
| 1 | 12 | 15.5 | 0.86 | 0.97 |
| 2 | 12 | 32.5 | 0.80 | 0.00 |
| 3 | 52 | 15.5 | 0.86 | 1.90 |
| 4 | 52 | 32.5 | 5.26 | 5.22 |
| 5 | 4 | 24 | 0.31 | 0.94 |
| 6 | 60 | 24 | 6.06 | 5.36 |
| 7 | 32 | 12 | 0.86 | 0.07 |
| 8 | 32 | 36 | 0.86 | 1.60 |
| 9 | 32 | 24 | 4.42 | 3.59 |
| 10 | 32 | 24 | 3.70 | 3.59 |
| 11 | 32 | 24 | 2.66 | 3.59 |

Table S5. Matched proteins from *S. cerevisiae* database.

| Accession | Description |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| P22943 | 12 kDa heat shock protein OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=HSP12 PE=1 SV=1 |
| P00358 | Glyceraldehyde-3-phosphate dehydrogenase 2 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=TDH2 PE=1 SV=3 |
| Q01855 | 40S ribosomal protein S15 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPS15 PE=1 SV=1 |
| P53334 | Probable family 17 glucosidase SCW4 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=SCW4 PE=1 SV=1 |
| P46955 | Beta-glucosidase-like protein NCA3, mitochondrial OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=NCA3 PE=3 SV=1 |
| P38248 | Cell wall protein ECM33 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=ECM33 PE=1 SV=3 |
| P02994 | Elongation factor 1-alpha OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=TEF1 PE=1 SV=1 |
| P04911 | Histone H2A.1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=HTA1 PE=1 SV=2 |
| P47001 | Cell wall mannoprotein CIS3 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=CIS3 PE=1 SV=1 |
| P60010 | Actin OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=ACT1 PE=1 SV=1 |
| Q08969 | Protein GRE1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=GRE1 PE=1 SV=1 |
| Q12355 | Cell wall mannoprotein PST1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=PST1 PE=1 SV=1 |
| P05759 | Ubiquitin-40S ribosomal protein S31 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPS31 PE=1 SV=3 |
| P36135 | Probable secreted beta-glucosidase UTH1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=UTH1 PE=1 SV=2 |
| P05737 | 60S ribosomal protein L7-A OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPL7A PE=1 SV=3 |
| P0CX46 | 60S ribosomal protein L2-B OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPL2B PE=1 SV=1 |
| P53301 | Probable glycosidase CRH1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=CRH1 PE=1 SV=1 |
| P22146 | 1,3-beta-glucanosyltransferase GAS1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=GAS1 PE=1 SV=2 |
| P00044 | Cytochrome c iso-1 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=CYC1 PE=1 SV=2 |
| P00445 | Superoxide dismutase [Cu-Zn] OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=SOD1 PE=1 SV=2 |
| P32340 | Rotenone-insensitive NADH-ubiquinone oxidoreductase, mitochondrial OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=NDI1 PE=1 SV=1 |
| P15703 | Glucan 1,3-beta-glucosidase OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=BGL2 PE=1 SV=1 |
| P07280 | 40S ribosomal protein S19-A OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPS19A PE=1 SV=2 |
| P53629 | Sterol O-acyltransferase 2 OS= <i>Saccharomyces cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=ARE2 PE=1 SV=1 |

| | |
|--------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| P00560 | Phosphoglycerate kinase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=PGK1 PE=1 SV=2 |
| P05743 | 60S ribosomal protein L26-A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPL26A PE=1 SV=3 |
| Q12692 | Histone H2A.Z OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=HTZ1 PE=1 SV=3 |
| P04037 | Cytochrome c oxidase subunit 4, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=COX4 PE=1 SV=1 |
| P0CX83 | 60S ribosomal protein L19-B OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPL19B PE=1 SV=1 |
| P35997 | 40S ribosomal protein S27-A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPS27A PE=1 SV=1 |
| P05750 | 40S ribosomal protein S3 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPS3 PE=1 SV=5 |
| P40482 | Protein transport protein SEC24 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=SEC24 PE=1 SV=1 |
| P28319 | Cell wall protein CWP1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=CWP1 PE=1 SV=2 |
| P40212 | 60S ribosomal protein L13-B OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPL13B PE=1 SV=1 |
| P0CX26 | 60S ribosomal protein L43-B OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPL43B PE=1 SV=1 |
| P0CX30 | 40S ribosomal protein S23-B OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPS23B PE=1 SV=1 |
| P18239 | ADP,ATP carrier protein 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=PET9 PE=1 SV=2 |
| P38616 | Protein YGP1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=YGP1 PE=1 SV=2 |
| P52489 | Pyruvate kinase 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=PYK2 PE=1 SV=1 |
| P23776 | Glucan 1,3-beta-glucosidase I/II OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=EXG1 PE=1 SV=1 |
| P40472 | Probable secreted beta-glucosidase SIM1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=SIM1 PE=1 SV=2 |
| P32836 | GTP-binding nuclear protein GSP2/CNR2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=GSP2 PE=1 SV=1 |
| P40442 | Putative uncharacterized protein YIL169C OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=YIL169C PE=3 SV=1 |
| P00761 SWISS-PROT:P00761 | Trypsin - Sus scrofa (Pig). |
| O76013 | SWISS-PROT:O76013 Keratin, type I cuticular HA6 (Hair keratin, type I HA6) |
| P28496 | Sphingosine N-acyltransferase LAC1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=LAC1 PE=1 SV=2 |
| P0CX53 | 60S ribosomal protein L12-A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=RPL12A PE=1 SV=1 |
| P39998 | Enhancer of mRNA-decapping protein 3 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=EDC3 PE=1 SV=1 |

Table S6. Matched proteins from pectinase database.

| Accession | Description |
|------------|------------------------------------------------------------------------------------------------------------------|
| A0A0G4N991 | Uncharacterized protein OS=Verticillium longisporum OX=100787 GN=BN1708_008936 PE=4 SV=1 |
| A0A1Y2U259 | Glycoside hydrolase family 55 protein OS=Hypoxylon sp. EC38 OX=1001937 GN=K449DRAFT_410365 PE=4 SV=1 |
| A0A370JPG0 | Uncharacterized protein OS=Venturia inaequalis OX=5025 GN=Vi05172_g1685 PE=3 SV=1 |
| A0A395GPQ8 | Pectin lyase-like protein (Fragment) OS=Aspergillus ibericus CBS 121593 OX=1448316 GN=BO80DRAFT_321211 PE=4 SV=1 |
| A0A0L1JA42 | Pectin lyase F OS=Aspergillus nomius NRRL 13137 OX=1509407 GN=ANOM_002499 PE=3 SV=1 |
| A0A1V6V1X2 | Uncharacterized protein OS=Penicillium coprophilum OX=36646 GN=PENCOP_c002G06905 PE=4 SV=1 |
| A0A1Y2DZG1 | Uncharacterized protein OS=Neocallimastix californiae OX=1754190 GN=LY90DRAFT_668057 PE=4 SV=1 |
| A0A367IZ94 | Uncharacterized protein (Fragment) OS=Rhizopus stolonifer OX=4846 GN=CU098_001619 PE=3 SV=1 |
| X8JKF7 | Pectate lyase OS=Rhizoctonia solani AG-3 Rhs1AP OX=1086054 GN=RSOL_437720 PE=4 SV=1 |
| A0A2J6QBA4 | Glycoside hydrolase family 55 protein OS=Pezoloma ericae OX=1745343 GN=NA56DRAFT_643654 PE=4 SV=1 |
| A0A167AVW5 | Pectate lyase OS=Colletotrichum incanum OX=1573173 GN=CI238_10558 PE=4 SV=1 |