Article

Characterization of yeasts isolated from Parmigiano Reggiano cheese natural whey starter: from spoilage agents to potential cell factories for whey valorization

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**Abstract:** Whey is the main by-product of the dairy industry and contains sugars (lactose) and proteins (especially serum proteins and, at lesser extent, residual caseins), which can be valorized by the fermentative action of yeasts. In the present study, we characterized the spoilage yeast fraction inhabiting natural whey starter (NWS), the undefined starter culture of thermophilic lactic acid bacteria used in Parmigiano Reggiano (PR) cheesemaking, and evaluated thermotolerance, mating type, and the aptitude to produce ethanol and bioactive peptides from whey lactose and proteins, respectively, in a selected pool of strains. We found that PR NWS yeast population consists of other species (*Saccharomyces cerevisiae*, *Wickerhamiella pararugosa*, and *Torulaspora delbrueckii*) in addition to the well-documented *Kluyveromyces marxianus*, with multiple biotypes scored within each species. Haploid and diploid *K. marxianus* strains were identified through *MAT* genotyping, while thermotolerance assay allowed the selection of strains suitable to grow up to 48 °C. In whey fermentation assay, one thermotolerant strain was suitable to release ethanol with yield of 86.5%, while another candidate was able to produce the highest amounts of both ethanol and bioactive peptides with potentially anti-hypertensive function. The present work demonstrated that PR NWS is a reservoir of ethanol and bioactive peptides producer yeasts, which can be exploited to valorize whey, in agreement with the principles of circularity and sustainability.

**Keywords:** Parmigiano Reggiano cheese; whey; natural whey starter; ethanol; bioactive peptide; yeasts;*Kluyveromyces marxianus*; *Wickerhamiella pararugosa*; *Torulaspora delbrueckii.*

**Supplementary Data**



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**Figure S1.** Multiple sequence alignment of 26S rRNA D1/D2 domain nucleotide sequences of *W. pararugosa* LA118 and LA206 with reference strain CBS1010T. Nucleotide sequences were aligned using Muscle program [1] in MEGA X software [2] and the resulting alignment was visualized using JalView v2.11 [3].

**Table S1**. Kinetic growth parameters of selected 12 *K. marxianus* strains, 2 *W. pararugosa* and 1 *T. delbrueckii* strain. Growth data obtained by gravimetric assays in whey were fitted with different mathematical models in Grofit [4] to calculate growth rate µ (h-1), growth efficiency A (maximum % of CO2 released), and lag phase (h). When available, for each strains *MAT* genotype and temperature score are reported. Data are represented as mean (n=3) ± standard deviation. Abbreviations: T, temperature; Km, *K. marxianus*; Wp, *W. pararugosa*; Td, *T. delbrueckii*; nd, not detected.

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| --- | --- | --- | --- | --- |
| **Species** | **Strains** | **Mating type** | **T score** | **Growth parameters** |
| **Model** | **µ** | **A** | **λ** |
| Km | CA104 | *MATa*/*MATα* | 13 | Richards | 0.0206 ±0.0009 | 2.3614±0.0460 | 25.1589±1.7176 |
|  | CA105 | *MATα* | 13 | Richards | 0.0192±0.0009 | 2.1657±0.3203 | 67.7658±3.6328 |
|  | CA111 | *MATa*/*MATα* | 13 | Gompertz | 0.0233±0.0047 | 2.6124±0.2264 | 15.10322.3275 |
|  | CA116 | *MATa*/*MATα* | 15 | Richards | 0.0170±0.0002 | 1.9749±0.6838 | 69.2531±7.3062 |
|  | CA204 | *MATa*/*MATα* | 16 | Richards | 0.0197±0.0009 | 2.0128±0.4480 | 31.8511±2.2288 |
|  | CA207 | *MATa*/*MATα* | 9 | logistics | 0.0230±0.0004 | 1.7240±0.1685 | 45.4162±10.7960 |
|  | CA213 | *MATa*/*MATα* | 12 | Richards | 0.0241±0.0006 | 2.3682±0.3023 | 30.8106±9.0832 |
|  | CA214 | *MATa*/*MATα* | 12 | logistics | 0.0222±0.0018 | 2.0853±0.4113 | 35.1206±12.0657 |
|  | LA102 | *MATα* | 12 | Gompertz | 0.0241±0.0011 | 2.2359±0.3692 | 19.0651±2.5031 |
|  | LA202 | *MATα* | 15 | logistics | 0.0219±0.0019 | 2.2831±0.0103 | 78.8519±11.8921 |
|  | LA210 | *MATα* | 12 | Gompertz | 0.0218±0.0008 | 2.5022±0.3536 | 21.2292±6.1693 |
|  | RO101 | *MATa* | 12 | Richards | 0.0215±0.0022 | 2.0656±0.2168 | 43.9611±1.8880 |
| Wp | LA118 | nd | nd | Gompertz | 0.0077±0.0000 | 0.1493±0.0000 | 3.434038±0.0000 |
|  | LA218 | nd | nd | modified Gompertz | 0.007±0.00004 | 0.4814±0.0000 | 10.67506±0.0000 |
| Td | RO204-3 | nd | nd | Gompertz | 0.0040±0.0021 | 2.8294±0.3888 | 326.5984±10.8513 |

**Table S2.** β-casein-derived peptides identified after whey fermentation by selected yeast strains. Details on strain isolation are in Table 2.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Peptide sequence | Fragment | *m/z* observed | *m/z* expected | ppm | Charge |   | CA105 | CA116 | CA214 | LA202 | RO101 | RO204-3 |
| PGEIVE | 9-14 | 643.3300 | 643.3297 | -0.5 | 1 |   |   |   | X |   | X |   |
| SLSssEESITR | 15-25 | 678.2598 | 678.2600 | 0.3 | 2 |   |   |   | X | X |   |   |
| SLsssEESITR | 15-25 | 718.2449 | 718.2432 | -2.4 | 2 |   | X | X | X | X | X |   |
| sLsssEESITR | 15-25 | 758.2262 | 758.2263 | 0.1 | 2 |   |   |   | X | X | X |   |
| SLSssEESITRINK | 15-28 | 570.9159 | 570.9164 | 0.9 | 3 |   |   |   | X |   | X |   |
| SLsssEESITRINK | 15-28 | 895.8542 | 895.8542 | 0.0 | 2 and 3 |   | X | X | X |   | X | X |
| sLsssEESITRINK | 15-28 | 935.8392 | 935.8373 | -2.0 | 2 |   | X | X | X | X | X | X |
| SLsssEESITRINKK | 15-29 | 640.2699 | 640.2702 | 0.5 | 3 |   |   |   |   |   | X |   |
| sLsssEESITRINKK | 15-29 | 999.8848 | 999.8848 | 0.0 | 2 |   |   |   |   |   |   | X |
| sssEESITRINK | 17-28 | 795.7975 | 795.7961 | -1.8 | 2 |   |   |   |   |   | X |   |
| KKIEKFQsE | 28-36 | 608.8021 | 608.8023 | 0.3 | 2 |   |   | X |   |   | X |   |
| KIEKFQsEE | 29-37 | 609.2757 | 609.2761 | 0.7 | 2 |   |   |   |   |   | X |   |
| DELQ | 43-46 | 504.2277 | 504.2300 | 4.6 | 1 |   |   |   |   |   | X |   |
| ELQ | 44-46 | 389.2014 | 389.2031 | 4.4 | 1 |   |   |   |   |   | X |   |
| AQTQSLVYPFPGPIPN | 53-68 | 864.9530 | 864.9514 | -1.8 | 1 |   | X |   |   |   |   |   |
| SLVYPFPGP | 57-65 | 976.5151 | 976.5138 | -1.3 | 1 and 2 |   |   |   | X |   | X |   |
| SLVYPFPGPI | 57-66 | 545.3047 | 545.3026 | -3.9 | 2 |   | X |   |   |   |   |   |
| SLVYPFPGPIPN | 57-68 | 1300.6944 | 1300.6936 | -0.6 | 1 |   | X |   |   |   |   |   |
| VYPFPGPIPN | 59-68 | 1100.5787 | 1100.5775 | -1.1 | 1 |   | X | X |   | X | X |   |
| SLPQ | 69-72 | 444.2446 | 444.2453 | 1.6 | 1 |   |   |   | X |   |   |   |
| NIPPLTQTPVVVPP | 73-86 | 736.4298 | 736.4296 | -0.3 | 2 |   |   |   |   |   | X |   |
| NIPPLTQTPVVVPPF | 73-87 | 809.9645 | 809.9638 | -0.9 | 2 |   | X |   |   |   |   |   |
| IPPL | 74-77 | 439.2925 | 439.2915 | -2.3 | 1 |   | X |   |   |   |   |   |
| PPL | 75-77 | 326.2076 | 326.2074 | -0.6 | 1 |   |   | X |   |   |   |   |
| PPLTQTPV | 75-82 | 852.4809 | 852.4825 | 1.9 | 1 |   |   |   |   | X |   |   |
| PPLTQTPVVVPP | 75-86 | 622.8670 | 622.8661 | -1.4 | 2 |   |   |   | X |   | X |   |
| PPLTQTPVVVPPF | 75-87 | 696.4004 | 696.4003 | -0.1 | 2 |   | X |   |   |   |   |   |
| PVVVPP | 81-86 | 607.3817 | 607.3814 | -0.5 | 1 |   | X | X | X |   | X |   |
| PVVVPPF | 81-87 | 754.4492 | 754.4498 | 0.8 | 1 and 2 |   | X | X | X | X | X | X |
| PVVVPPFLQP | 81-90 | 1092.6431 | 1092.6452 | 1.9 | 1 and 2 |   | X |   |   |   |   |   |
| VVPP | 83-86 | 411.2597 | 411.2602 | 1.2 | 1 |   |   |   | X |   |   |   |
| VMGVSK | 92-97 | 310.6752 | 310.6754 | 0.6 | 2 |   |   |   | X |   | X | X |
| VSKVKEA | 95-101 | 380.7317 | 380.7318 | 0.3 | 2 |   |   |   |   | X |   |   |
| EAMAPK | 100-105 | 646.3225 | 646.3229 | 0.6 | 1 |   |   |   | X |   |   |   |
| AMAPK | 101-105 | 517.2801 | 517.2803 | 0.4 | 1 |   |   |   | X | X | X |   |
| APKHKEMPFPKYPVEPFT | 103-120 | 715.0393 | 715.0397 | 0.6 | 4 and 3 |   |   |   |   |   |   | X |
| LTD | 127-129 | 348.1761 | 348.1765 | 1.1 | 1 |   | X |   | X |   | X |   |
| TDV | 128-130 | 334.1603 | 334.1609 | 1.8 | 1 |   |   |   | X |   |   |   |
| DVENLHLPLP | 129-138 | 573.8141 | 573.8113 | -4.9 | 2 |   | X | X | X |   | X |   |
| VENLHLPLP | 130-138 | 516.2982 | 516.2978 | -0.8 | 2 |   | X | X | X |   | X |   |
| NLHLPLPLLQS | 132-142 | 622.8748 | 622.8717 | -5.0 | 2 |   | X |   |   |   |   |   |
| LHLPLP | 133-138 | 345.2207 | 345.2209 | 0.6 | 2 |   | X | X | X | X | X |   |
| LHLPLPLLQ | 133-141 | 522.3356 | 522.3342 | -2.7 | 2 |   | X |   |   |   |   |   |
| LHLPLPLLQS | 133-142 | 565.8508 | 565.8502 | -1.1 | 2 |   | X |   |   |   |   |   |
| MHQPHQPLPPT | 144-154 | 641.8222 | 641.8217 | -0.8 | 2 and 3 |   | X | X | X | X | X |   |
| MHQPHQPLPPTVMFPP | 144-159 | 618.6448 | 618.6445 | -0.5 | 3 |   |   |   | X |   | X |   |
| MHQPHQPLPPTVmFPP | 144-159 | 623.9769 | 623.9761 | -1.3 | 3 |   |   | X |   |   |   |   |
| HQPHQPLPPT | 145-154 | 576.3020 | 576.3014 | -1.0 | 2 |   | X | X |   |   |   |   |
| HQPHQPLPPTVMFPP | 145-159 | 574.9644 | 574.9643 | -0.2 | 3 |   |   |   |   | X |   |   |
| LPPT | 151-154 | 427.2534 | 427.2551 | 4.0 | 1 |   |   |   |   |   |   | X |
| TVm | 154-156 | 366.1694 | 366.1693 | -0.3 | 1 |   | X |   |   |   |   |   |
| SLSQSKVLPVP | 164-174 | 577.8436 | 577.8426 | -1.7 | 2 |   |   |   | X |   | X |   |
| SLSQSKVLPVPQ | 164-175 | 641.8722 | 641.8719 | -0.5 | 2 |   | X | X |   | X | X | X |
| SLSQSKVLPVPQKAVPYPQ | 164-182 | 689.3940 | 689.3930 | -1.5 | 3 |   |   | X |   |   |   | X |
| SLSQSKVLPVPQKAVPYPQR | 164-183 | 556.3224 | 556.3218 | -1.1 | 4 |   | X | X |   | X |   | X |
| SLSQSKVLPVPQKAVPYPQRDMPI | 164-187 | 670.3743 | 670.3729 | -2.1 | 4 |   |   | X |   |   |   |   |
| SLSQSKVLPVPQKAVPYPQRDMPIQ | 164-188 | 936.1814 | 936.1810 | -0.4 | 3 |   |   |   |   |   |   | X |
| SQSKVLPVP | 166-174 | 954.5622 | 954.5619 | -0.3 | 1 |   |   |   | X |   | X |   |
| SQSKVLPVPQ | 166-175 | 541.8137 | 541.8139 | 0.4 | 2 |   | X | X | X | X | X | X |
| SQSKVLPVPQK | 166-176 | 605.8611 | 605.8613 | 0.3 | 2 |   | X |   | X | X | X |   |
| SQSKVLPVPQKAVPYPQ | 166-182 | 622.6869 | 622.6876 | 1.1 | 2 and 3 |   |   |   |   |   |   | X |
| SQSKVLPVPQKAVPYPQR | 166-183 | 506.2928 | 506.2928 | 0.0 | 3 and 4 |   | X | X |   | X |   |   |
| SQSKVLPVPQKAVPYPQRDMP | 166-186 | 592.0749 | 592.0729 | -3.4 | 4 |   |   |   |   |   | X |   |
| SKVLPVP | 168-174 | 739.4700 | 739.4713 | 1.8 | 1 |   |   |   | X |   |   |   |
| SKVLPVPQ | 168-175 | 867.5309 | 867.5298 | -1.3 | 1 |   |   |   | X |   | X |   |
| SKVLPVPQK | 168-176 | 498.3161 | 498.3160 | -0.2 | 2 |   |   |   |   |   | X |   |
| KVLPVP | 169-174 | 652.4377 | 652.4392 | 2.3 | 1 and 2 |   |   |   | X |   | X |   |
| KVLPVPQ | 169-175 | 780.4978 | 780.4978 | 0.0 | 1 |   | X |   | X |   | X |   |
| KAVPYP | 176-181 | 674.3879 | 674.3872 | -1.0 | 1 |   | X |   |   |   |   |   |
| KAVPYPQ | 176-182 | 802.4453 | 802.4458 | 0.6 | 1 |   | X | X | X | X | X |   |
| KAVPYPQR | 176-183 | 479.7782 | 479.7771 | -2.3 | 2 |   | X | X | X | X | X |   |
| LYQEPVLGPVRGPFP | 192-206 | 834.9592 | 834.9590 | -0.2 | 2 |   |   |   |   |   | X |   |
| YQEPVLGPVR | 193-202 | 579.3149 | 579.3139 | -1.7 | 2 |   | X |   | X | X | X |   |
| YQEPVLGPVRGPF | 193-205 | 486.9296 | 486.9295 | -0.2 | 2 |   | X |   | X |   | X |   |
| YQEPVLGPVRGPFP | 193-206 | 778.4170 | 778.4170 | 0.0 | 2 |   | X | X |   | X | X |   |
| GPVRGPFP | 199-206 | 413.7315 | 413.7321 | 1.5 | 2 |   | X | X | X | X | X |   |
| GPVRGPFPI | 199-207 | 470.2746 | 470.2742 | -0.9 | 2 |   |   | X |   |   | X |   |
| GPVRGPFPII | 199-208 | 526.8174 | 526.8162 | -2.3 | 2 |   |   |   |   |   | X | X |
| IIV | 207-209 | 344.2546 | 344.2544 | -0.6 | 1 |   | X | X | X | X | X |   |

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| **Table S3**. αS1-casein-derived peptides identified after whey fermentation by selected yeast strains. Details on strain isolation are in Table 2.  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Peptide sequence** | **Fragment** | ***m/z* observed** | ***m/z* expected** | **ppm** | **Charge** |  | **CA105** | **CA116** | **CA214** | **LA202** | **RO101** | **RO204-3** |
| RPKHPIKH | 1-8 | 338.2100 | 338.2103 | 0.9 | 3 |   |  |   | **X** |  |  |  |
| RPKHPIKHQ | 1-9 | 380.8963 | 380.8965 | 0.5 | 3 |   | **X** |   | **X** | **X** |  | **X** |
| RPKHPIKHQGLPQEV | 1-15 | 441.7562 | 441.7561 | -0.2 | 4 |   |  | **X** |   |   |  |  |
| RPKHPIKHQGLPQEVL | 1-16 | 470.0269 | 470.0272 | 0.6 | 4 and 5 |   |  |  |   |   |  | **X** |
| LLR | 20-22 | 401.2866 | 401.2871 | 1.2 | 1 |   |  |  | **X** | **X** | **X** |  |
| LSKDIGsEsTEDQ | 40-52 | 784.7913 | 784.7919 | 0.8 | 2 |   |  |  |  |  | **X** |  |
| SKDIGsEsTE | 41-50 | 606.7067 | 606.7071 | 0.7 | 2 |   |  |  | **X** | **X** |  |  |
| sKDIGsEsTE | 41-50 | 646.6900 | 646.6902 | 0.3 | 2 |   |  |  |  | **X** |  |  |
| SKDIGsEsTED | 41-51 | 664.2193 | 664.2206 | 2.0 | 2 |   |  |  | **X** |  | **X** |  |
| SKDIGsEStED | 41-51 | 664.2195 | 664.2206 | 1.7 | 2 |   |  |  |  |  |  |  |
| SKDIGsEsTEDQ | 41-52 | 728.2496 | 728.2498 | 0.3 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| sKDIGsEsTEDQ | 41-52 | 768.2322 | 768.2330 | 1.0 | 2 |   |  |  |  |  |  |  |
| SKDIGsEsTEDQA | 41-53 | 763.7695 | 763.7684 | -1.4 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| IGsEsTEDQ | 44-52 | 563.1722 | 563.1729 | 1.2 | 2 |   |  |  |  | **X** |  |  |
| DQAMEDIKQ | 51-59 | 539.2471 | 539.2477 | 1.1 | 2 |   |  |  |  |  | **X** |  |
| DIK | 56-58 | 375.2233 | 375.2238 | 1.3 | 1 |   |  |  |  |  | **X** | **X** |
| sVEQKHIQ | 75-82 | 524.7439 | 524.7448 | 1.7 | 2 |   | **X** |  | **X** |  | **X** |  |
| sVEQKHIQK | 75-83 | 588.7920 | 588.7923 | 0.5 | 2 |   |  | **X** |  |  |  |  |
| HIQ | 80-82 | 397.2186 | 397.2194 | 2.0 | 1 |   |  |  | **X** |  | **X** | **X** |
| LRLKKYKVPQ | 99-108 | 424.9426 | 424.9432 | 1.4 | 3 |   | **X** | **X** |  | **X** |  | **X** |
| LRLKKYKVPQL | 99-109 | 347.2304 | 347.2302 | -0.6 | 4 |   |  | **X** |  | **X** |  | **X** |
| LRLKKYKVPQLEIVPN | 99-114 | 646.7342 | 646.7348 | 0.9 | 3 and 4 |   |  | **X** |  | **X** |  | **X** |
| LRLKKYKVPQLEIVPNsAEERLH | 99-122 | 568.9167 | 568.9167 | 0.0 | 5 |   |  |  |  |  |  | **X** |
| LRLKKYKVPQLEIVPNsAEERLHSMKEGIHAQ | 99-130 | 637.8435 | 637.8435 | 0.0 | 6 |   |  |  |  |  |  | **X** |
| LRLKKYKVPQLEIVPNsAEERLHSMKEGIHAQQKEPMIGVNQEL | 99-142 | 742.1083 | 742.1086 | 0.4 | 7 |   |  |  |  |  |  | **X** |
| RLKKYKVPQ | 100-108 | 387.2484 | 387.2485 | 0.3 | 2 and 3 |   | **X** | **X** |  | **X** | **X** |  |
| RLKKYKVPQL | 100-109 | 318.9603 | 318.9592 | -3.4 | 4 |   |  |  |  | **X** |  |  |
| KKYKVPQ | 102-108 | 445.7763 | 445.7765 | 0.4 | 2 |   | **X** | **X** | **X** |   | **X** |  |
| KKYKVPQLEIVPN | 102-114 | 389.7360 | 389.7356 | -1.0 | 4 |   |  |  |  |   |  | **X** |
| LEIVPN | 109-114 | 684.3916 | 684.3927 | 1.6 | 1 |   |  |  |   | **X** | **X** | **X** |
| KEGIHAQQK | 124-132 | 519.7874 | 519.7882 | 1.5 | 2 |   | **X** | **X** |   |   | **X** |  |
| sAEER | 115-119 | 336.1229 | 336.1234 | 1.5 | 2 |   |  |  | **X** |   |  |  |
| PMI | 134-136 | 360.1935 | 360.1952 | 4.7 | 1 |   |  |  |  |   |  | **X** |
| PEL | 147-149 | 358.1959 | 358.1973 | 3.9 | 1 |   |  |  |  |   |  | **X** |
| YYV | 165-167 | 444.2135 | 444.2129 | -1.4 | 1 |   |  |  | **X** | **X** |  |  |
| APSFSDIPNPIGSEN | 176-190 | 772.8647 | 772.8650 | 0.4 | 2 |   |  |  | **X** |  |  |  |
| APSFSDIPNPIGSENSEK | 176-193 | 944.9503 | 944.9498 | -0.5 | 2 |   |  |  | **X** |  | **X** |  |
| SFSDIPNPIGSENSE | 178-192 | 796.8569 | 796.8574 | 0.6 | 2 |   |  |  | **X** |  |  |  |
| FSDIPNPIG | 179-187 | 480.2458 | 480.2453 | -1.0 | 2 |   | **X** |   | **X** |  |  | **X** |
| FSDIPNPIGSE | 179-189 | 588.2843 | 588.2826 | -2.9 | 2 |   |  | **X** |   | **X** |  |  |
| FSDIPNPIGSENSE | 179-192 | 753.3406 | 753.3414 | 1.1 | 2 |   | **X** | **X** |   | **X** | **X** | **X** |
| FSDIPNPIGSENSEK | 179-193 | 817.3906 | 817.3888 | -2.2 | 2 |   |  | **X** |   |  |  | **X** |
| SDIPNPIGSENSE | 180-192 | 679.8064 | 679.8072 | 1.2 | 2 |   |  |  |   | **X** |  |  |
| SDIPNPIGSENSEK | 180-193 | 743.8548 | 743.8546 | -0.3 | 2 |   |  |  |   |  |  | **X** |
| IPNPIGSEN | 182-190 | 470.7412 | 470.7404 | -1.7 | 2 |   | **X** |   |   |  |  |  |
| IPNPIGSENSE | 182-192 | 578.7783 | 578.7777 | -1.0 | 2 |   | **X** |   |   | **X** |  |  |

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| **Table S4**. αS2-casein-derived peptides identified after whey fermentation by selected yeast strains. Details on strain isolation are in Table 2.  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Peptide sequence** | **Fragment** | ***m/z* observed** | ***m/z* expected** | **ppm** | **Charge** |  | **CA105** | **CA116** | **CA214** | **LA202** | **RO101** | **RO204-3** |
| SIIsQETYK | 13-21 | 574.7645 | 574.7654 | 1.6 | 2 |  |  |  | **X** |  |  |  |
| AINPsKENL | 27-35 | 533.2531 | 533.2524 | -1.3 | 2 |  |  |  | **X** |  |  |  |
| YSI | 52-54 | 382.1975 | 382.1973 | -0.5 | 1 |  |  |  |  |  |  | **X** |
| YLYQGPIVLNPWDQVKR | 98-114 | 697.0474 | 697.0458 | -2.3 | 3 |  |  | **X** | **X** |  | **X** |  |
| LYQGPIVLNPWDQVKR | 99-114 | 963.5336 | 963.5334 | -0.2 | 2. 3 and 4 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| YQGPIVLNPWDQVK | 100-113 | 828.9404 | 828.9408 | 0.5 | 2 |   | **X** |   |   |   | **X** |  |
| YQGPIVLNPWDQVKR | 100-114 | 906.9917 | 906.9914 | -0.3 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| QGPIVLNPWDQVK | 101-113 | 747.4103 | 747.4092 | -1.5 | 2 |   |  |  |  |  |  |  |
| QGPIVLNPWDQVKR | 101-114 | 550.6424 | 550.6423 | -0.2 | 3 |   |  |  | **X** | **X** |  |  |
| RNAVPITPT | 114-122 | 484.7813 | 484.7798 | -3.1 | 2 |   |  | **X** |  |  |  |  |
| NAVPITPTLNR | 115-125 | 598.3433 | 598.3433 | 0.0 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| NAVPITPTLNRE | 115-126 | 662.8637 | 662.8646 | 1.4 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| NAVPITPtLNREQLSTsEENSKKTVDMEsTEVFTKKTKLTEEEKNRLNF | 115-163 | 842.9743 | 842.9755 | 1.4 | 7 |   |  |  |  |  |  |  |
| AVPITPTLNR | 116-125 | 541.3234 | 541.3218 | -3.0 | 2 |   | **X** |   | **X** | **X** | **X** |  |
| AVPITPTLNRE | 116-126 | 605.8429 | 605.8431 | 0.3 | 2 |   |  |   | **X** |  |  |  |
| PITP | 118-121 | 427.2534 | 427.2551 | 4.0 | 1 |   |  |   |  |  |  | **X** |
| NREQLsTsEE | 124-133 | 676.7419 | 676.7420 | 0.1 | 2 |   |  |   | **X** |  |  |  |
| YLK | 179-181 | 423.2607 | 423.2602 | -1.2 | 1 |   | **X** | **X** | **X** |  | **X** | **X** |
| TKVIPYVR | 198-205 | 325.8712 | 325.8710 | -0.6 | 3 |   | **X** | **X** |  |  |  |  |
| YVRY | 203-206 | 600.3153 | 600.3140 | -2.2 | 1 |   |  |  |  | **X** |  |  |

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| --- |
| **Table S5**. κ-casein-derived peptides identified after whey fermentation by selected yeast strains. Details on strain isolation are in Table 2. |
|  |
| **Peptide sequence** | **Fragment** | ***m/z* observed** | ***m/z* expected** | **ppm** | **Charge** |  | **CA105** | **CA116** | **CA214** | **LA202** | **RO101** | **RO204-3** |
| KYIPIQYVL | 30-38 | 568.8385 | 568.8393 | 1.4 | 2 |  |  |  |   |   |  | **X** |
| YGL | 44-46 | 718.2449 | 718.2432 | -2.4 | 2 |   | **X** |   |   |   | **X** |   |
| PVALINN | 53-59 | 740.4292 | 740.4301 | 1.2 | 1 |   |  |   | **X** |   | **X** |   |
| FLPYPYYAKPA | 61-71 | 665.3473 | 665.3475 | 0.3 | 2 |   |  |   |  |   | **X** |   |
| AVRSPA | 72-77 | 300.6766 | 300.6768 | 0.7 | 2 |   |  |   | **X** |   | **X** |   |
| AVRSPAQ | 72-78 | 364.7059 | 364.7061 | 0.5 | 2 |   |  |   | **X** |   |  |   |
| AVRSPAQI | 72-79 | 421.2485 | 421.2482 | -0.7 | 2 |   | **X** |   | **X** | **X** | **X** |   |
| AVRSPAQIL | 72-80 | 477.7898 | 477.7902 | 0.8 | 2 |   |  |   |  | **X** |   |   |
| SNTVPAK | 86-92 | 358.7003 | 358.7005 | 0.6 | 2 |   |  |   | **X** |  |   |   |
| MAIPPKKNQ | 112-120 | 342.8637 | 342.8637 | 0.0 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| MAIPPKKNQD | 112-121 | 571.3056 | 571.3053 | -0.5 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| MAIPPKKNQDK | 112-122 | 635.3530 | 635.3528 | -0.3 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| AIPPKKNQDK | 113-122 | 569.8315 | 569.8326 | 1.9 | 2 |   |  | **X** |  |   | **X** |  |
| PPKKNQDK | 115-122 | 477.7714 | 477.7720 | 1.3 | 2 |   | **X** | **X** | **X** |   | **X** |  |
| DKTEIPTINT | 121-130 | 566.2986 | 566.2982 | -0.7 | 2 |   | **X** |   | **X** |   | **X** | **X** |
| KTEIPTIN | 122-129 | 915.5132 | 915.5146 | 1.5 | 1 |   |  |   | **X** |   | **X** |   |
| TEI | 123-125 | 362.1917 | 362.1922 | 1.4 | 1 |   |  |   | **X** |   |  |   |
| TEIPTIN | 123-129 | 787.4177 | 787.4196 | 2.4 | 1 |   | **X** |   |   |   | **X** |   |
| TIASGE | 130-135 | 577.2818 | 577.2828 | 1.7 | 1 |   |  |   | **X** |   |   |   |
| TIAsGEPT | 130-137 | 855.3493 | 855.3496 | 0.4 | 1 |   | **X** | **X** |  | **X** | **X** | **X** |
| TIAsGEPTSTPT | 130-141 | 1241.5314 | 1241.5297 | -1.4 | 1 |   |  | **X** |  | **X** | **X** |  |
| TIASGEPTSTPTTE | 130-143 | 1391.6528 | 1391.6536 | 0.6 | 1 |   |  |  |  |  | **X** |  |
| TIAsGEPTSTPTTE | 130-143 | 736.3129 | 736.3136 | 1.0 | 2 |   |  |  |  |  | **X** |  |
| TIASGEPTSTPTTEAVESTVATLEDsPE | 130-157 | 967.4340 | 967.4322 | -1.9 | 3 |   |  |  |  |  | **X** |  |
| GEPTSTPTTE | 134-143 | 1019.4517 | 1019.4528 | 1.1 | 1 |   |  |  | **X** | **X** |  |  |
| STPTTE | 138-143 | 635.2872 | 635.2883 | 1.7 | 1 |   |  |  |  | **X** | **X** |  |
| AVESTVAtL | 144-152 | 970.4490 | 970.4493 | 0.3 | 1 |   |  |   |  |  | **X** |  |
| AVESTVATLEDsP | 144-156 | 699.8041 | 699.8054 | 1.9 | 2 |   |  |   |  |  | **X** |  |
| AVEStVATLEDSPE | 144-157 | 1527.6467 | 1527.6462 | -0.3 | 1 |   |  |   |  |  | **X** |  |
| AVESTVATLEDsPE | 144-157 | 764.3280 | 764.3267 | -1.7 | 2 |   |  |   |  |  | **X** |  |
| AVEStVATLEDsPE | 144-157 | 804.3109 | 804.3099 | -1.2 | 2 |   |  |   |  |  | **X** |  |
| VESTVAtL | 145-152 | 450.2102 | 450.2097 | -1.1 | 2 |   |  |   | **X** |  | **X** |  |
| VESTVATLEDsPE | 145-157 | 728.8079 | 728.8082 | 0.4 | 2 |   |  |   |  |  | **X** |  |
| STVATLEDsP | 147-156 | 550.2322 | 550.2314 | -1.5 | 2 |   |  |   |  |  | **X** |  |
| STVATLEDsPE | 147-157 | 614.7537 | 614.7527 | -1.6 | 3 |   | **X** |   | **X** | **X** | **X** |  |
| STVAtLEDsPE | 147-157 | 654.7380 | 654.7358 | -3.4 | 2 |   | **X** |   | **X** | **X** | **X** |  |
| TVATLEDsPE | 148-157 | 571.2373 | 571.2367 | -1.1 | 2 |   |  |   | **X** |  | **X** |  |
| VATLEDsPE | 149-157 | 1040.4160 | 1040.4184 | 2.3 | 1 and 2 |   |  |   | **X** |  | **X** |  |
| TLEDsPE | 151-157 | 870.3108 | 870.3128 | 2.3 | 1 |   |  |   | **X** |  | **X** |  |
| sPEVIESPP | 155-163 | 1034.4446 | 1034.4442 | -0.4 | 1 |   |  |   |  |  | **X** |  |
| sPEVIESPPEI  | 155-165 | 638.7897 | 638.7891 | -0.9 | 2 |   |  |   |  |  | **X** |  |
| VIESPPEIN | 158-166 | 499.2644 | 499.2637 | -1.4 | 2 |   |  | **X** | **X** | **X** |  |  |
| SPPEIN | 161-166 | 328.6655 | 328.6661 | 1.8 | 2 |   |  |  | **X** |  | **X** |  |
| SPPEINT | 161-167 | 757.3737 | 757.3727 | -1.3 | 1 |   | **X** |   | **X** | **X** |  |  |
| PEI | 163-175 | 358.1959 | 358.1973 | 3.9 | 1 |  |  |  |  |  |  | **X** |

|  |
| --- |
| **Table S6**. α-lactalbumin-derived peptides identified after whey fermentation by selected yeast strains. Details on strain isolation are in Table 2. |
|  |
| **Peptide sequence** | **Fragment** | ***m/z* observed** | ***m/z* expected** | **ppm** | **Charge** |  | **CA105** | **CA116** | **CA214** | **LA202** | **RO101** | **RO204-3** |
| LTK | 7-9 | 361.2440 | 361.2445 | 1.4 | 1 |  |  |  | **X** |  | **X** |  |
| TKCEVFR | 8-14 | 441.7285 | 441.7287 | 0.5 | 2 |  |  |  |  | **X** |  |  |
| RELKDLKGYGGVSLPE | 14-29 | 587.6575 | 587.6562 | -2.2 | 3 and 4  |  |  | **X** |  |  |  | **X** |
| ELKDLKGYGG | 15-24 | 540.2909 | 540.2902 | -1.3 | 2 |  |  |  |  |  | **X** |  |
| ELKDLKGYGGVSLPE | 15-29 | 802.9291 | 802.9301 | 1.2 | 2 and 3 |   | **X** | **X** |  |  |  |  |
| KDLKGY | 17-22 | 362.2047 | 362.2054 | 1.9 | 2 |   | **X** |  |  |  |  |  |
| KDLKGYGGVS | 17-26 | 512.2777 | 512.2771 | -1.2 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| KDLKGYGGVSL | 17-27 | 379.5489 | 379.5485 | -1.1 | 3 |   | **X** |  |  |  |  |  |
| KDLKGYGGVSLP | 17-28 | 411.9002 | 411.8994 | -1.9 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** |  |
| KDLKGYGGVSLPE | 17-29 | 454.9143 | 454.9136 | -1.5 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| DLK | 18-20 | 375.2233 | 375.2238 | 1.3 | 1 |   |  |  |  |  | **X** | **X** |
| DLKGYGG | 18-24 | 355.1796 | 355.1794 | -0.6 | 2 |   | **X** |  |  | **X** | **X** |  |
| DLKGYGGVSLP | 18-28 | 553.3002 | 553.2980 | -4.0 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| DLKGYGGVSLPE | 18-29 | 617.8192 | 617.8193 | 0.2 | 2 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| KGYGGVS | 20-26 | 667.3404 | 667.3410 | 0.9 | 1 |   | **X** |  | **X** |  | **X** |  |
| KGYGGVSLPE | 20-29 | 503.7634 | 503.7639 | 0.9 | 2 |   | **X** | **X** |  | **X** |  |  |
| FHTSGYDT | 35-42 | 464.1959 | 464.1958 | -0.2 | 2 |   | **X** |  |  |  |  |  |
| FHTSGYDTQ | 35-43 | 528.2257 | 528.2251 | -1.1 | 2 |   |  | **X** | **X** | **X** | **X** |  |
| IVQNNDSTEYGL | 45-56 | 676.8218 | 676.8201 | -2.5 | 2 |   |  |  |  |  |  | **X** |
| YGL | 54-56 | 352.1875 | 352.1867 | -2.3 | 1 |   | **X** |  |  |  | **X** |  |
| LDD | 85-87 | 362.1551 | 362.1558 | 1.9 | 1 |   | **X** | **X** |  | **X** |  | **X** |
| LTD | 89-91 | 348.1761 | 348.1765 | 1.1 | 1 |   | **X** |  | **X** |  | **X** |  |
| KKIL | 97-100 | 501.3756 | 501.3759 | 0.6 | 1 |   |  | **X** |  |  |  |  |
| ILD | 99-101 | 360.2131 | 360.2129 | -0.6 | 1 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| LDK | 100-102 | 375.2240 | 375.2238 | -0.5 | 1 |   | **X** |  |  | **X** | **X** | **X** |
| DKVGINY | 101-107 | 808.4184 | 808.4199 | 1.9 | 1 |   |  |  | **X** |  | **X** |  |
| DKVGINYW | 101-108 | 497.7530 | 497.7533 | 0.6 | 2 |   |  |  |  | **X** |  |  |
| KVGINYW | 102-108 | 440.2388 | 440.2398 | 2.3 | 2 |   |  |  | **X** | **X** |  |  |
| VGINYWLAHKA | 103-113 | 424.5687 | 424.5680 | -1.6 | 3 |   |  | **X** |  |  |  |  |
| LAH | 109-111 | 340.1972 | 340.1979 | 2.1 | 1 |   |  |  | **X** | **X** |  |  |
| KLDQ | 118-121 | 503.2803 | 503.2824 | 4.2 | 1 |   |  |  |  |  |  | **X** |

|  |
| --- |
| **Table S7**. β-lactoglobulin-derived peptides identified after whey fermentation by selected yeast strains. Details on strain isolation are in Table 2. |
|  |
| **Peptide sequence** | **Fragment** | ***m/z* observed** | ***m/z* expected** | **ppm** | **Charge** |  | **CA105** | **CA116** | **CA214** | **LA202** | **RO101** | **RO204-3** |
| LIV | 2-4 | 344.2546 | 344.2544 | -0.6 | 1 |   | **X** | **X** | **X** | **X** | **X** |  |
| LIVTQ | 2-6 | 573.3606 | 573.3606 | 0.0 | 1 |   | **X** |  |   |   |   |   |
| LIVTQTMK | 2-9 | 933.5435 | 933.5438 | 0.3 | 1 and 2 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| LIVTQTMKG | 2-10 | 495.7869 | 495.7863 | -1.2 | 2 |   | **X** | **X** | **X** |  |  |  |
| LIVTQTMKGL | 2-11 | 552.3286 | 552.3283 | -0.5 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |
| LIVTQTMKGLD | 2-12 | 609.8426 | 609.8418 | -1.3 | 2 |   | **X** |  | **X** | **X** | **X** |  |
| LIVTQTMKGLDIQ | 2-14 | 730.4143 | 730.4131 | -1.6 | 2 |   |  | **X** |  |  | **X** |  |
| LIVTQTMKGLDIQK | 2-15 | 794.4595 | 794.4606 | 1.4 | 2 and 3 |   |  |  |  |  |  | **X** |
| LIVTQTmKGLDIQKVAGTW | 2-20 | 706.7270 | 706.7258 | -1.7 | 3 |   | **X** | **X** |  |  |  | **X** |
| KGL | 9-11 | 317.2185 | 317.2183 | -0.6 | 1 |   | **X** | **X** | **X** | **X** | **X** |  |
| KGLDIQKVAGTW | 9-20 | 439.2506 | 439.2505 | -0.2 | 3 |   |  | **X** |  |  |  |  |
| LDI | 11-13 | 360.2128 | 360.2129 | 0.3 | 1 |   |  |  |  |  | **X** |  |
| YSL | 21-23 | 382.1975 | 382.1973 | -0.5 | 1 |   |  |  |  |  |  | **X** |
| AMAASDISL | 24-32 | 439.7176 | 439.7180 | 0.9 | 2 |   |  |  |  |  |  | **X** |
| AmAASDISLLDAQSAPLRVY | 24-43 | 703.3625 | 703.3613 | -1.7 | 3 |   |  |  |  |  |  | **X** |
| SDISLLDAQSAPLRV | 28-42 | 792.9347 | 792.9332 | -1.9 | 2 |   |  |  |  |  |  | **X** |
| SDISLLDAQSAPLRVY | 28-43 | 874.4660 | 874.4649 | -1.3 | 2 and 3 |   |  |  |  |  |  | **X** |
| ISLL | 30-33 | 445.3024 | 445.3021 | -0.7 | 1 |   |  |  |  |  | **X** |  |
| ISLLDAQSAPLRV | 30-42 | 691.9055 | 691.9037 | -2.6 | 2 |   |  |  |  |  |  | **X** |
| ISLLDAQSAPLRVY | 30-43 | 773.4354 | 773.4354 | 0.0 | 2 |   |  |  |  |  |  | **X** |
| LLDAQSAPLRVY | 32-43 | 673.3780 | 673.3774 | -0.9 | 2 |   |  |  |  |  |  | **X** |
| LDAQSAPLRVY | 33-43 | 616.8360 | 616.8353 | -1.1 | 2 |   |  | **X** |  |  |  | **X** |
| LDAQSAPLRVYVE | 33-45 | 730.8909 | 730.8908 | -0.1 | 2 |   |  |  |  |  |  | **X** |
| DAQSAPLRV | 34-42 | 478.7625 | 478.7616 | -1.9 | 2 |   |  | **X** |  |  | **X** | **X** |
| DAQSAPLRVYVE | 34-45 | 674.3496 | 674.3488 | -1.2 | 2 |   | **X** | **X** |  | **X** | **X** |  |
| AQSAPLR | 35-41 | 371.7136 | 371.7139 | 0.8 | 2 |   | **X** |  |  |  |  |  |
| AQSAPLRV | 35-42 | 421.2496 | 421.2482 | -3.3 | 2 |   | **X** |  |  |  | **X** |  |
| AQSAPLRVY | 35-42 | 502.7804 | 502.7798 | -1.2 | 2 |   | **X** | **X** |  | **X** |  | **X** |
| AQSAPLRVYVE | 35-44 | 616.8373 | 616.8353 | -3.2 | 2 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| YVEELKPTPE | 42-52 | 602.8094 | 602.8084 | -1.7 | 2 |   |  |  |  |  | **X** |  |
| YVEELKPTPEGDLEIL | 42-58 | 922.9819 | 922.9800 | -2.1 | 2 |   |  |  |  |  |  | **X** |
| VEELKPTPE | 44-52 | 521.2761 | 521.2768 | 1.3 | 2 |   | **X** | **X** |  | **X** |  |  |
| VEELKPTPEGDLEIL | 44-58 | 841.4501 | 841.4484 | -2.0 | 2 and 3 |   |  |  |  |  |  | **X** |
| KPT | 47-49 | 345.2129 | 345.2132 | 0.9 | 1 |   |  |  |  |  |  |  |
| KKII | 70-73 | 501.3756 | 501.3759 | 0.6 | 1 |   |  | **X** |  |  |  |  |
| IAE | 73-75 | 332.1808 | 332.1816 | 2.4 | 1 |   |  |  | **X** |  |  |  |
| KTKIPAVFK | 76-84 | 516.3357 | 516.3342 | -2.9 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** | **X** |
| KTKIPAVFKI | 76-85 | 382.2538 | 382.2533 | -1.3 | 3 |   | **X** | **X** |  |  |  |  |
| KTKIPAVFKID | 76-86 | 420.5964 | 420.5956 | -1.9 | 3 |   | **X** | **X** | **X** | **X** | **X** |  |
| AVF | 81-83 | 336.1923 | 336.1918 | -1.5 | 1 |   |  | **X** |  |  |  | **X** |
| IDALNENK | 85-92 | 458.7397 | 458.7404 | 1.5 | 2 |   |  |  |  |  |  |  |
| VLVL | 93-96 | 443.3233 | 443.3228 | -1.1 | 1 |   |  |  | **X** | **X** | **X** |  |
| VLVLDTDYK | 93-101 | 533.2955 | 533.2950 | -0.9 | 2 |   |  |  | **X** |  | **X** |  |
| LVR | 123-125 | 387.2711 | 387.2714 | 0.8 | 1 |   |  |  |  | **X** |  |  |
| VDDEALEKF | 129-137 | 533.2599 | 533.2586 | -2.4 | 2 |   | **X** |  | **X** |  |  |  |
| EALEKFDKALKALPMHIRL | 132-150 | 445.4613 | 445.4595 | -4.0 | 5 |   |  |  |  |  |  | **X** |
| DKALKALPMHIRL | 138-150 | 377.2297 | 377.2301 | 1.1 | 4 |   |  |  |  |  |  | **X** |
| ALKALP | 140-145 | 306.7073 | 306.7076 | 1.0 | 2 |   |  |  | **X** |  | **X** |  |
| ALKALPMHIRL | 140-150 | 316.4497 | 316.4496 | -0.3 | 3 and 4 |   | **X** | **X** | **X** | **X** | **X** |  |
| KALPMHIR | 142-149 | 322.5289 | 322.5286 | -0.9 | 3 |   | **X** |  |  |  |  |  |
| KALPMHIRL | 142-150 | 539.8311 | 539.8313 | 0.4 | 2 and 3 |   | **X** | **X** | **X** | **X** | **X** |  |
| PMHIRLS | 145-151 | 427.2397 | 427.2393 | -0.9 | 2 |   | **X** |  | **X** |  |  |  |
| HIRLSFNPT | 147-155 | 542.7983 | 542.7985 | 0.4 | 2 |   | **X** | **X** | **X** | **X** | **X** |  |

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