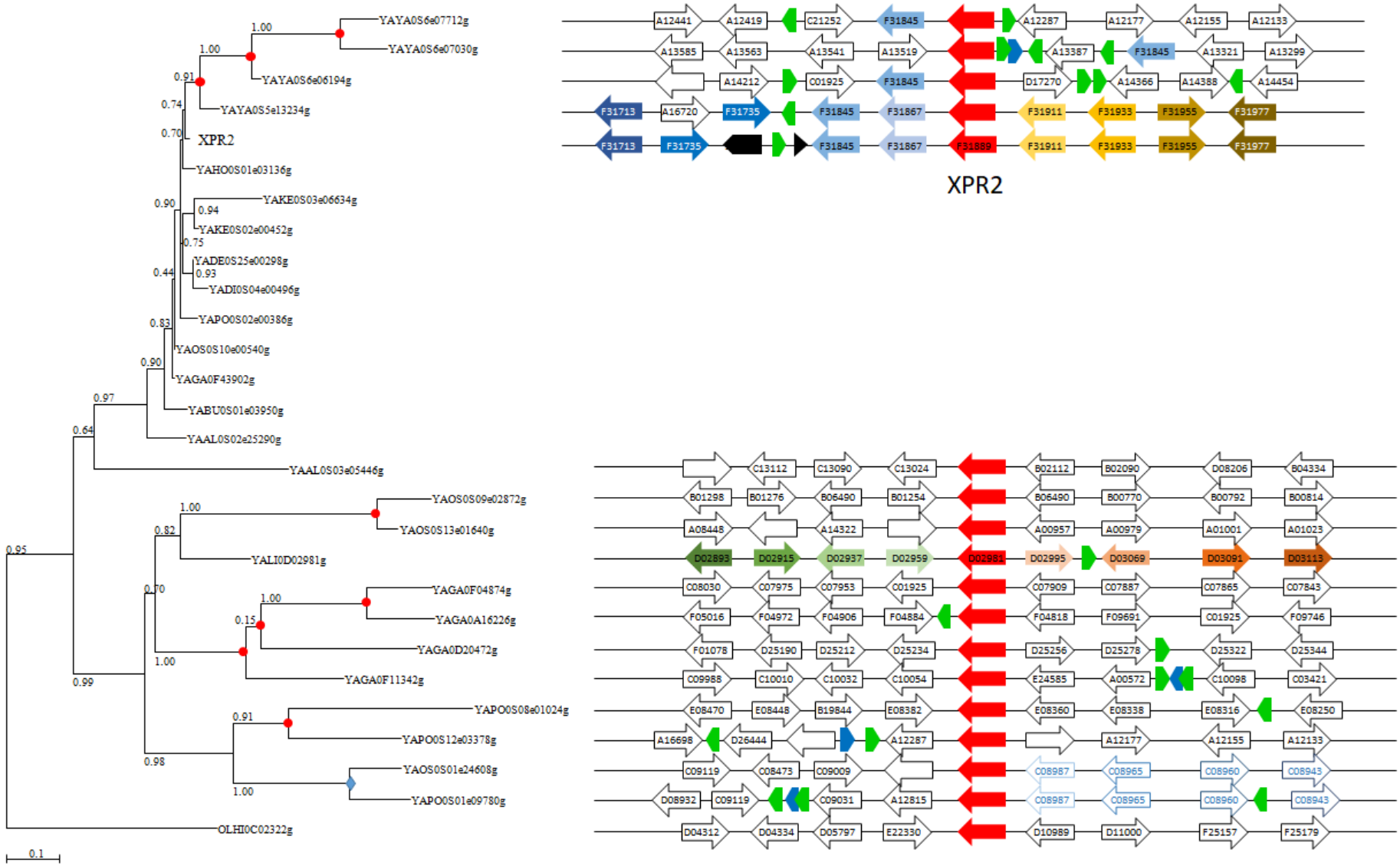


**Figure S1.** Synteny analysis of alkaline proteases, closely related to *XPR2* homologues emanating from successive duplication events in *Yarrowia* clade species. The phylogenetic tree was constructed from an alignment of 228 amino acids with phyML and a LG evolutionary model. *XPR2* orthologues are in red and flanking genes are in different blue or yellow to brown colours reflecting orthologues in the different species, as in Figure 1. Genes with no homology to each other are represented by white arrows and the name of their homologue in *Y. lipolytica* is written inside. A single exception concerns blue genes inside blue arrows in *Y. osloensis* and *Y. porcina* (see the blue diamond at the ancestral node). Red dots indicate species specific duplications.

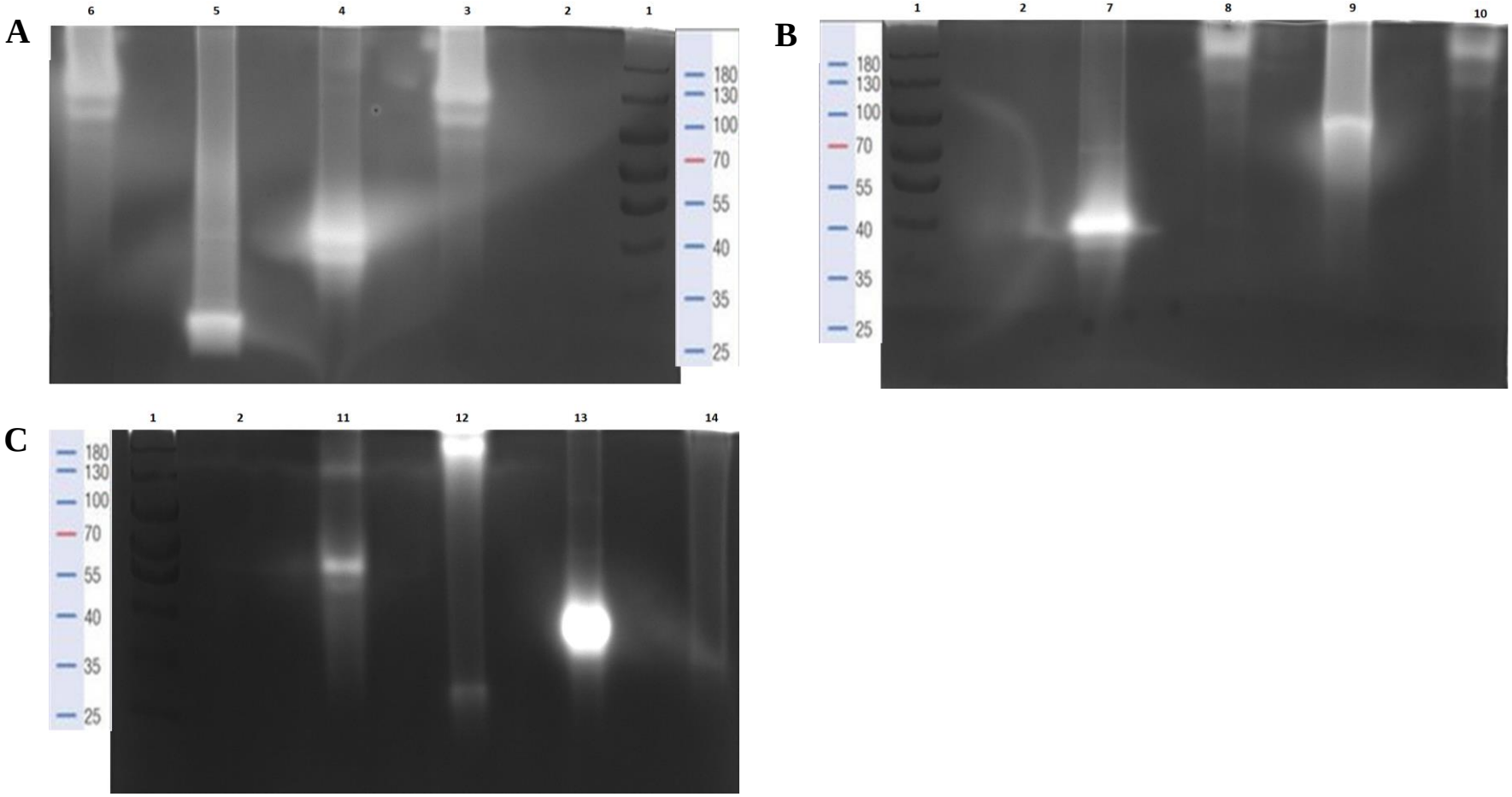


**Figure S2.** Gelatin zymogram of *Yarrowia* clade's proteolytic enzymes performed on 10% SDS-PAGE. (A) (Lane 1) Molecular mass marker, (lane 2) Control, (lane 3) *Y. lipolytica*, (lane 4) *Y. galli*, (lane 5) *Y. keelungensis*, (lane 6) *Y. alimentaria*. (B) (Lane 1) Molecular mass marker, (lane 2) Control, (lane 7) *Y. parophonii*, (lane 8) *Y. brassicae*, (lane 9) *Y. bubula*, (lane 10) *Y. divulgata*. (C) (Lane 1) Molecular mass marker, (lane 2) Control, (lane 11) *Y. hollandica*, (lane 12) *Y. porcina*, (lane 13) *Y. osloensis*, (lane 14) *Y. yakushimensis*.

**Table S1**. Results of comparative proteomic analysis of *Yarrowia* clade proteolytic enzymes secreted in the BSG medium.

| No. | *Host* | Accession number | Gene name | Protein name | Size observed on gelatin gel | Taxonomy | Protein sequence coverage [%] | Matched peptide sequence | Peptide location Start-End Point |
|---|---|---|---|---|---|---|---|---|---|
| ***1.*** | *Yarrowia lipolytica* W29 | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | 130 | *Yarrowia lipolytica* CLIB122 | 40 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | KAQTGNYAYVR | 176 – 186 |
| | | | | | | | | AQTGNYAYVR | 177 – 186 |
| | | | | | | | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTQNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANTK | 243 – 251 |
| | | | | | | | | TYGVDANTKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GVLNFSGGGPKSASQDALWSR | 289 – 309 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | | | | | ATQEGLLVAIAAGNDAVDACNDSPGNIGGSTSGIITVGSIDSSDK | 310 – 354 |
| ***2.*** | *Yarrowia lipolytica* W29 | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | 110 | *Yarrowia lipolytica* CLIB122 | 44 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | KAQTGNYAYVR | 176 – 186 |
| | | | | | | | | AQTGNYAYVR | 177 – 186 |
| | | | | | | | | ETVGKHPTVSYVVDSGIR | 187 – 204 |
| | | | | | | | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTQNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANTK | 243 – 251 |
| | | | | | | | | TYGVDANTKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | | | | | ATQEGLLVAIAAGNDAVDACNDSPGNIGGSTSGIITVGSIDSSDK | 310 – 354 |
| | | | | | | | | GSPNAVAYNGVGI | 442 – 454 |
| ***3.*** | *Yarrowia keelungensis* | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular | 30 | *Yarrowia lipolytica* | 35 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | AQTGNYAYVR | 177 – 186 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CBS 11062 | | | protease (AEP) | | CLIB122 | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTQNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | | | | | ATQEGLLVAIAAGNDAVDACNDSPGNIGGSTSGIITVGSIDSSDK | 310 – 354 |
| | | Q6C080 | YALI0_F27071g | Aspartyl protease | | *Yarrowia lipolytica* CLIB122 | 22 | YDSAASSSYK | 125 – 134 |
| | | | | | | | | FDGILGLAYDTISVNK | 186 – 201 |
| | | | | | | | | IVPPVYNAVNR | 202 – 212 |
| | | | | | | | | NQFSFFLGDTNK | 218 – 229 |
| | | | | | | | | GTDGGVATFGGVDEDYFEGK | 230 – 249 |
| | | | | | | | | ITWLPVR | 250 – 256 |
| | | | | | | | | GWSGQYTVECDK | 310 – 321 |
| | | Q6CFP5 | YALI0_B05104g | Peptide hydrolase | | *Yarrowia lipolytica* CLIB122 | 21 | SNLAEFTDFFTR | 130 – 141 |
| | | | | | | | | QVNQLAHGYK | 158 – 167 |
| | | | | | | | | KPTVEYFAHPWGQPSIIAR | 172 – 190 |
| | | | | | | | | QYFTETK | 272 – 278 |
| | | | | | | | | YGYPSSFVIESGFQYTSK | 349 – 366 |
| | | | | | | | | LSFEHMR+Oxidation (M) | 378 – 384 |
| | | | | | | | | LTIGYAYELAFAK | 389 – 401 |
| ***4.*** | *Yarrowia osloensis* CBS 10146 | | | Alkaline extracellular protease | 65 | *Yarrowia sp*. E02 | 32 | AVETTPVTQWGLSR | 158 – 171 |
| | | | | | | | | ISHKQAQSGNYAYVR | 172 – 186 |
| | | | | | | | | QAQSGNYAYVR | 176 – 186 |
| | | | | | | | | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | TYGVDANAKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | Alkaline | | *Yarrowia* | 26 | ETVGKYPTVAYVVDSGIR | 187 – 204 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | extracellular protease | | *sp.*B02 | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | TYGVDANAKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GSPNAVAYNGVGL | 442 – 454 |
| ***5.*** | *Yarrowia parophonii* CBS 12427 | | | Alkaline extracellular protease | 65 | *Yarrowia sp*. E02 | 32 | AVETTPVTQWGLSR | 158 – 171 |
| | | | | | | | | ISHKQAQSGNYAYVR | 172 – 186 |
| | | | | | | | | QAQSGNYAYVR | 176 – 186 |
| | | | | | | | | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | SAALSVINQGFTWALNDFISKR | 262 – 283 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp.* B02 | 26 | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | SAALSVINQGFTWALNDFISKR | 262 – 283 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GSPNAVAYNGVGL | 442 – 454 |
| ***6.*** | *Yarrowia porcina* CBS 12935 | A0A371CCQ4 | B0I71DRAFT_162965 | Carboxypeptidase | 30 | *Yarrowia lipolytica* YB392 | 25 | AKDPSSLGLDK | 75 – 85 |
| | | | | | | | | DPSSLGLDK | 77 – 85 |
| | | | | | | | | QYSGYLDVEDEDK | 88 – 100 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | QYDEYEPMACGK | 254 – 265 |
| | | | | | | | | GGAPPVLDEPTCENMR | 266 – 281 |
| | | | | | | | | KECDSGTSLCYK | 330 – 341 |
| | | | | | | | | ECDSGTSLCYK | 331 – 341 |
| | | | | | | | | DFICNWLGNK | 409 – 418 |
| | | | | | | | | WTDELEWFGK | 420 – 429 |
| | | | | | | | | ELSDWVVDGK | 436 – 445 |
| | | | | | | | | VYEAGHMVPYDQPK | 461 – 474 |
| | | | | | | | | NSLEMLNSWLAK | 475 – 486 |
| | | | | | | | | DYSYGSK | 487 – 493 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp.* B02 | 20 | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWAR | 300 – 309 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp.* E02 | 20 | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWAR | 300 – 309 |
| | | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | | *Yarrowia lipolytica* CLIB122 | 21 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | AVWGANFADTQNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| ***7.*** | *Yarrowia alimentaria* CBS 10151 | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | 130 | *Yarrowia lipolytica* CLIB122 | 42 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | KAQTGNYAYVR | 176 – 186 |
| | | | | | | | | AQTGNYAYVR | 177 – 186 |
| | | | | | | | | ETVGKHPTVSYVVDSGIR | 187 – 204 |
| | | | | | | | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTQNADLLGHGTHVAGTVGGK | 214 – 242 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | TYGVDANTK | 243 – 251 |
| | | | | | | | | TYGVDANTKLVAVK | 243 – 256 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GVLNFSGGGPKSASQDALWSR | 289 – 309 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | | | | | ATQEGLLVAIAAGNDAVDACNDSPGNIGGSTSGIITVGSIDSSDK | 310 – 354 |
| | | | | | | | | GSPNAVAYNGVGI | 442 – 454 |
| ***8.*** | *Yarrowia alimentaria* CBS 10151 | | | Alkaline extracellular protease | 110 | *Yarrowia sp*. E02 | 29 | ISHKQAQSGNYAYVR | 172 – 186 |
| | | | | | | | | QAQSGNYAYVR | 176 – 186 |
| | | | | | | | | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | TYGVDANAKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp*. B02 | 28 | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTTNADLLGHGTHVAGTVGGK | 214 – 242 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | TYGVDANAKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWAR | 300 – 309 |
| | | | | | | | | GSPNAVAYNGVGL | 442 – 454 |
| | | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | | *Yarrowia lipolytica* CLIB122 | 28 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | AVWGANFADTQNADLLGHGTHVAGTVGGK | 214 – 242 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | | | | | GSPNAVAYNGVGI | 442 – 454 |
| ***9.*** | *Yarrowia galli* CBS 9722 | | | Alkaline extracellular protease | 40 | *Yarrowia sp.* E02 | 17 | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | SAALSVINQGFTWALNDFISKR | 262 – 283 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GVLNFSGGGPKSASQDALWSR | 289 – 309 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp.* B02 | 18 | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | SAALSVINQGFTWALNDFISKR | 262 – 283 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GSPNAVAYNGVGL | 442 – 454 |
| ***10.*** | *Yarrowia galli* CBS 9722 | | | Alkaline extracellular protease | 37-39 | *Yarrowia sp.* B02 | 22 | TTPVTQWGLSR | 161 – 171 |
| | | | | | | | | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | TYGVDANAKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GSPNAVAYNGVGL | 442 – 454 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp*. E02 | 19 | ETVGKYPTVAYVVDSGIR | 187 – 204 |
| | | | | | | | | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | TYGVDANAKLVAVK | 243 – 256 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | GVLNFSGGGPK | 300 – 309 |
| | | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | | *Yarrowia lipolytica* CLIB122 | 19 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | LVAVKVFAGR | 252 – 261 |
| | | | | | | | | SAALSVINQGFTWALNDYISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | | | | | GSPNAVAYNGVGI | 442 – 454 |
| ***11.*** | *Yarrowia brassicae* CBS 15225 | P09230 | YALI0F31889g (XPR2) | Alkaline extracellular protease (AEP) | 180 | *Yarrowia lipolytica* CLIB122 | 16 | AIQTTPVTQWGLSR | 158 – 171 |
| | | | | | | | | AQTGNYAYVR | 177 – 186 |
| | | | | | | | | HPTVSYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | TYGVDANTK | 243 – 251 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWSR | 300 – 309 |
| | | | | Alkaline extracellular protease | | *Yarrowia sp.* B02 | 16 | YPTVAYVVDSGIR | 192 – 204 |
| | | | | | | | | TTHSEFGGR | 205 – 213 |
| | | | | | | | | TYGVDANAK | 243 – 251 |
| | | | | | | | | SAALSVINQGFTWALNDFISK | 262 – 282 |
| | | | | | | | | GVLNFSGGGPK | 289 – 299 |
| | | | | | | | | SASQDALWAR | 300 – 309 |