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**Experimental and bioinformatics study for production of L-asparaginase from *Bacillus licheniformis*: a promising enzyme for medical application**

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**Table S1: Levels of reaction conditions of process parameters as independent variables studied in RSM experimental design for optimization of L-asparaginase production by the selected test mutant.**

|  |  |  |
| --- | --- | --- |
| **Test variable** | **Variable code** | **Variables levels** |
| **Incubation temperature (°C)** | **A** | **-1 0 +1** |
| **35 37.5 40** |
| **Initial pH** | **B** | **6 7 8** |
| **Incubation time (h)** | **C** | **18 33 48** |
| **Agitation (RPM)** | **D** | **150 175 200** |

**Table S2: Experiments that were deduced by the RSM experimental design and performed for L-asparaginase production by the mutant**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment** | **Temperature (°C)** | | **pH** | | **Time (h)** | | **Agitation (rpm)** | |
|  | **Value** | **Level code** | **Value** | **Level code** | **Value** | **Level code** | **Value** | **Level code** |
| 1 | 40 | 1 | 7 | 0 | 48 | 1 | 175 | 0 |
| 2 | 37.5 | 0 | 8 | 1 | 33 | 0 | 200 | 1 |
| 3 | 37.5 | 0 | 7 | 0 | 48 | 1 | 200 | 1 |
| 4 | 40 | 1 | 8 | 1 | 33 | 0 | 175 | 0 |
| 5 | 37.5 | 0 | 8 | 1 | 48 | 1 | 175 | 0 |
| 6 | 37.5 | 0 | 8 | 1 | 18 | -1 | 175 | 0 |
| 7 | 35 | -1 | 7 | 0 | 33 | 0 | 150 | -1 |
| 8 | 37.5 | 0 | 7 | 0 | 33 | 0 | 175 | 0 |
| 9 | 37.5 | 0 | 7 | 0 | 18 | -1 | 200 | 1 |
| 10 | 35 | -1 | 6 | -1 | 33 | 0 | 175 | 0 |
| 11 | 35 | -1 | 8 | 1 | 33 | 0 | 175 | 0 |
| 12 | 40 | 1 | 7 | 0 | 33 | 0 | 200 | 1 |
| 13 | 37.5 | 0 | 7 | 0 | 48 | 1 | 150 | -1 |
| 14 | 35 | -1 | 7 | 0 | 48 | 1 | 175 | 0 |
| 15 | 37.5 | 0 | 6 | -1 | 48 | 1 | 175 | 0 |
| 16 | 37.5 | 0 | 7 | 0 | 18 | -1 | 150 | -1 |
| 17 | 37.5 | 0 | 8 | 1 | 33 | 0 | 150 | -1 |
| 18 | 40 | 1 | 7 | 0 | 18 | -1 | 175 | 0 |
| 19 | 40 | 1 | 7 | 0 | 33 | 0 | 150 | -1 |
| 20 | 37.5 | 0 | 6 | -1 | 33 | 0 | 150 | -1 |
| 21 | 37.5 | 0 | 7 | 0 | 33 | 0 | 175 | 0 |
| 22 | 35 | -1 | 7 | 0 | 18 | -1 | 175 | 0 |
| 23 | 40 | 1 | 6 | -1 | 33 | 0 | 175 | 0 |
| 24 | 37.5 | 0 | 6 | -1 | 18 | -1 | 175 | 0 |
| 25 | 35 | -1 | 7 | 0 | 33 | 0 | 200 | 1 |
| 26 | 37.5 | 0 | 7 | 0 | 33 | 0 | 175 | 0 |
| 27 | 37.5 | 0 | 6 | -1 | 33 | 0 | 200 | 1 |

**Table S3: Pairwise distances among L-asparaginases of bacterial species presented in the phylogenetic tree shown in Figure 1.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | L-asparaginase\_*Bacillus\_subtilis* |  |  |  |  |  |  |  |  |  |
| 2 | MULTISPECIES:\_L-asparaginase\_*Bacillus* | 0.00267 |  |  |  |  |  |  |  |  |
| 3 | MULTISPECIES:\_type\_II\_asparaginase\_ *Bacillus\_amyloliquefaciens*\_group | 2.54891 | 2.54891 |  |  |  |  |  |  |  |
| 4 | Type\_II\_asparaginase\_*Bacillus\_halotolerans* | 2.05573 | 2.05573 | 2.20263 |  |  |  |  |  |  |
| 5 | Type\_II\_asparaginase\_*Bacillus\_licheniformis* | 2.07678 | 2.07678 | 2.13201 | 0.11280 |  |  |  |  |  |
| 6 | Type\_II\_asparaginase\_*Bacillus\_mojavensis* | 2.03511 | 2.03511 | 2.25264 | 0.01613 | 0.11579 |  |  |  |  |
| 7 | Type\_II\_asparaginase\_*Bacillus\_subtilis*\_subsp.\_spizizenii. | 0.00535 | 0.00803 | 2.54891 | 2.05573 | 2.07678 | 2.03511 |  |  |  |
| 8 | Type\_II\_asparaginase\_*Bacillus\_subtilis* | 2.07678 | 2.07678 | 2.13201 | 0.10981 | 0.00267 | 0.11280 | 2.07678 |  |  |
| 9 | Type\_II\_asparaginase\_*Bacillus\_tequilensis* | 2.05573 | 2.05573 | 2.15500 | 0.13391 | 0.08920 | 0.14003 | 2.05573 | 0.08628 |  |
| 10 | Type\_II\_asparaginase\_*Bacillus\_velezensis* | 2.48221 | 2.48221 | 0.01905 | 2.15500 | 2.13201 | 2.20263 | 2.48221 | 2.13201 | 2.15500 |

**Table S4: Pairwise distances among L-asparaginases of *Bacillus licheniformis*, *E. coli* and *Erwinia chrysanthemi* presented in the phylogenetic tree shown in Figure 2.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | 1 | 2 | 3 |
| 1 | ansB\_*Escherichia\_coli*\_B354 |  |  |  |
| 2 | Lasparaginase\_Dickeya\_(formerly\_*Erwinia*)\_*chrysanthemi* | 0.732 |  |  |
| 3 | L-asparaginase\_*Bacillus\_licheniformis* | 1.253 | 1.161 |  |

**Table S5: ANOVA of the quadratic model for the process parameters optimization of** **L-asparaginase productivity by *Bacillus licheniformis***  
**mutant using Box-Behnken central composite design**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **SS** | | **DF** | **MS** | | **F-value** | **p-value** | | **Comments** | | |
| **Model** | 0.34 | | 14 | 0.024 | | 19.59 | <0.0001 | | Significant | | |
| **Residual** | 0.015 | | 12 | 1.223E-003 | |  |  | |  | | |
| **Lack of Fit** | 0.015 | | 10 | 1.453E-003 | | 19.23 | 0.0504 | | not significant | | |
| **Total Regression** | 0.35 | | 26 |  | |  |  | |  | | |
| **Analysis reliability parameters** | | | | | | | | | | | |
| **R-Squared** | | 0.9581 | |  |  | | |  | |  |  |
| **Adjusted R-Squared** | | 0.9092 | |  |  | | |  | |  |  |
| **Predicted R-Squared** | | 0.7600 | |  |  | | |  | |  |  |
| **Adequate Precision** | | 15.913 | |  |  | | |  | |  |  |

\*Where, SS, Sum of Squares; DF, Degrees of Freedom; MS, Mean of Squares.