

## Supplementary Materials

**Table S1.** Relative amino acid compositions of mesophilic and Thermo-ASNases

Residue(s)	Proteins		L-ASNase												
Residue(s)	MP [91]	TP/HP [91]	<b>EcAI</b>	<i>TkAI</i>	<i>TsAI</i>	<i>TzAI</i>	<i>PhAI</i>	<i>PfAI</i>	<i>PyAI</i>	<b>EcAII</b>	<i>MrAII</i>	<i>StAII</i>	<b>EcAIII</b>	<i>TkAIII</i>	<i>MrAIII</i>
Ala	8.09 ± 1.54	6.82 ± 1.42	<b>8.58</b>	8.84	6.65	8.49	6.10	6.14	7.01	<b>10.92</b>	5.83	17.75	<b>14.64</b>	12.42	10.56
Cys	1.10 ± 0.18	0.86 ± 0.27	<b>0.30</b>	0.00	0.30	0.00	0.00	0.61	0.00	<b>0.58</b>	0.92	1.18	<b>1.56</b>	0.33	0.62
Asp	5.06 ± 0.18	4.63 ± 0.54	<b>5.33</b>	5.79	5.74	5.46	5.18	6.44	4.88	<b>7.76</b>	5.52	5.62	<b>4.36</b>	4.90	5.59
Glu	6.45 ± 0.54	8.55 ± 0.95	<b>5.33</b>	6.71	6.34	7.27	7.01	6.44	8.23	<b>2.01</b>	8.59	5.62	<b>7.79</b>	9.48	5.28
Phe	4.61 ± 0.78	4.40 ± 0.82	<b>2.96</b>	2.44	2.1	3.03	3.05	2.76	2.13	<b>3.16</b>	4.60	1.78	<b>2.18</b>	3.27	5.59
Gly	6.70 ± 0.96	7.16 ± 0.68	<b>8.28</b>	9.76	8.16	10.91	9.45	9.20	9.76	<b>8.62</b>	8.59	9.76	<b>12.15</b>	12.09	11.80
His	2.04 ± 0.21	1.57 ± 0.16	<b>3.55</b>	1.52	1.21	2.12	0.92	0.92	1.22	<b>0.86</b>	1.53	2.96	<b>1.87</b>	1.31	1.55
Ile	7.40 ± 1.69	7.82 ± 1.64	<b>6.51</b>	7.62	11.18	6.97	11.28	9.20	5.79	<b>3.74</b>	9.51	2.37	<b>4.05</b>	6.21	7.45
Lys	6.81 ± 2.00	7.61 ± 2.16	<b>2.96</b>	3.96	10.27	6.06	7.01	6.14	3.66	<b>6.90</b>	7.98	1.48	<b>2.80</b>	8.50	7.45
Leu	10.43 ± 0.55	10.21 ± 0.68	<b>10.06</b>	10.37	8.46	9.70	9.76	10.12	11.59	<b>7.47</b>	7.98	11.24	<b>9.66</b>	7.52	8.70
Met	2.42 ± 0.28	2.29 ± 0.25	<b>3.55</b>	3.05	4.23	2.73	3.66	3.68	3.96	<b>2.30</b>	2.76	2.07	<b>4.05</b>	3.60	3.73
Asn	4.90 ± 1.20	3.52 ± 0.94	<b>5.03</b>	3.05	4.23	2.42	2.74	4.29	2.44	<b>6.61</b>	6.44	1.18	<b>3.12</b>	2.29	6.21
Pro	3.77 ± 0.77	4.36 ± 0.99	<b>6.51</b>	5.49	4.53	5.46	3.96	3.99	5.49	<b>3.74</b>	4.29	7.10	<b>3.12</b>	2.61	2.17
Gln	3.99 ± 0.75	1.78 ± 0.22	<b>4.73</b>	1.22	0.91	1.21	1.22	1.23	0.92	<b>3.74</b>	0.92	2.37	<b>2.49</b>	0.65	1.86

Table S1. Cont.

Residue(s)	Proteins		L-ASNase												
	MP [91]	TP/HP [91]	<b>EcAI</b>	<i>TkAI</i>	<i>TsAI</i>	<i>TzAI</i>	<i>PhAI</i>	<i>PfAI</i>	<i>PyAI</i>	<b>EcAII</b>	<i>MrAII</i>	<i>StAII</i>	<b>EcAIII</b>	<i>TkAIII</i>	<i>MrAIII</i>
Arg	4.33 ± 0.98	5.57 ± 1.16	<b>4.44</b>	<i>7.01</i>	<i>3.02</i>	<i>4.24</i>	<i>5.18</i>	<i>4.60</i>	<i>6.71</i>	<b>2.30</b>	<i>2.15</i>	<i>7.99</i>	<b>4.67</b>	<i>4.25</i>	<i>3.73</i>
Ser	6.08 ± 0.57	5.54 ± 1.01	<b>4.73</b>	<i>4.27</i>	<i>4.23</i>	<i>5.46</i>	<i>6.71</i>	<i>4.91</i>	<i>3.66</i>	<b>4.89</b>	<i>5.22</i>	<i>4.73</i>	<b>4.98</b>	<i>3.27</i>	<i>4.66</i>
Thr	5.09 ± 0.57	4.34 ± 0.23	<b>6.81</b>	<i>7.93</i>	<i>7.55</i>	<i>6.67</i>	<i>5.18</i>	<i>6.75</i>	<i>6.40</i>	<b>10.06</b>	<i>6.14</i>	<i>2.96</i>	<b>5.92</b>	<i>5.56</i>	<i>3.73</i>
Val	6.35 ± 0.75	8.05 ± 0.68	<b>5.92</b>	<i>7.32</i>	<i>6.04</i>	<i>8.18</i>	<i>7.01</i>	<i>8.59</i>	<i>11.59</i>	<b>10.63</b>	<i>7.06</i>	<i>10.06</i>	<b>7.79</b>	<i>8.82</i>	<i>6.21</i>
Trp	1.02 ± 0.31	1.06 ± 0.20	<b>0.30</b>	<i>0.61</i>	<i>0.60</i>	<i>0.61</i>	<i>0.92</i>	<i>0.61</i>	<i>1.52</i>	<b>0.29</b>	<i>0.61</i>	<i>0.89</i>	<b>0.31</b>	<i>1.31</i>	<i>0.00</i>
Tyr	3.30 ± 0.43	3.82 ± 0.33	<b>4.14</b>	<i>3.05</i>	<i>4.23</i>	<i>3.03</i>	<i>3.66</i>	<i>3.37</i>	<i>3.05</i>	<b>3.45</b>	<i>3.37</i>	<i>0.89</i>	<b>2.49</b>	<i>1.63</i>	<i>3.11</i>
Charged residues (DEKRH)	24.69	27.93	<b>21.60</b>	<i>25.00</i>	<i>26.59</i>	<i>25.15</i>	<i>25.31</i>	<i>24.54</i>	<i>24.70</i>	<b>19.83</b>	<i>25.77</i>	<i>23.67</i>	<b>21.50</b>	<i>28.43</i>	<i>23.60</i>
Polar/uncharged residues (GSTNQYC)	31.26	27.02	<b>34.02</b>	<i>29.28</i>	<i>29.61</i>	<i>29.33</i>	<i>28.96</i>	<i>30.36</i>	<i>23.18</i>	<b>37.94</b>	<i>31.59</i>	<i>23.08</i>	<b>32.71</b>	<i>25.81</i>	<i>31.99</i>
Hydrophobic residues (LMIVWPAF)	44.09	45.01	<b>44.39</b>	<i>45.74</i>	<i>43.81</i>	<i>45.16</i>	<i>45.74</i>	<i>45.12</i>	<i>49.08</i>	<b>42.25</b>	<i>42.63</i>	<i>53.26</i>	<b>45.80</b>	<i>45.76</i>	<i>44.41</i>
Aromatic residues (F+H+W+Y)	10.97	10.85	<b>10.95</b>	<i>7.63</i>	<i>8.16</i>	<i>8.79</i>	<i>8.54</i>	<i>7.67</i>	<i>7.93</i>	<b>7.76</b>	<i>10.12</i>	<i>6.51</i>	<b>6.85</b>	<i>7.52</i>	<i>10.25</i>

mesophilic L-ASNases (EcAI, EcAII, EcAIII) are in bold, thermo-ASNases are in italics. Decreased and increased amino acid content in thermo-ASNases compared to mesophilic counterparts is marked in red and green, respectively.