

## Supplementary Information

### **Impact of structural stability of cold adapted *Candida antarctica* lipase B (CaLB): In relation to pH, chemical and thermal denaturation**

Gulam Rabbani<sup>1</sup>, Ejaz Ahmad<sup>1</sup>, Mohsin Vahid Khan<sup>1</sup>, Mohd. Tashfeen Ashraf<sup>2</sup>, Rajiv Bhat<sup>3</sup> and Rizwan Hasan Khan<sup>1\*</sup>

<sup>1</sup> Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh-202 002, India

<sup>2</sup> School of Biotechnology, Gautam Buddha University, Greater Noida-201308, India

<sup>3</sup> School of Biotechnology, Jawaharlal Nehru University, New Mehrauli Road, New Delhi-110067, India

**\*Address for correspondence:**

Interdisciplinary Biotechnology Unit

Aligarh Muslim University,

Aligarh 202 002, India

Telefax: +91-571-2721776

E-mail: [rizwanhkhan@hotmail.com](mailto:rizwanhkhan@hotmail.com), [rizwanhkhan1@gmail.com](mailto:rizwanhkhan1@gmail.com)

**Running Title:** Characterization of molten globule state

CLUSTAL 2.1 multiple sequence alignment

```

1TCC|B      --LPSGSDPAFSQPKSVLDAGLTCQGASPSVSKPILLVPGTGTTPQSFDSMWIPLSTQ 58
4K6H      -ALPSGSDPAFSQPKSVLDAGLTCQGASPSVSKPILLVPGTGTTPQSFDSMWIPLSTQ 59
ACI06118.1|MALPSGSDPAFSQPKSVLDAGLTCQGASPSVSKPILLVPGTGTTPQSFDSMWIPLSTQ 60
            *****

1TCC|B      LGYTPCWISPPPFMLNDTQVNT EYMVNAITALYAGSGNNKLPVLTWSQGGLVAQNGLTFF 118
4K6H      LGYTPCWISPPPFMLNDTQVNT EYMVNAITALYAGSGNNKLPVLTWSQGGLVAQNGLTFF 119
ACI06118.1|LGYTPCWISPPPFMLNDTQVNT EYMVNAITALYAGSGNNKLPVLTWSQGGLVAQNGLTFF 120
            *****

1TCC|B      PSIRSKVDRMLMAFAPDYKGT VLAGPLDALAVSAPSVWQQTGSAITLALRNAGGLTQIVP 178
4K6H      PSIRSKVDRMLMAFAPDYKGT VLAGPLDALAVSAPSVWQQTGSAITLALRNAGGLTQIVP 179
ACI06118.1|PSIRSKVDRMLMAFAPDYKGT VLAGPLDALAVSAPSVWQQTGSAITLALRNAGGLTQIVP 180
            *****

1TCC|B      TTNLYSATDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVIDHAGSLTSQFSYVVGR 238
4K6H      TTNLYSATDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVIDHAGSLTSQFSYVVGR 239
ACI06118.1|TTNLYSATDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVIDHAGSLTSQFSYVVGR 240
            *****

1TCC|B      SALRSTTGQARSADYGITDCNPLPANDLTPEQKVAAAALLAPAAAAIVAGPKQNCPEPLM 298
4K6H      SALRSTTGQARSADYGITDCNPLPANDLTPEQKVAAAALLMAPAAAAIVAGPKQNCPEPLM 299
ACI06118.1|SALRSTTGQARSADYGITDCNPLPANDLTPEQKVAAAALLAPAAAAIVAGPKQNCPEPLM 300
            *****;*****

1TCC|B      PYARPFVAVGKRTCSGIVTP----- 317
4K6H      PYARPFVAVGKRTCSGIVTPLEHHHHH 326
ACI06118.1|PYARPFVAVGKRTXSGIVTPSL----- 321
            *****

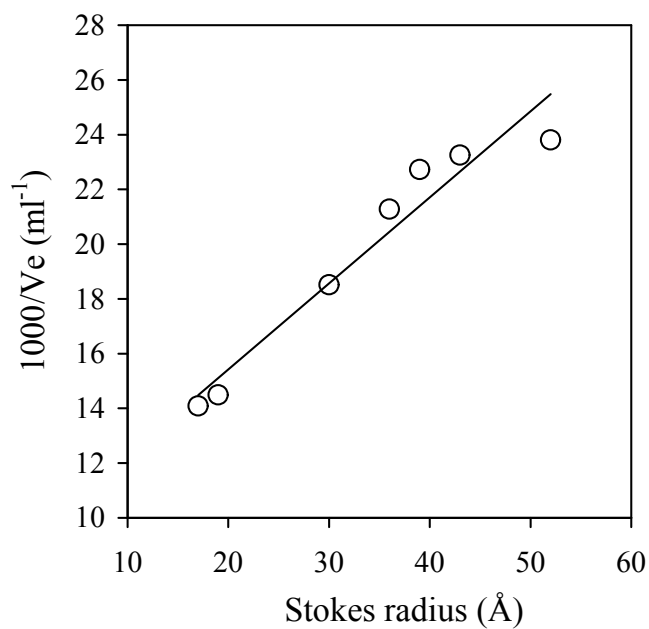
```

**Fig. S1:** Comparison of *Candida antarctica* lipase B (CaLB) amino acid sequence.

1TCC: *Candida antarctica* lipase B

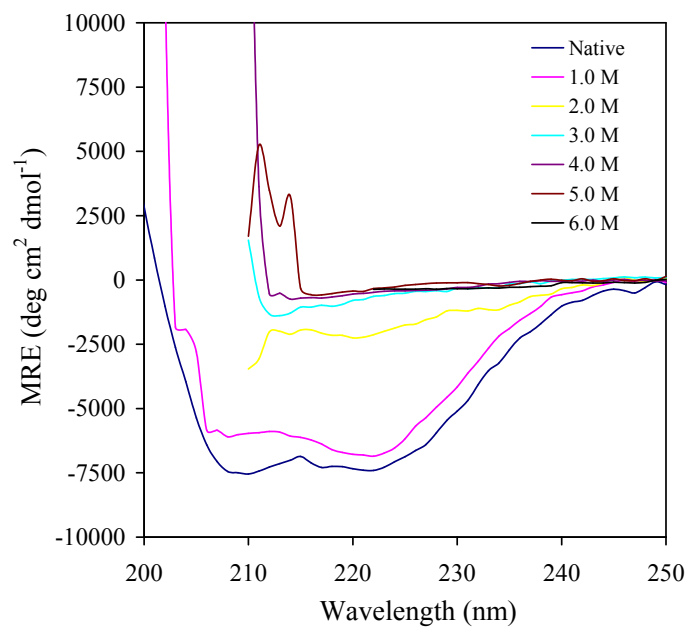
4K6H: CaLB mutant L278M from *Candida antarctica*

ACI06118.1: *Candida antarctica* (Yeast) (*Trichosporon oryzae*)



**Fig. S2:** The migration rates ( $1000/V_e$ ) versus Stokes radius ( $\text{\AA}$ ) of used calibrants are:

S. No.	Standard	Stokes radius ( $\text{\AA}$ )	Elution volume (ml)
1.	cytochrome <i>c</i>	17	71
2.	lysozyme	19	69
3.	ovalbumin	30	54
4.	BSA monomer and dimer	36 and 43	47 and 43
5.	conalbumin	39	44
6.	glucose oxidase	52	42



**Fig. S3:** Far-UV CD spectra of CaLB acquired in 1.0, 2.0, 3.0, 4.0, 5.0 and 6 M GuHCl respectively. CaLB samples were incubated at pH 7.4. Only selected spectra have been shown for the sake of clarity.