**Supporting Information**

**Table S1** **Halogenated substrates used in the activity determination.**

**Table S2 Oligonucleotides used for gene cloning and vector construction.**

**Table S3** **Conditions used in determination of steady-state kinetic constants by gas chromatography.**

**Table S4** **Similarity matrix of DadB and other HLDs identified.**

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**Figure S2** **Phylogenetic analyses** of DadA, DadB and other 18 **identified** **HLDs.** Multiple sequence alignment was conducted by MUSCLE[[17](#_ENREF_17)] and the tree was constructed with Neighbor-Joining method[[18](#_ENREF_18)] by MEGA 5.05 [[19](#_ENREF_19)]. Robustness of output trees were estimated by bootstrapping the data 1000 times. This phylogenetic tree is basically the same with the tree of Chovancova (Chovancova et al. 2007).

**Figure S3 Substrate specificity profile of DadB toward chlorinated (blue), brominated (red), and iodinated (green) substrates.** The activities of 4 chlorinated alkenes and the corresponding chlorinated alkanes are indicated in the black box.

**Figure S4** **Activity comparison of DadB with other HLDs.** The values, except for DadB, were obtained from the results published by Koudelakova et al. [[20](#_ENREF_20)]. Two activities greater than 250 nmol∙s−1∙mg−1 are cut off and labeled with the values.

**Figure S5** **Effect of temperature and pH on the activity of DadB.** Both experiments chose 1,3-dibromopropane as substrate and the data are expressed as relative activities. The data in the left picture are determined in 100 mM glycine buffer, pH 8.6 under different temperatures. The data in the right picture are determined at 37oC in different buffers (▲, 100 mM potassium acetate buffers with pH 4.0, 5.0, 5.5 and 6.0; ■, 100 mM imidazole buffers with pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0; ●, 100 mM MOPS buffers with pH 6.0, 6.5, 7.0, 7.5 and 8.0; ◆, 100 mM potassium phosphate buffers with pH 6.0, 6.5, 7.0, 7.5 and 8.0; ▼, 100 mM glycine buffers with pH 8.0, 8.5, 9.0 and 10.0).

**Figure S6** **Secondary structure elements prediction of HLDs.** Sequences of DadB and other 7 HLDs with crystal structures [[8](#_ENREF_8),[11](#_ENREF_11),[21-25](#_ENREF_21)] were submitted to PRIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/). And multiple sequence alignment was conducted by ClustalX2.1[[1](#_ENREF_1)]. The fragments in blue, magenta and yellow background represent the realistic (7 HLDs with solved structures) or predicted (homology modeling of DadB) β-sheets, α- helices and coiled coils respectively. The blue, magenta and yellow letters means they are belong to β-sheets, α- helices and coiled coils according to the Secondary structure elements prediction.

**Figure S7** **Three-dimensional structure model of DadB.** The cyan and green elements constitute the cap domain and the main domain, respectively. The yellow, red, magenta, blue represents the halide-binding residues, the nucleophile residue, the acid residue, and the base residue, respectively.

**Table S1** **Halogenated substrates used in the activity determination.**

|  |  |  |
| --- | --- | --- |
| substrates | brand | purity |
| 1-chlorobutane | Sigma-Aldrich | 99.5% |
| 1-chlorohexane | Aldrich | 99% |
| 1-bromobutane | Sigma-Aldrich | 99% |
| 1-bromohexane | Aldrich | 98% |
| 1-iodopropane | Aldrich | 99% |
| 1-iodobutane | Aldrich | 99% |
| 1-iodohexane | Aldrich | ≥98% |
| 1,2-dichloroethane | Fluka | ≥99.5% |
| 1,3-dichloropropane | Aldrich | 99% |
| 1,5-dichloropentane | Aldrich | 99% |
| 1,2-dibromoethane | TCI | >99% |
| 1,3-dibromopropane | Dr.Ehrenstorfer | 100% |
| 1-bromo-3-chloropropane | Sigma | 99% |
| 1,3-diiodopropane | Aldrich | 99% |
| 2-iodobutane | Aldrich | 99% |
| 1,2-dichloropropane | Fluka | ≥99% |
| 1,2-dibromopropane | Aldrich | 97% |
| 2-bromo-1-chloropropane | Dr.Ehrenstorfer | 99% |
| 1,2,3-trichloropropane | Aldrich | 99% |
| 1-chloro-2-(2-chloroethoxy)ethane | Fluka | ≥99%(GC) |
| chlorocyclohexane | Aldrich | 99% |
| bromocyclohexane | Aldrich | 98% |
| (bromomethyl)cyclohexane | Aldrich | 99% |
| 1-bromo-2-chloroethane | Aldrich | 98% |
| chlorocyclopentane | Aldrich | 99% |
| 4-bromobutanenitrile | Alfa Aesar | 97% |
| 1,2,3-tribromopropane | Aldrich | 97% |
| 1,2-dibromo-3-chloropropane | TCI | >98%(GC) |
| 3-chloro-2-methylprop-1-ene | Aldrich | 98% |
| 2,3-dichloroprop-1-ene | Aldrich | 98% |
| dichloromethane | SCRC | ≥99.9% |
| 1-chloro-2-methylpropane | TCI | >95.0%(GC) |
| 1,3-dichloropropene | TCI | >92.0%(GC) |
| 1,2,3-trichloropropene | TCI | >95.0%(GC) |
| 1-chlorooctane | Aldrich | 99% |
| 1-chlorodecane | Aldrich | 98% |
| 1-chlorododecane | Aldrich | ≥97%(GC) |
| 1-chlorotetradecane | Aldrich | 98% |
| 1-chlorohexadecane | Aldrich | 95% |
| 1-bromohexadecane | Aldrich | 97% |
| 1-chlorooctadecane | Aldrich | 96% |
| trichloromethane | SCRC | ≥99.8% |
| 1-chloro-3-nitrobenzene | Aldrich | >98% |
| 4-bromodiphenyl ether | Aldrich | 99% |
| decabromodiphenyl | Aldrich | 98% |
| trichloroacetic acid | Sigma | >99% |

**Table S2 Oligonucleotides used for gene cloning and vector construction.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer | gene | vector | Sequence (5'-3') | enzyme |
| 22b4127-f | *dadB* | pET22b | AAGGAGATATACATATGCTCAGAGAACAACTCCCC | NdeI |
| 22b4127-r | *dadB* | pET22b | GGTGGTGGTGCTCGAGCGAATTGGATAGGGCCT | XhoI |
| 22b2238-f | *dadA* | pET22b | AAGGAGATATACATATGGGCTTCGCGGACTGTCC | NdeI |
| 22b2238-r | *dadA* | pET22b | GGTGGTGGTGCTCGAGTCGCGGATTCGCCAAGCG | XhoI |
| 32a2238-f | *dadA* | pET32a | GGTGCCACGCGGATCCATGGGCTTCGCGGACTGTC | BamHI |
| 32a2238-r | *dadA* | pET32a | GCTCGAATTCGGATCCTCATCGCGGATTCGCCAAGCG | BamHI |
| 28a2238-f | *dadA* | pET28a | CGCGCGGCAGCCATATGGGCTTCGCGGACTGTCC | NdeI |
| 28a2238-r | *dadA* | pET28a | GTCATGCTAGCCATATGTTATCGCGGATTCGCCAAG | NdeI |
| pGEX2238-f | *dadA* | pGEX-4T-1 | AAGGAGATATACATATGCTCAGAGAACAACTCCCC | BamHI |
| pGEX2238-r | *dadA* | pGEX-4T-1 | GGTGGTGGTGCTCGAGCGAATTGGATAGGGCCT | BamHI |

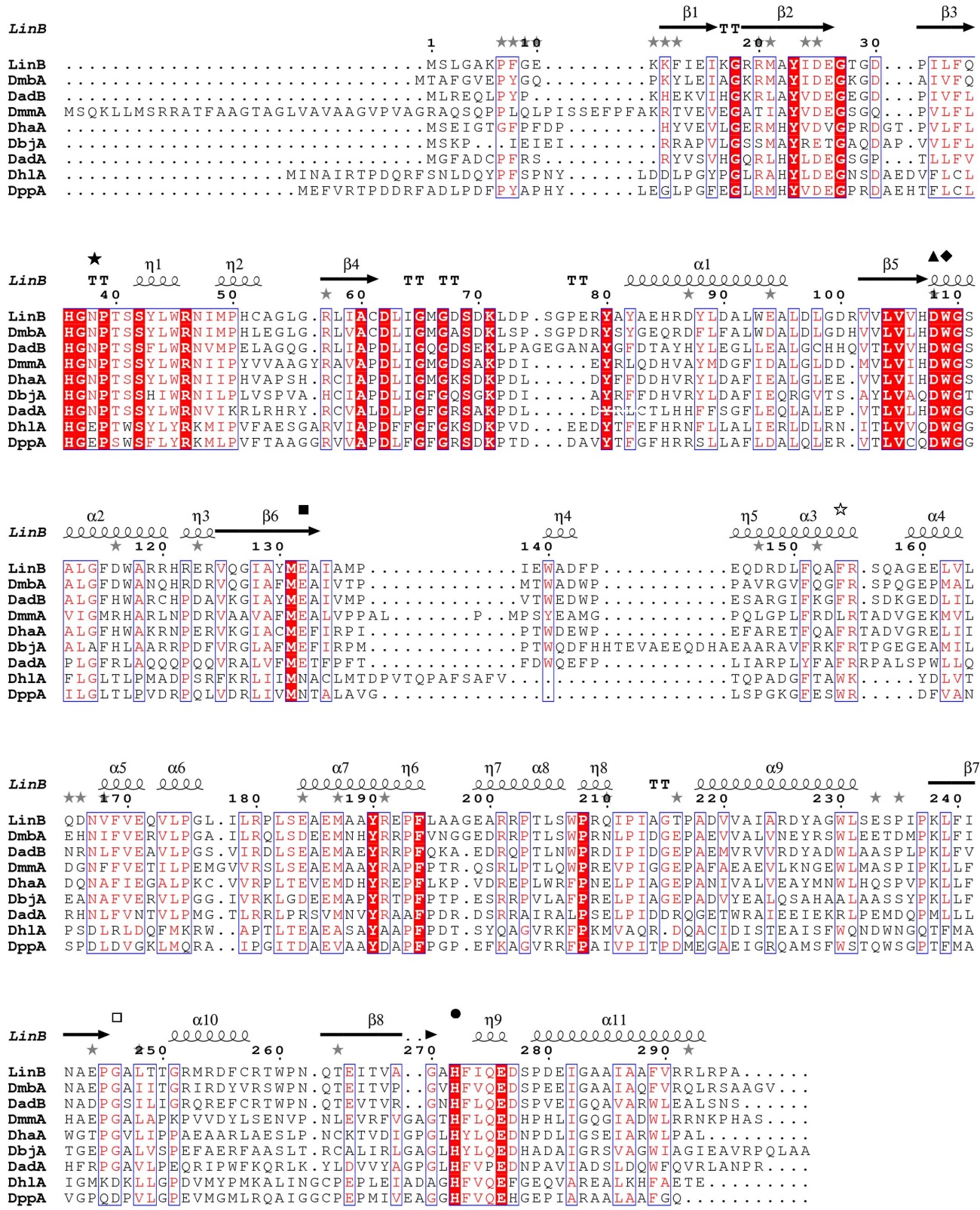
**Table S3** **Conditions used in determination of** **steady-state kinetic constants by gas chromatography.**

|  |  |  |
| --- | --- | --- |
| substrates | column temperature (oC) | testing time (min) |
| 1-chlorobutane | 40 | 4 |
| 1,3-dibromopropane | 130 | 5 |
| 1,2-dibromoethane | 100 | 5 |
| 4-bromobutanenitrile | 170 | 5 |
| 3-chloro-2-methylprop-1-ene | 35 | 5 |
| 2,3-dichloroprop-1-ene | 60 | 6 |

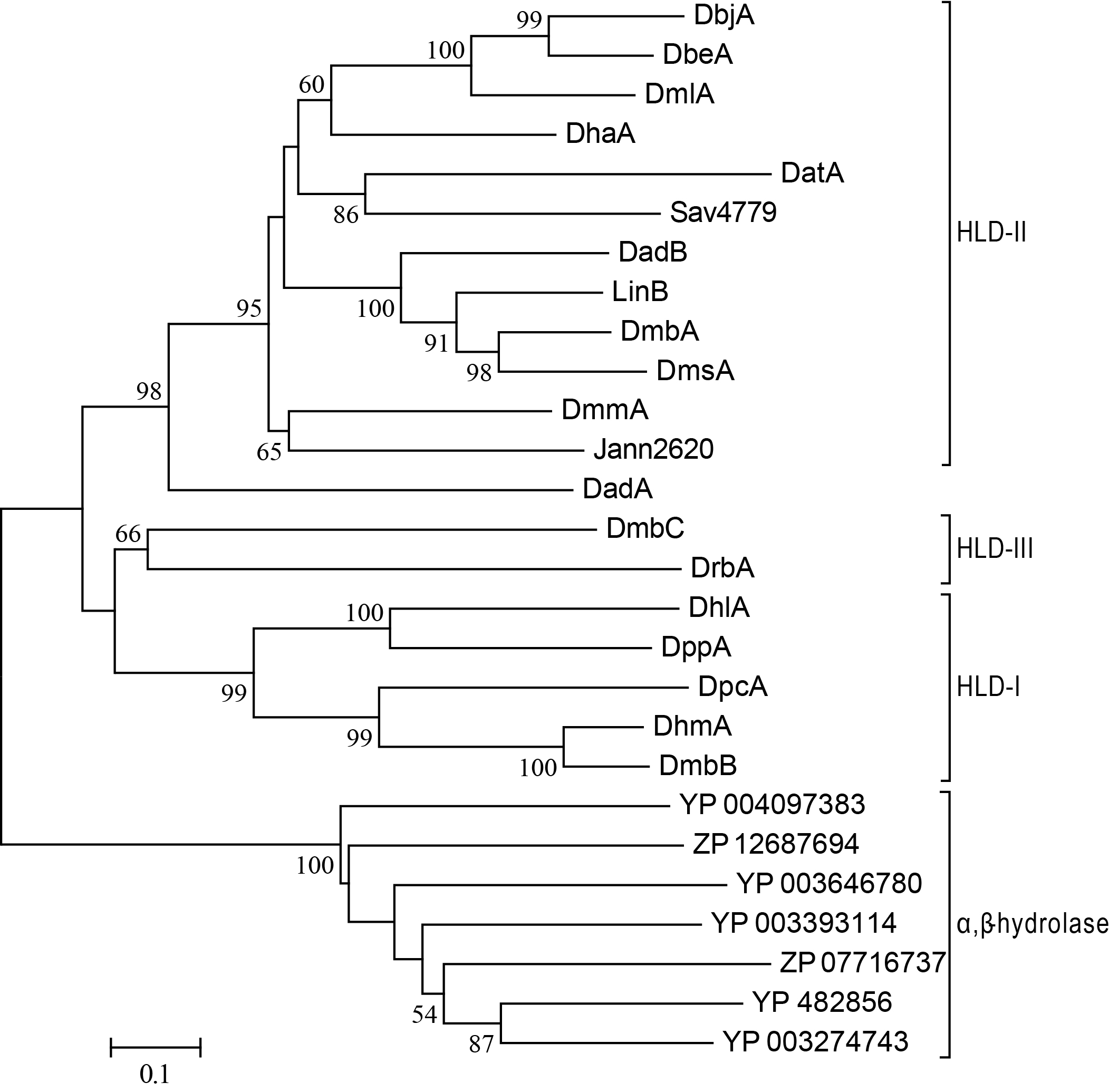
**Table S4** **Similarity matrix of DadB and other HLDs identified.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| DmbA | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DmsA | 70 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LinB | 69 | 63 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DadB | 56 | 53 | 60 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DbjA | 40 | 39 | 41 | 40 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DbeA | 42 | 41 | 45 | 43 | 73 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DmlA | 40 | 41 | 46 | 41 | 60 | 61 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DhaA | 45 | 44 | 49 | 48 | 51 | 50 | 52 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |
| DmmA | 42 | 43 | 46 | 44 | 43 | 46 | 44 | 50 | 100 |  |  |  |  |  |  |  |  |  |  |  |
| Jann2620 | 43 | 43 | 46 | 42 | 44 | 47 | 44 | 48 | 50 | 100 |  |  |  |  |  |  |  |  |  |  |
| DatA | 35 | 35 | 35 | 32 | 35 | 36 | 36 | 34 | 37 | 35 | 100 |  |  |  |  |  |  |  |  |  |
| Sav4779 | 42 | 44 | 47 | 41 | 41 | 45 | 46 | 46 | 42 | 39 | 44 | 100 |  |  |  |  |  |  |  |  |
| DadA | 35 | 34 | 32 | 34 | 35 | 36 | 34 | 37 | 36 | 37 | 34 | 32 | 100 |  |  |  |  |  |  |  |
| DhlA | 21 | 22 | 25 | 22 | 21 | 21 | 21 | 26 | 22 | 26 | 21 | 22 | 24 | 100 |  |  |  |  |  |  |
| DppA | 23 | 25 | 25 | 24 | 24 | 23 | 26 | 28 | 25 | 26 | 22 | 24 | 25 | 52 | 100 |  |  |  |  |  |
| DhmA | 23 | 24 | 25 | 21 | 22 | 22 | 24 | 27 | 26 | 22 | 22 | 22 | 27 | 36 | 38 | 100 |  |  |  |  |
| DmbB | 22 | 24 | 25 | 22 | 23 | 22 | 24 | 27 | 26 | 23 | 23 | 24 | 26 | 36 | 39 | 83 | 100 |  |  |  |
| DpcA | 21 | 24 | 21 | 20 | 22 | 22 | 22 | 25 | 27 | 26 | 23 | 21 | 26 | 38 | 43 | 50 | 50 | 100 |  |  |
| DmbC | 27 | 27 | 29 | 27 | 27 | 25 | 27 | 27 | 31 | 27 | 22 | 26 | 30 | 20 | 23 | 26 | 24 | 24 | 100 |  |
| DrbA | 27 | 25 | 28 | 25 | 22 | 24 | 26 | 25 | 23 | 24 | 24 | 24 | 25 | 21 | 22 | 21 | 22 | 22 | 28 | 100 |

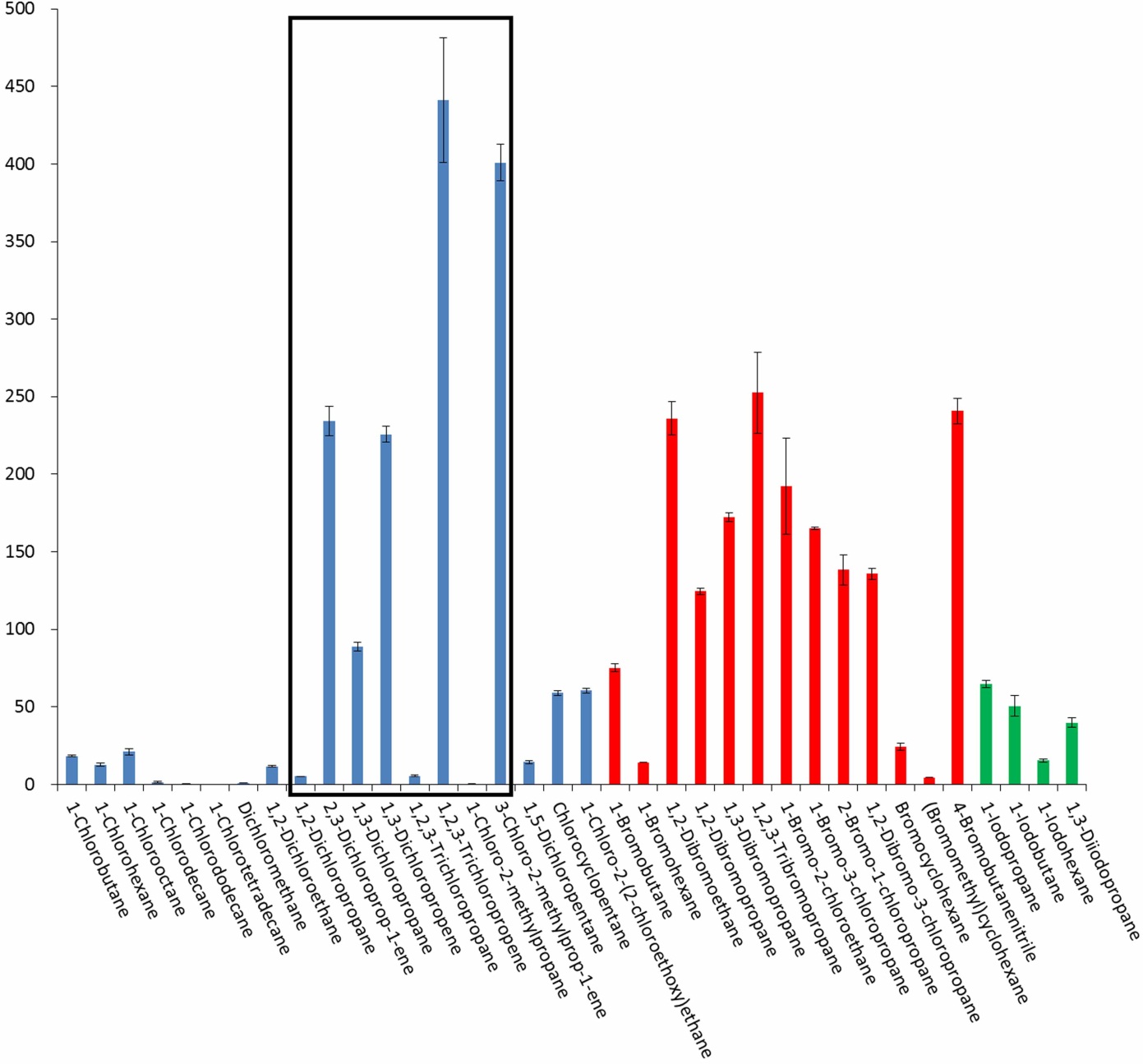
Values in the table represent the similarity (percentage) of amino acid sequences between the two HLDs at top and left. The bold numbers is the values of DadB. This matrix is produced by ClustalX 2.1[[1](#_ENREF_1),[2](#_ENREF_2)]. LinB(P51698), *Spinghobium japonicum* UT26[[3](#_ENREF_3)]; DmbA(AJ784272), DmbB(AJ784273) and DmbC(AM696288), *Mycobacterium bovis* 5033/66[[4](#_ENREF_4),[5](#_ENREF_5)]; DbjA(NP\_767727), *Bradyrhizobium japonicum* USDA110[[6](#_ENREF_6)]; DbeA(BAJ23986), *Bradyrhizobium elkani* USDA94(Prudnikova et al., unpublished data); DmlA(NP\_106032), *Mesorhizobium loti* MAFF303099[[6](#_ENREF_6)]; DhaA(AAC15838), *Rhodococcus rhodochrous* NCIMB 13064[[7](#_ENREF_7)]; DmmA(AAT70109), the metagenomic DNA of a marine microbial consortium[[8](#_ENREF_8)]; DatA(AB478945), *Agrobacterium tumefaciens* C58[[9](#_ENREF_9)]; DhlA(AAA88691), *Xanthobacter autotrophicus* GJ10[[10](#_ENREF_10)]; DppA(ZP\_01908831), *Plesiocystis pacifica* SIR-1[[11](#_ENREF_11)]; DhmA(AJ314789), *Mycobacterium avium* N85[[12](#_ENREF_12)]; DpcA(YP\_580518), *Psychrobacter cryohalolentis* K5[[13](#_ENREF_13)]; DrbA(AM696289), *Rhodopirellula baltica* SH1[[5](#_ENREF_5)]; DmsA(AAL17946), *Mycobacterium smegmatis* ATCC700084[[14](#_ENREF_14)]; Jann2620(YP\_510562), *Jannaschia sp.* CCS1[[15](#_ENREF_15)]; Sav4779(NP\_825956), *Streptomyces avermitilis MA-4680*[[15](#_ENREF_15)].

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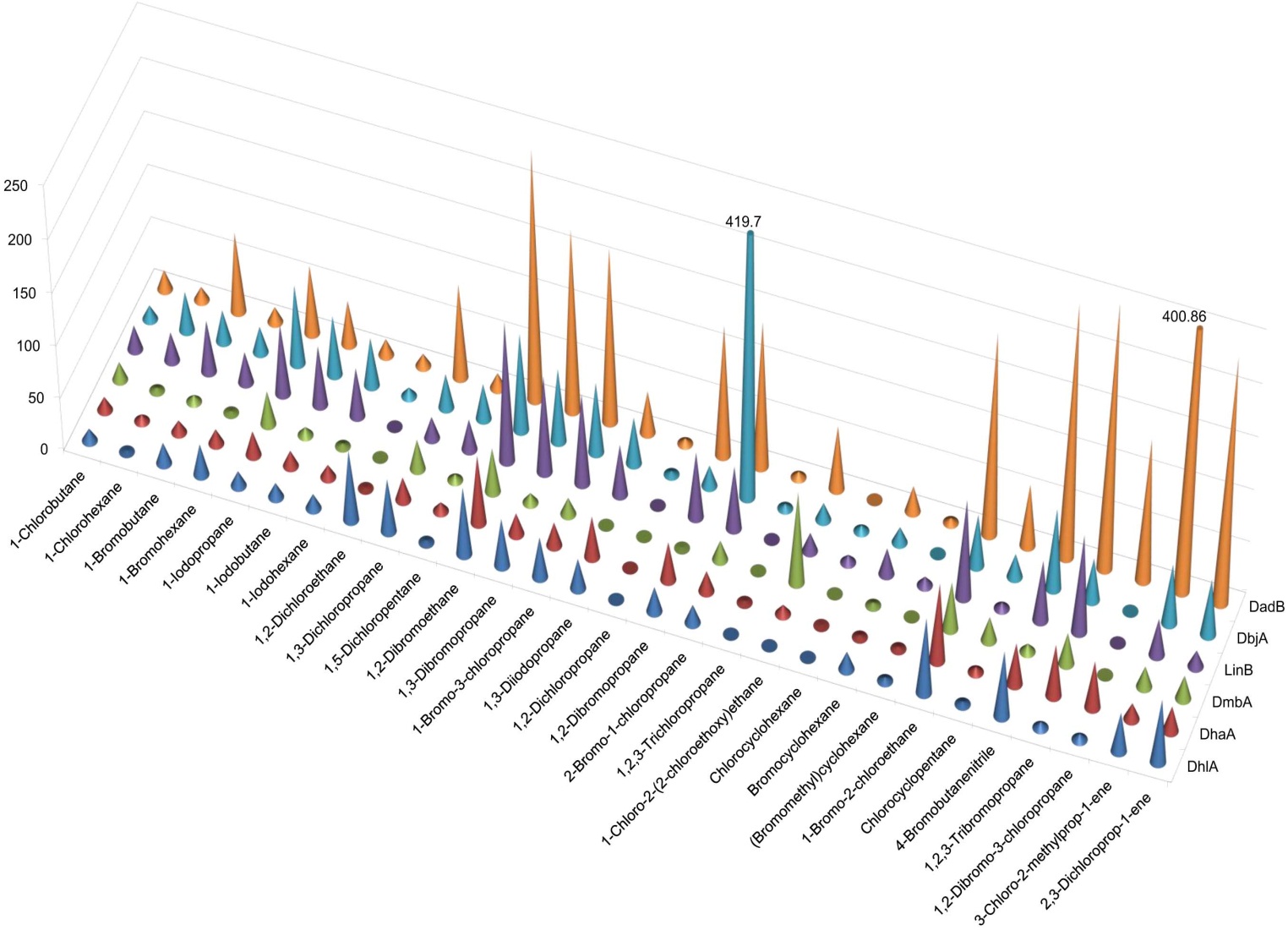
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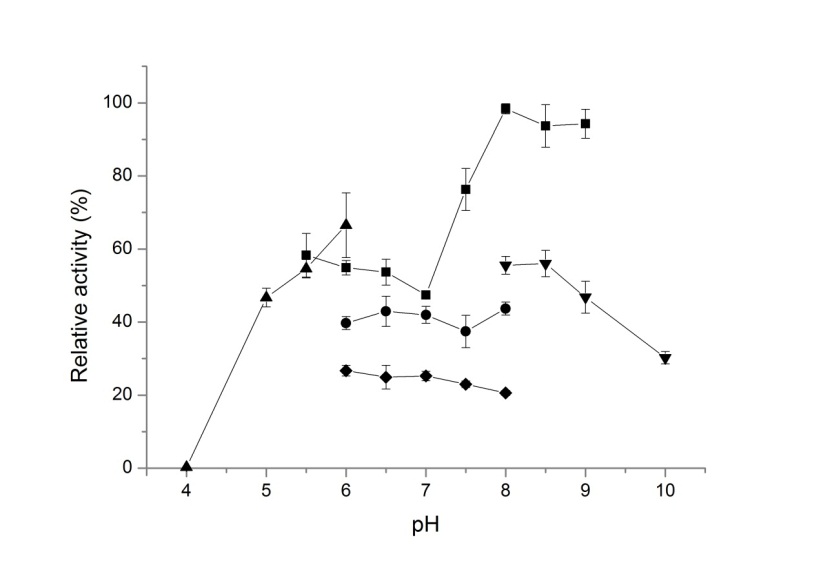
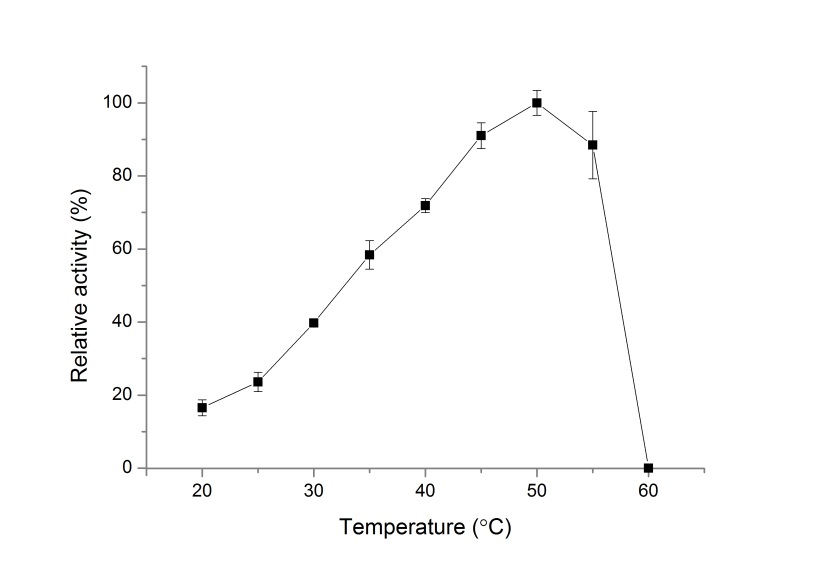
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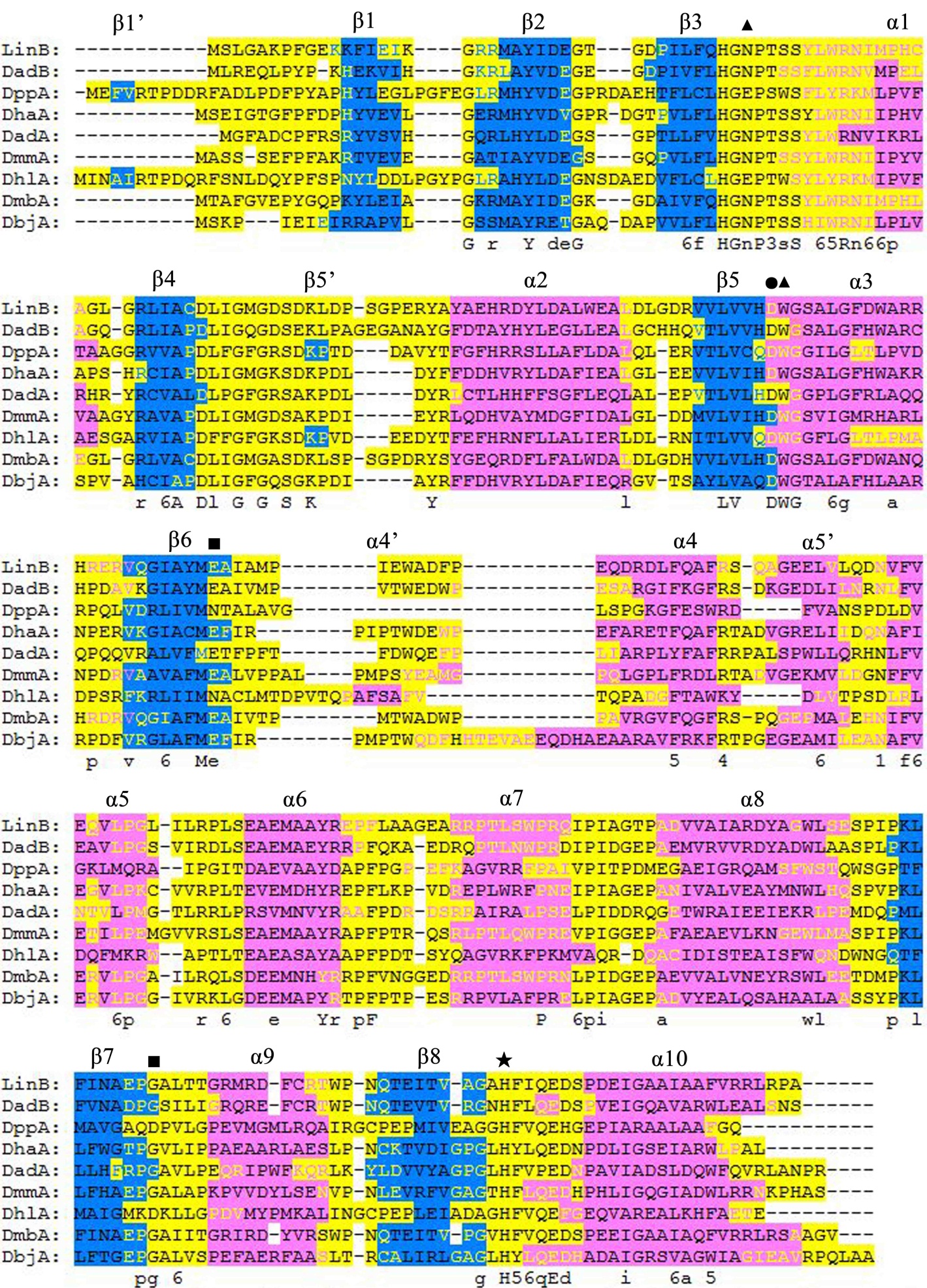
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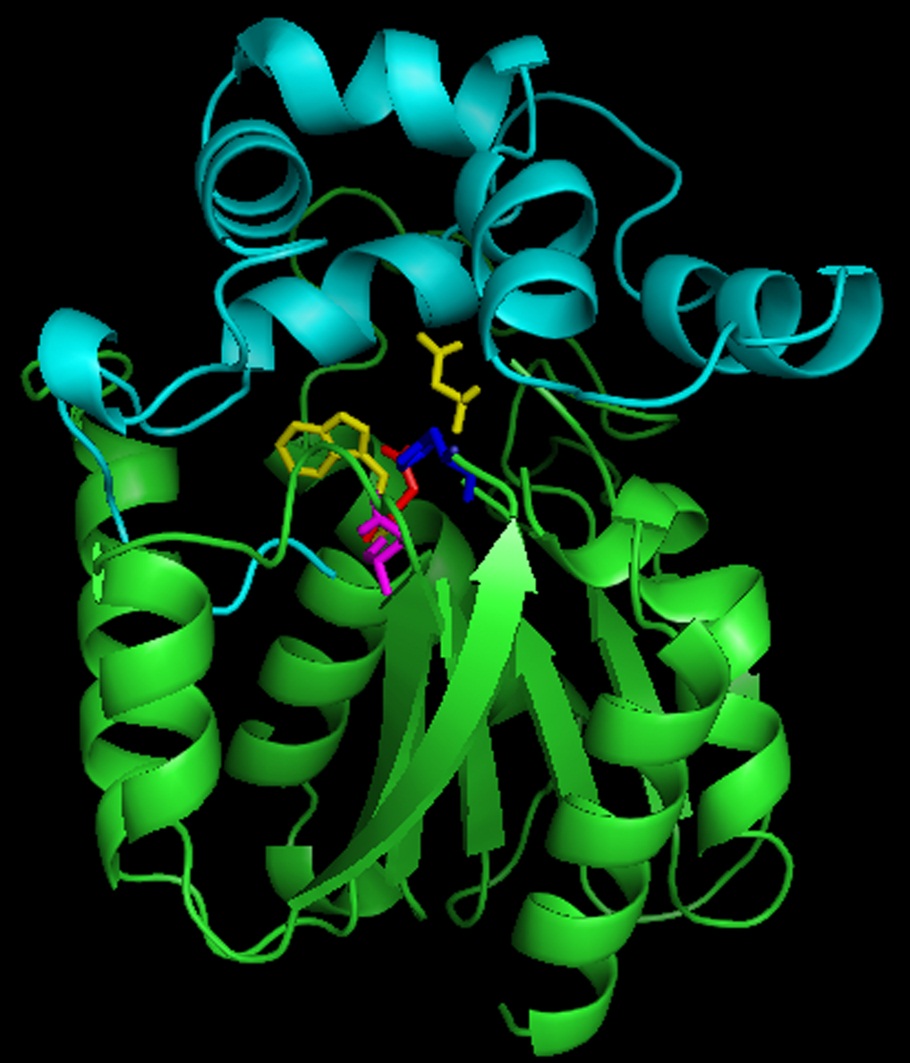
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**Figure S5** **Effect of temperature and pH on the activity of DadB.** Both experiments chose 1,3-dibromopropane as substrate and the data are expressed as relative activities. The data in the left picture are determined in 100 mM glycine buffer, pH 8.6 under different temperatures. The data in the right picture are determined at 37oC in different buffers (▲, 100 mM potassium acetate buffers with pH 4.0, 5.0, 5.5 and 6.0; ■, 100 mM imidazole buffers with pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0; ●, 100 mM MOPS buffers with pH 6.0, 6.5, 7.0, 7.5 and 8.0; ◆, 100 mM potassium phosphate buffers with pH 6.0, 6.5, 7.0, 7.5 and 8.0; ▼, 100 mM glycine buffers with pH 8.0, 8.5, 9.0 and 10.0).



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**Figure S7** **Three-dimensional structure model of DadB.** The cyan and green elements constitute the cap domain and the main domain, respectively. The yellow, red, magenta, blue represents the halide-binding residues, the nucleophile residue, the acid residue, and the base residue, respectively.

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