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1 # Supplementary Material

2 **Different fitness to arabinogalactan side chains is supported by diversified**
3 **specificity of glycoside hydrolase family 39 enzymes in the genus *Bifidobacterium***
4

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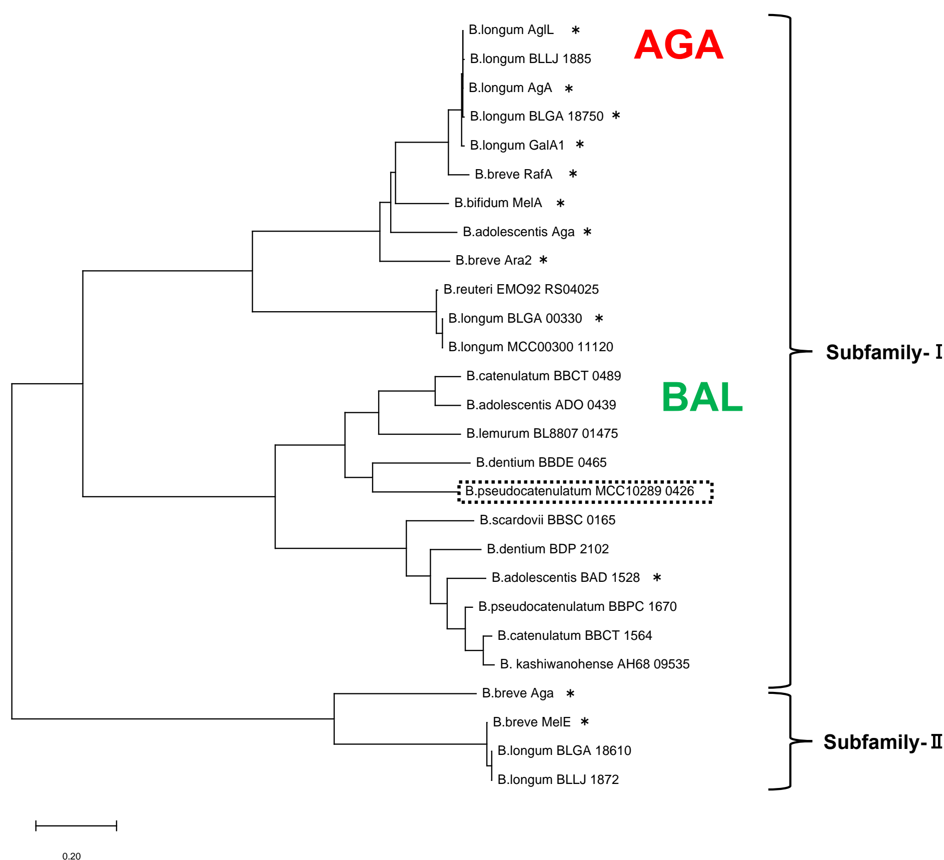

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18 **Supplementary Figure 1.** The phylogenetic relationships of bifidobacterial GH36 α-
19 galactosidases and β-L-arabinopyranosidases. The phylogenetic tree of *B.*
20 *pseudocatenulatum* MCC10289_0426 with homologous proteins from bifidobacteria
21 was constructed by the neighbor-joining method using the aligned sequences; for the
22 construction, the program Clustal W was implemented in the MEGA11 software. *B.*
23 *pseudocatenulatum*_MCC10289_0426 is indicated by a dashed-line box. The protein
24 names or locus tags are shown alongside *Bifidobacterium* strains as follows: *B.*
25 *adolescentis* Aga (GenBank ID: AAD30994.2), *B. bifidum* MelA (ABD96085.1), *B.*
26 *breve* Aga (AAK96217.2), *B. breve* Aga2 (ABB76662.1), *B. longum* GalA1
27 (ACD98928.1), *B. longum* AgA (AAN25312.1), *B. longum* AglL (AAG02023.1), *B.*
28 *breve* RafA (ABE96531.1), *B. breve* MelE (ABE96518.1), *B. longum* BLLJ_1872
29 (BAJ67536.1), *B. longum* BLLJ_1885 (BAJ67549.1), *B. longum* BlAga3
30 (BBV22622.1), *B. longum* BlAga1 (BBV24464.1), and *B. longum* BlAga2
31 (BBV24450.1), *B. longum* MCC00300_11120 (WP_077381863.1), *B. reuteri*
32 EMO92_RS04025 (WP_150335335.1), *B. catenulatum* BBCT_0489 (BAR01457.1),
33 *B. adolescentis* BADO_0439 (AII75864.1), *B. lemurum* BL8807_01475

34 (QOL34630.1), *B. dentium* BBDE_0465 (BAQ26459.1), *B. adolescentis* BAD_1528
35 (BBD14080.1), *B. pseudocatenulatum* BBPC_1670 (BAR04348.1), *B. catenulatum*
36 BBCT_1564 (BAR02532.1), *B. kashiwanohense* AH68_09535 (AIZ15229.1), *B.*
37 *dentium* BDP_2102 (ADB10663.1), and *B. scardovii* BBSC_0165 (BAQ30245.1).
38 Asterisk indicates characterized enzymes.

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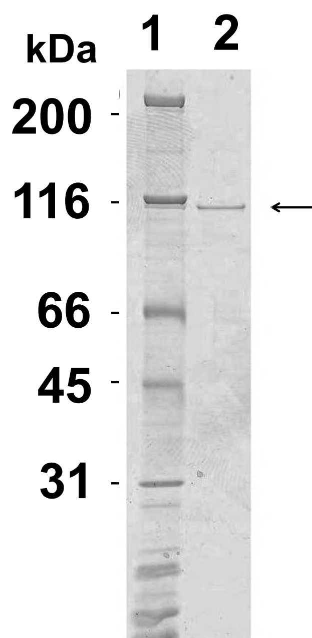

40 **Supplementary Figure 2.** SDS-PAGE analysis of recombinant AAfase. Purified
41 AAfase was electrophoresed on a 5–20% gradient polyacrylamide gel and stained with
42 Coomassie Brilliant Blue R-250. Lane 1, molecular size marker; lane 2, purified
43 AAfase. Arrow indicates target protein at expected molecular size.

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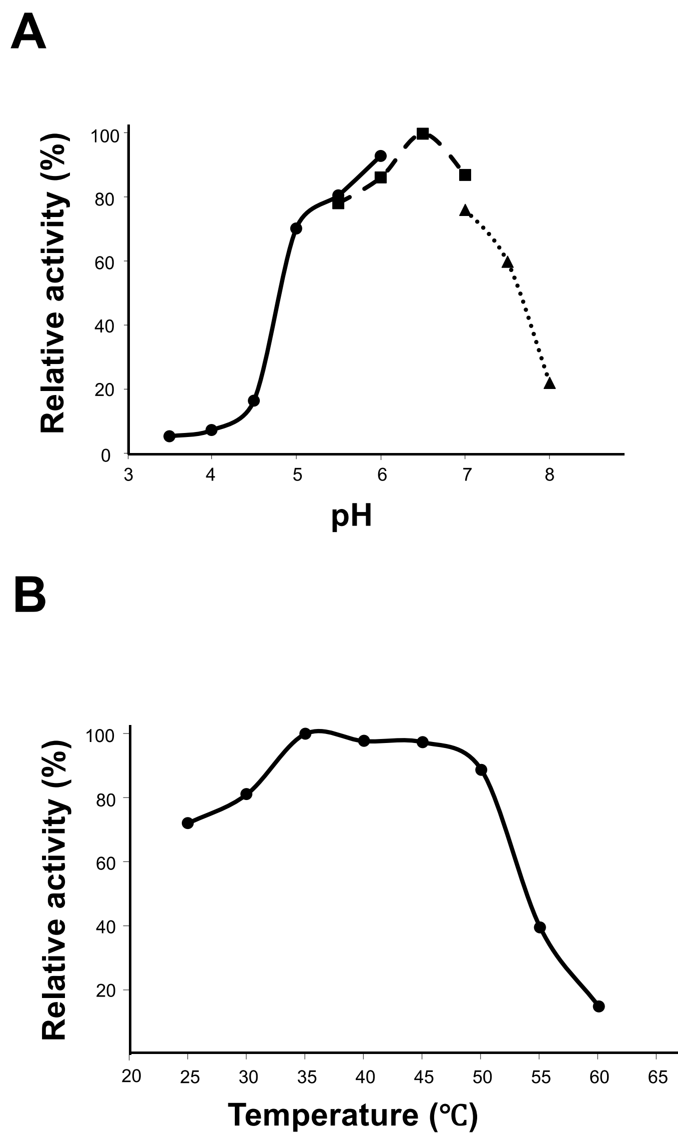

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45 **Supplementary Figure 3.** Optimal pH and temperature of AAfase. (A) pH dependence
46 of AAfase activity in various buffers at 45 °C for 20 min. Sodium acetate buffer (closed
47 circle and solid line), MES buffer (closed square and dashed line), and HEPES buffer
48 (closed triangle and dotted line) were used. Enzyme activities are expressed as a
49 percentage of the activity in MES buffer at pH 6.5. (B) The temperature dependence of
50 AAfase activity at pH 6.5 for 20 min. The enzymatic activities are expressed as the
51 percentage of the activity at 35 °C.

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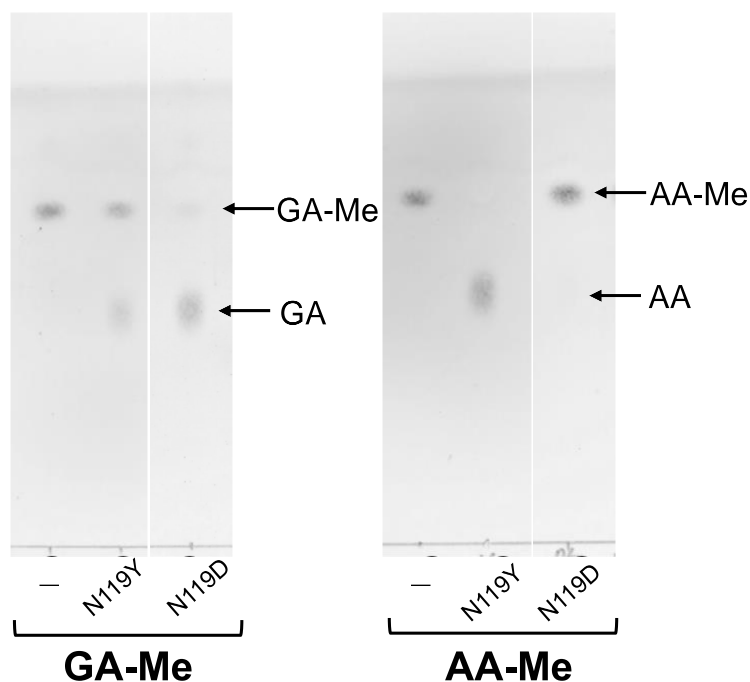

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55 **Supplementary Figure 4.** TLC analysis of the enzymatic activity of GAfase N119Y and GAfase
56 N119D. GA-Me and AA-Me are the abbreviation of α-D-Gal-(1→3)-α-L-Ara*f*-OMe and β-L-Ara*p*-
57 (1→3)-α-L-Ara*f*-OMe, respectively. 1 mM GA-Me and AA-Me were incubated with absence (−) or
58 presence (+) of the two types of mutants in 50 mM sodium acetate buffer (pH 5.0) at 37 ℃ for 18 h. As
59 enzymes, the elution fraction containing imidazole after His-tag affinity purification were used in this
60 study, because desalted GAfase N119D got lost enzymatic activity. The amount of enzyme was matched
61 when the same enzyme was used in order to see the activity on different substrates, although the quantity
62 of N119Y and N119D were different. The reaction mixtures were analyzed by TLC, as described in main
63 text.

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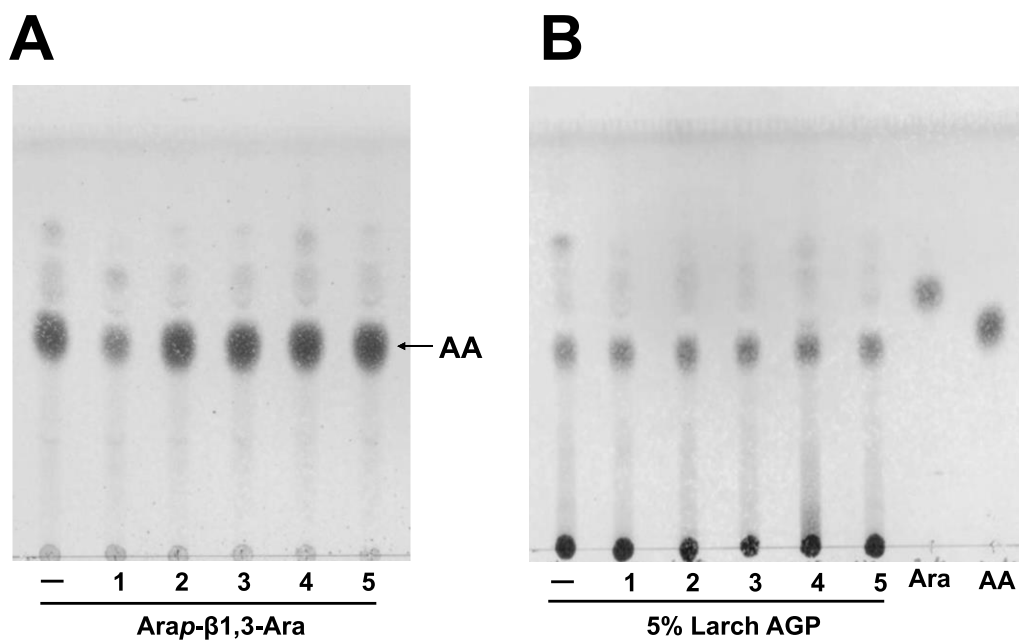

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67 **Supplementary Figure 5.** TLC analysis of the culture supernatants after incubation for

68 41 hours in β-L-Ara*p*-(1→3)-L-Ara (A) and and 48 hours in larch AGP (B). 1, *B.*

69 *pseudocatenulatum* MCC10289; 2, *B. pseudocatenulatum* MCC10285; 3, *B.*

70 *pseudocatenulatum* MCC10311; 4, *B. kashiwanohense* MCC10250; 5, *B.*

71 *pseudocatenulatum* JCM1200^{T}. As a control, the medium without any bacteria (−) was

72 used. AA is the abbreviation for β-L-Ara*p*-(1→3)-L-Ara.

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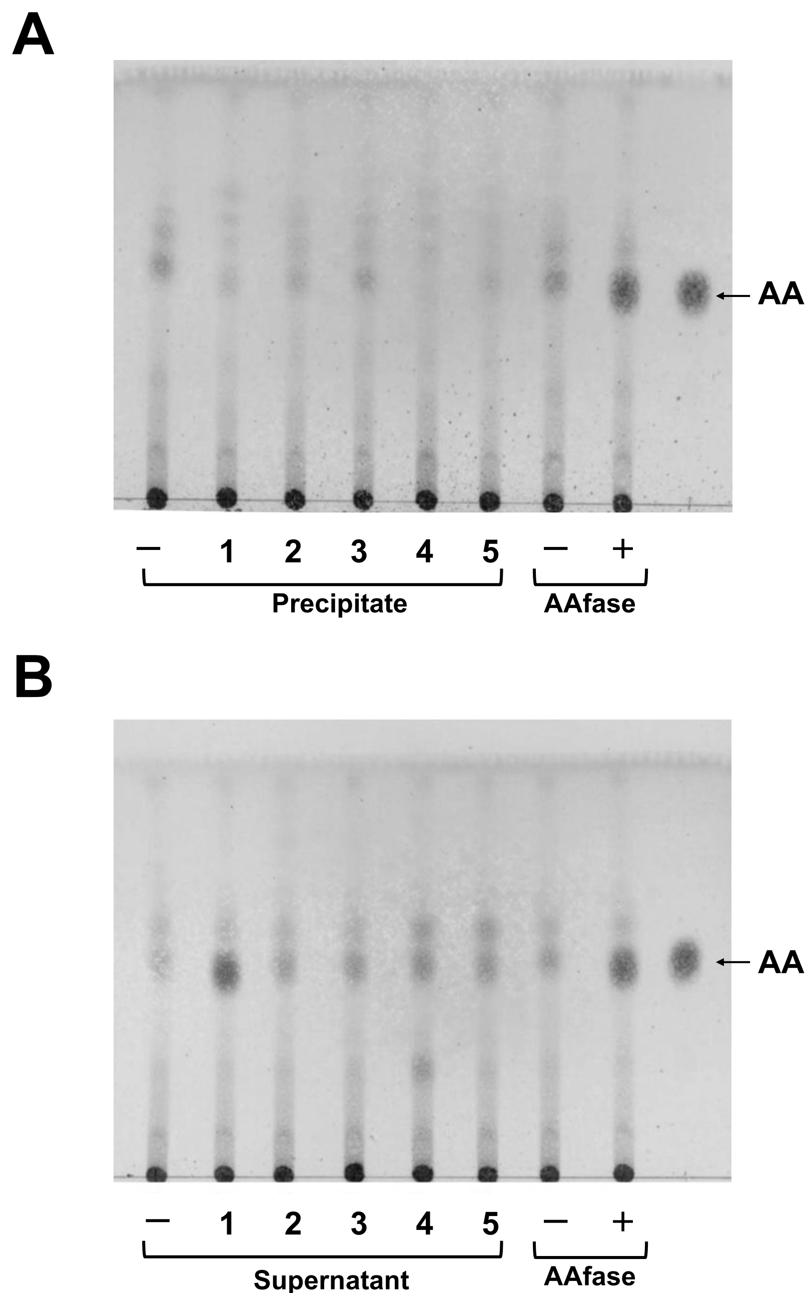

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76 **Supplementary Figure 6.** TLC analysis of the bacterial enzymatic activities with 2.5%
77 larch AGP. Larch AGP was incubated with bacterial cell fractions (A) and culture
78 supernatant (B) after cultivation of B. pseudocatenulatum MCC10289 (1), B.
79 pseudocatenulatum MCC10285 (2), B. pseudocatenulatum MCC10311 (3), B.
80 kashiwanohense MCC10250 (4), and B. pseudocatenulatum JCM1200T (5) grown in
81 larch AGP after 18 h incubation. As a control, the medium without any bacteria (−) was
82 used for enzymatic activity assay. As a control, larch AGP was incubated in the

83 absence (−) or presence (＋) of AAfase.

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