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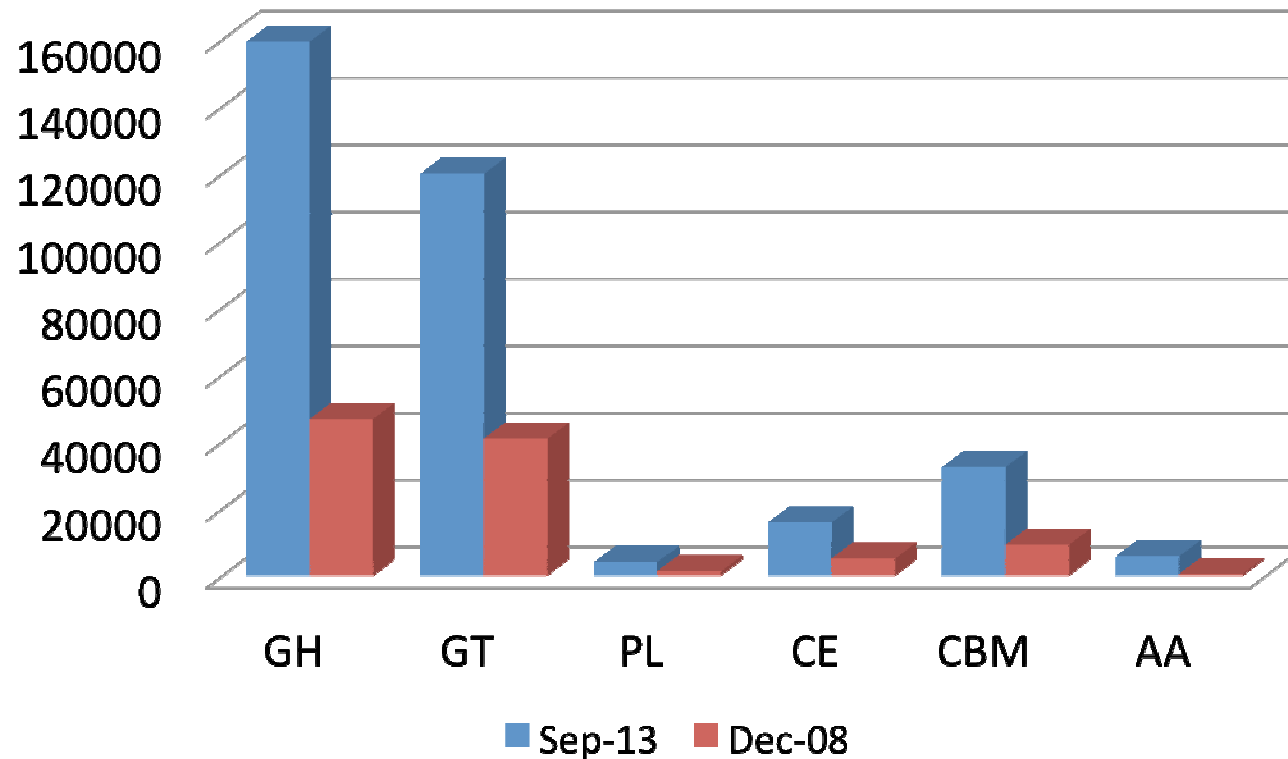
Exploring Carbohydrate Active Enzymes from Indonesian Microbial Diversity

Dr. Dessy Natalia

Center for Life Sciences
Biochemistry Division, FMIPA
Institut Teknologi Bandung



Carbohydrate Active Enzymes in www.cazy.org



Glycoside Hydrolases (GH); GlycosylTransferases (GT)
Polysaccharide Lyases (PL); Carbohydrate Esterase (CE)
Auxiliary Activities (AA)



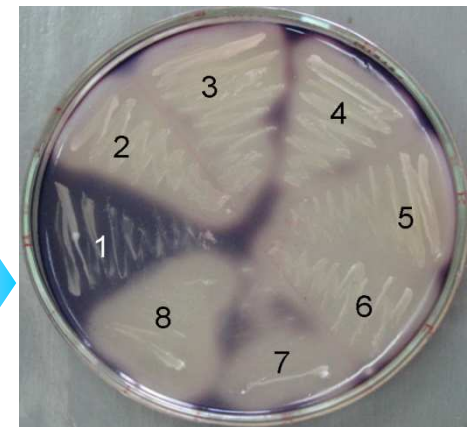
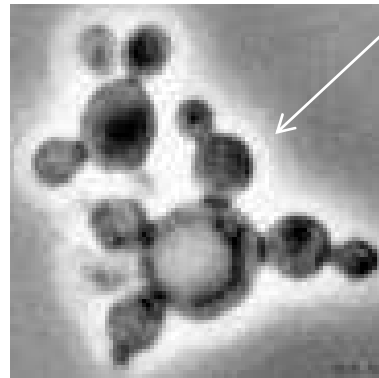
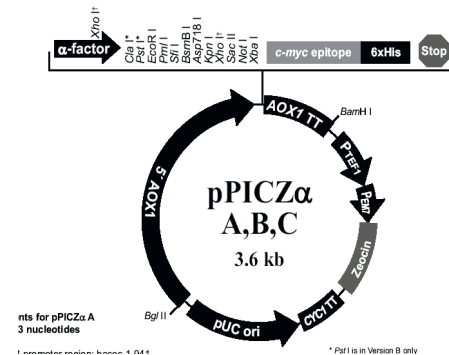
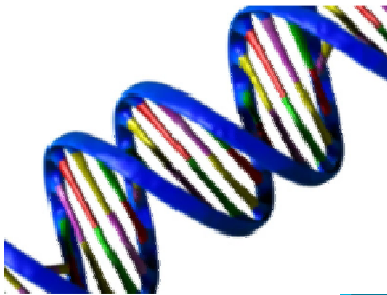
CAZy from Indonesia

No	Enzymes	Genes	Microorganisms	Sources	References
1	α -Amylase	SfamY	<i>Saccharomycopsis fibuligera</i> R64	Soil, West Java	Ismaya et al, 2013
2	α -Amylase	baqA	<i>Bacillus aquimaris</i> MKSC 6.2	Soft coral sinularia Sp.	Puspasari et al, 2013
3	Glucosylase	GLL1	<i>S. fibuligera</i> R64	Soil, Wes Java	Natalia et al, 2011
4	α -Amylase		<i>B. amyloliquifaciens</i> ABBD	Hard coral acropora sp.	Nurachman et al, 2010
5	α -Amylase	BmaN	<i>B. megaterium</i>	Sea anemon	Sanjaya et al. unpublished
6	α -Amylase		<i>Bacillus</i> sp. ALSHL3	Cilauteuren, Pameungpeuk, West Java	Vidilaseris et al, 2009
7	Glucosylase	GLL1	<i>S. fibuligera</i> R64	Soil, Wes Java	Natalia et al, 2011
8	CGTase		<i>Bacillus</i> sp.		Retnoningrum et al. Unpublished
9	β -D-Xylosidase	xyl43B	<i>Geobacillus thermoleovorans</i> IT-08	Hot spring, Gunung Pancar, Bogor, West Java	Ratnadewi et al, 2014
10	Thermoalkalophilic Xylanase		<i>B. halonduran</i> C-125	Cimanggu Hot Spring, West java, Indonesia	Ulfah et al, 2011
11	Arabinofuranosidase	abfAa1 dab abfAb3	<i>B. subtilis</i> DB104 and <i>B. licheniformis</i> CW 1		Nurcholis, et al., 2012
12	Fructansucrase		<i>Weissella confusa</i> MBFCNC-2(1)	Ice	Malik
13	Xylanase		<i>Bacillus</i> sp. AQ-1	Household aquarium sediment	Wahyuntari, et al. 2009
14	Xylanase		<i>Geobacillus stearothermophilus</i>	Tanjung Api, Poso	Saksono et al, 2011
15	Xylanase		<i>B. pumilus</i> PU4-2	Termite	Widjaja, et al. 2008
16	Endoglucanase	engA	<i>B. amiloliquefaciens</i> PSM 3.1	Hard coral	Nurachman et al, 2010
17	Xylanase		A xylanolytic-thermophilic bacterium	Gunung Pancar Hot Spring, West Java	Suwanto, et al., 2001
18	Cellulase		Metagenomic	Hot spring, West Sumatra	Moeis et al. 2011
19	Cellulase and Xylanase		<i>Rhizomucor variabilis</i> , <i>Fusarium</i> sp., <i>F. Solari</i> , <i>Aspergillus niger</i> , <i>B. thuringiensis</i> , <i>Sphingobacterium daejonense</i>	Tropical Forests in Java and Sumatra	Agustini, et al, 2012
20	Xylanase		<i>Streptomyces</i> 234P-16 and <i>Streptomyces</i> SKKI-8	Padang dan Sukabumi	Meryandini et al., 2008
21	Cellulase		<i>Trichoderma</i> spp.	Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development Indonesian Agency for Agricultural Research and Development	Lestari et al., 2014
22	Mannanase		Thermophilic Bacteria	Hot Springs from West Sumatra	Marlida, et al
23	Xylanase		<i>Bacillus</i> sp.	Pawan, Hot Spring Riau	Helianti, 2007
24	Xylanase		PC-01 Isolated bacterium	Pacet Hot Spring East Java	Yamani et al., 2012
25	chitinase		<i>Bacillus</i> sp. 13.26	Tompaso Hot Spring, North Sulawesi	Purwani, et al., 2004
26	Thermostable chitinase		<i>Bacillus licheniformis</i> Mb-2	Tompaso Geothermal Springs, North Sulawesi	Toharisman et al., 2005
27	Chitinase		<i>Bacillus</i> sp. ALSHL3	Kamojang Crater, West Java	Natsir et al. 2002
28	Chitinase		<i>Bacillus</i> sp. HSA, 3-1a	Sulili Hot Spring, South Sulawesi	Natsir et al, 2010
29	Chitinase and Chitosanase		unidentified (~100 micobrial strains)	Sponge Samples from diving in Nias and Binuangen	Uria et al. 2005
30	Chitin Deacetylase		<i>B. Thermoleovorans</i> LW -4-11	Langoan Hot Spring	Toharisman et al., 2008
31	Endochitinase		<i>Thricoderma virens</i> UKM-1		Alias et al, 2009
32	Chitinase		<i>Aeromonas caviae</i> WS7b	Soil sample from a black pepeer plantation on Bangka Island	Malik et al. 2003
33	Chitosanase		Inralaya Swamp Bacteria	South Sumatera	Baehaki et al, 2013
34	Chitosanase	Csn1	<i>Bacillus amyloliquifaciens</i> ABBD	Hard coral	Handa et al. 2013
35	Inulin fructotransferase		Actinomycete Nonumuraea sp.	Soil	Pudjiraharti et al,
36	Inulinase		<i>Bacillus licheniformis</i>	Hot spring, West Sumatra	Azhar et al. 2013



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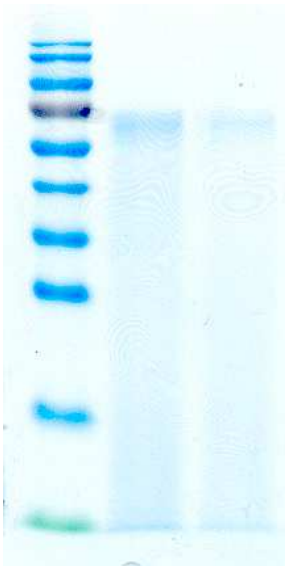
Production of Recombinant α -Amylases in *Pichia pastoris*



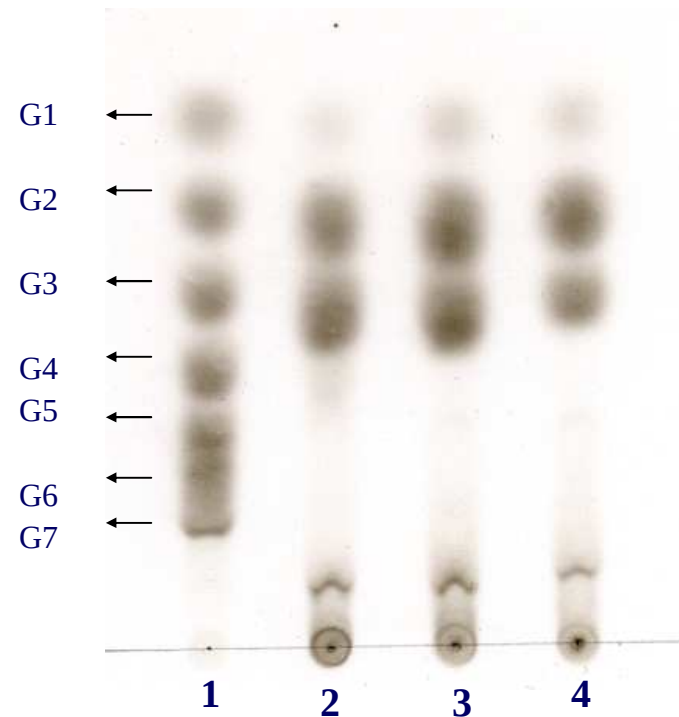


Saccharomycopsis fibuligera α -amylase

- pH : 5.5
- Temperature: 55°



SDS-PAGE Analysis



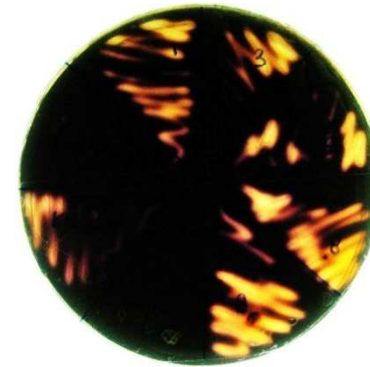
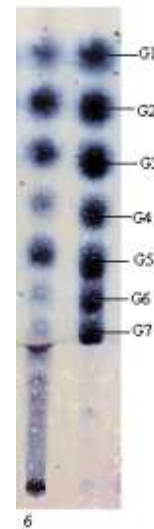
TLC Analysis





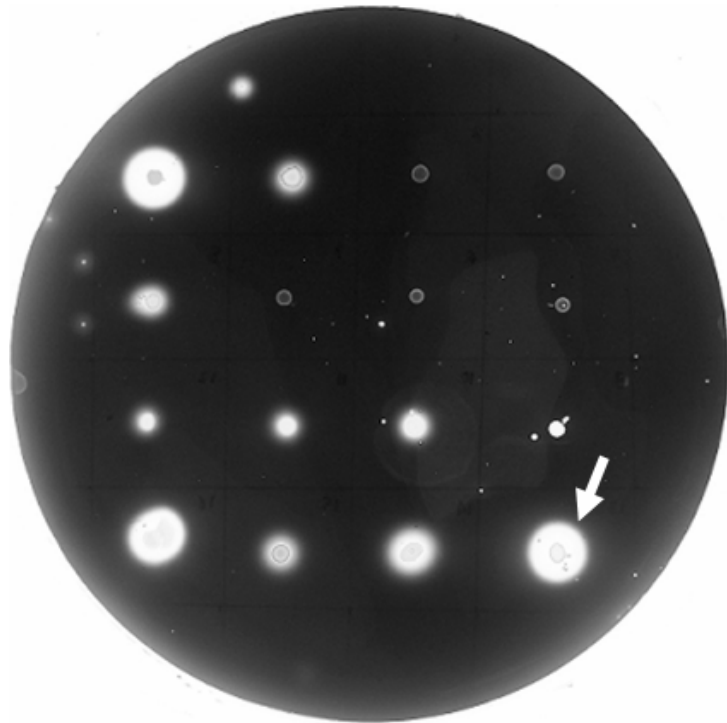
Bacillus licheniformis α -Amylases

- pH : 8
- Temperature: 70 ° C
- End Product:
maltopentaose,
maltotetraose,
maltotriose, maltose
and glucose

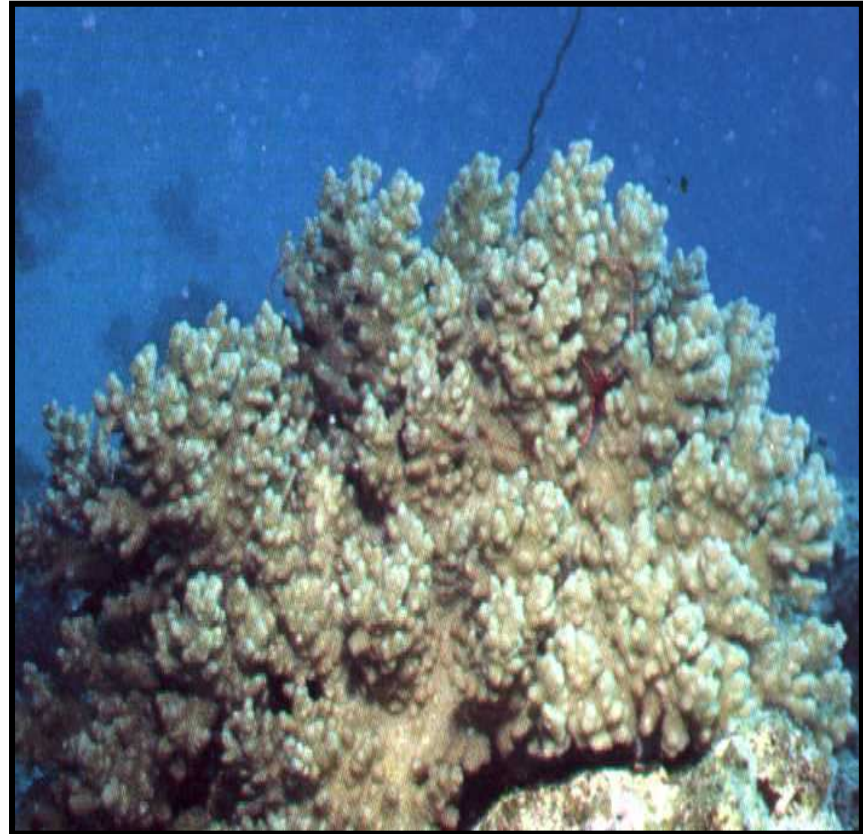




Secretion of Amylases by Marine Bacteria



***Bacillus aquimaris* MKSC6.2**

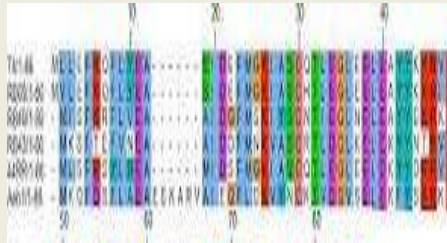


***Sinularia* sp**

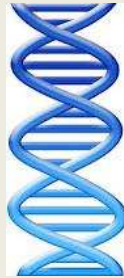


PCR Based Cloning of α -Amylase Gene from *B. aquimaris* MKSC 6.2

Degenerate Primers



Inverse PCR



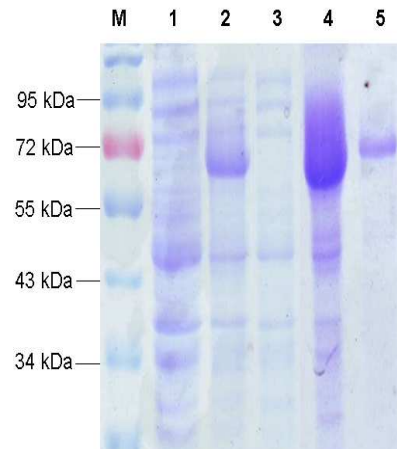
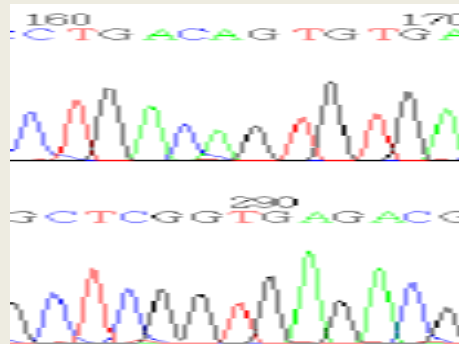
PCR



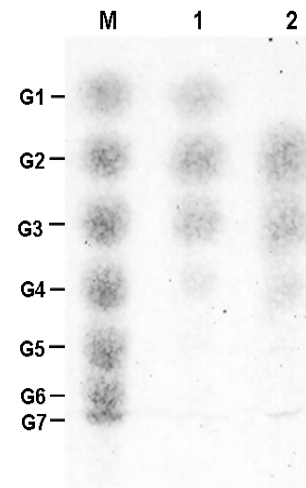
Clone PCR Fragments Into pGEMT vector



Subclone into pET30 Protein Expression in *E. coli*



SDS-PAGE
Refolded
BaqA



TLC
1.Casava
2.Corn

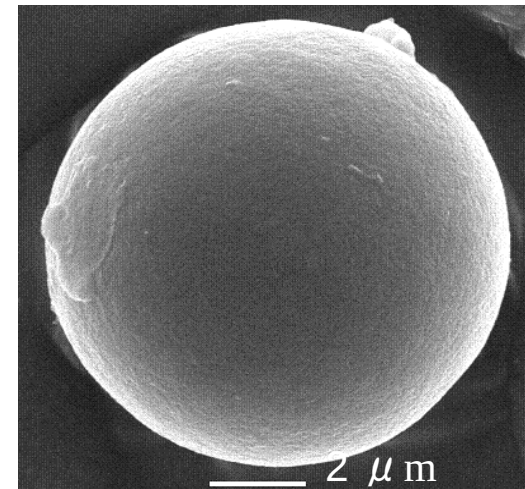
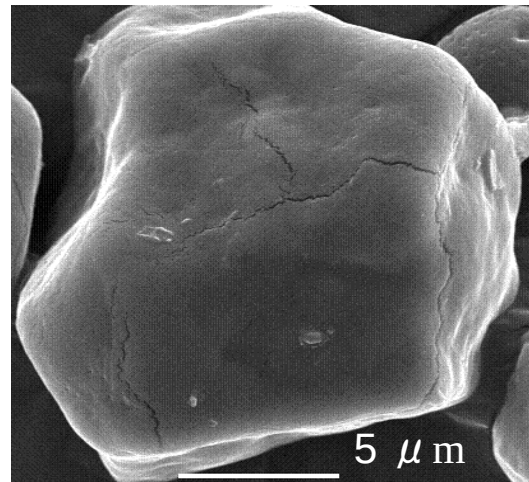




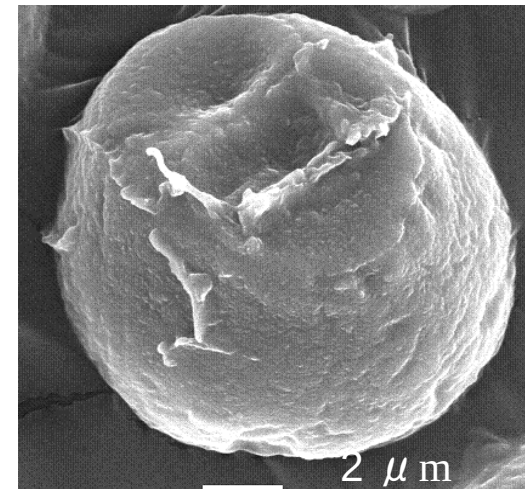
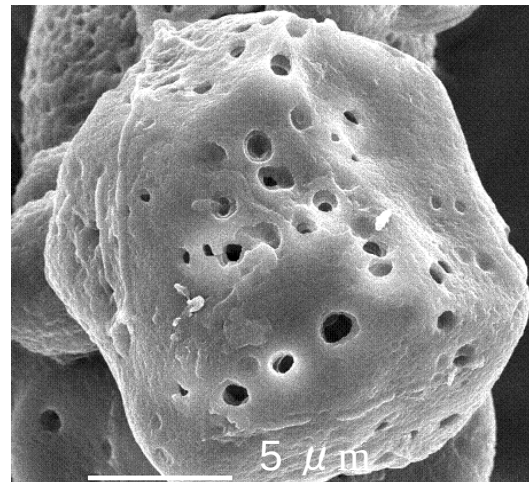
Corn

Casava

(-) BaqA

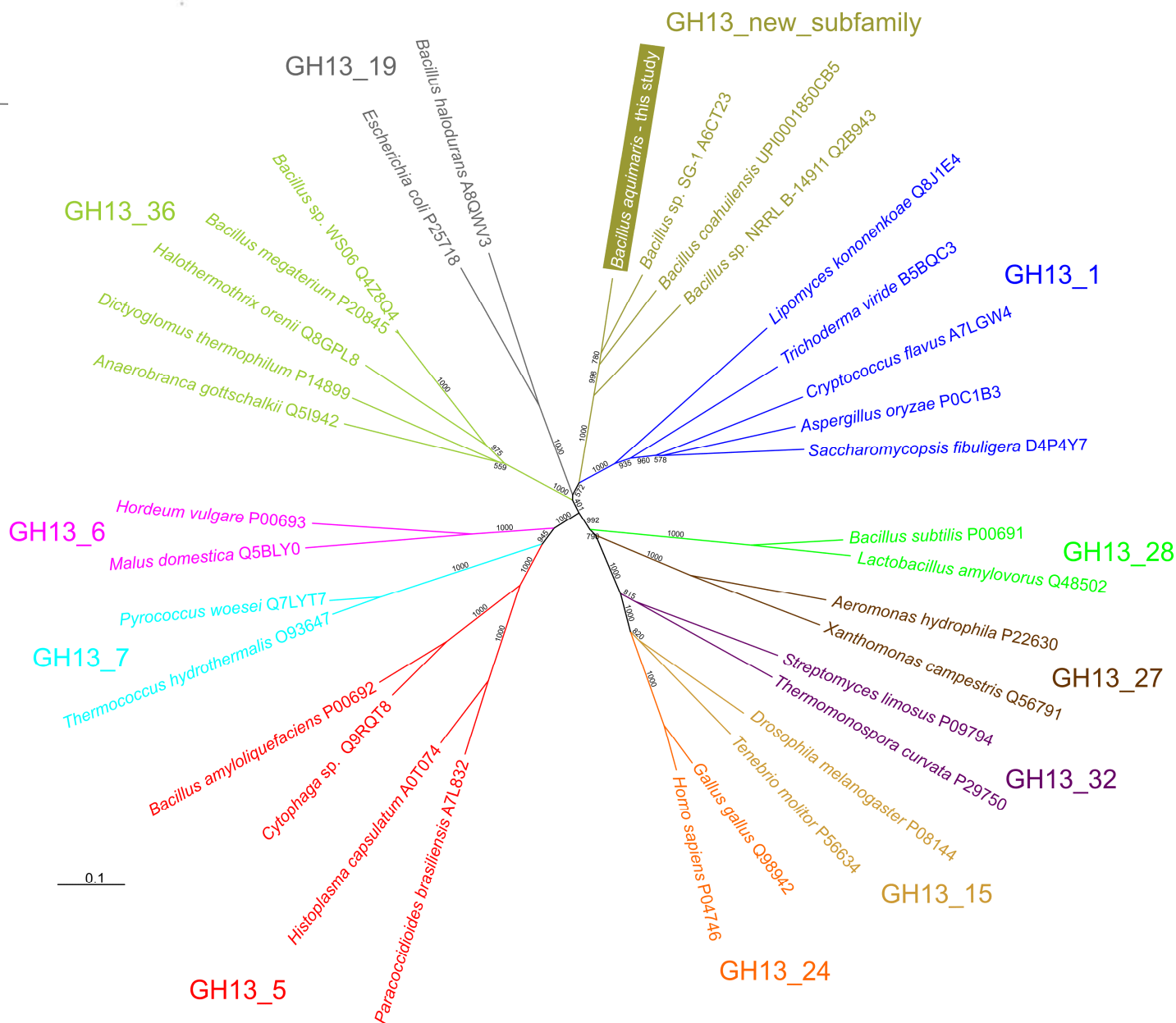


(+) BaqA





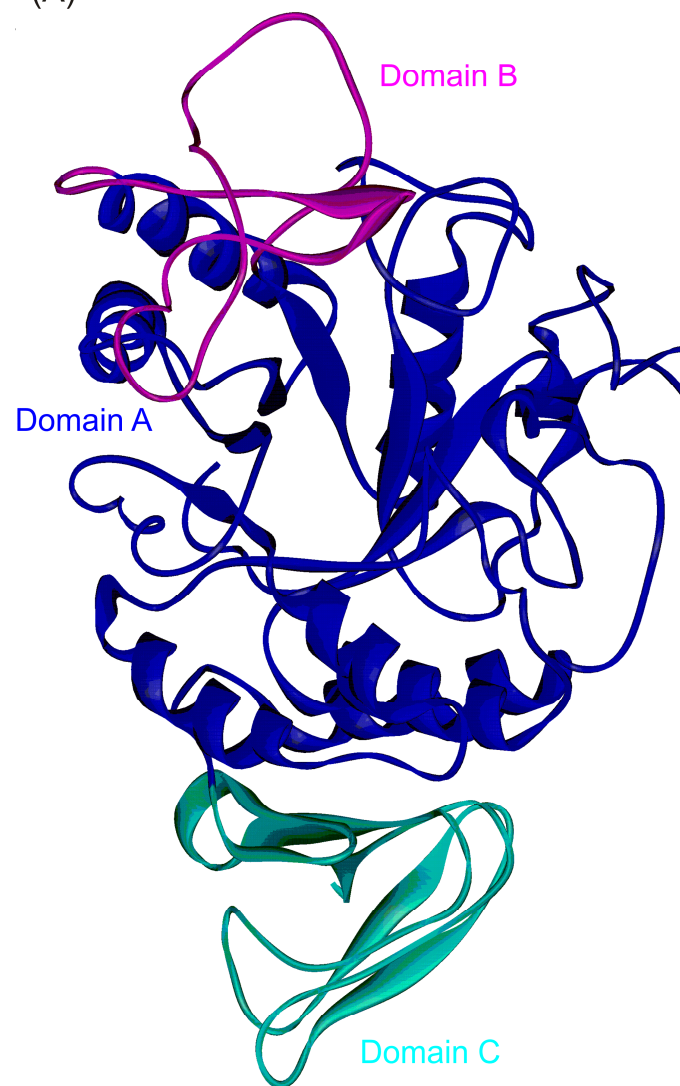
Evolutionary Tree of the Family GH13 α -Amylases



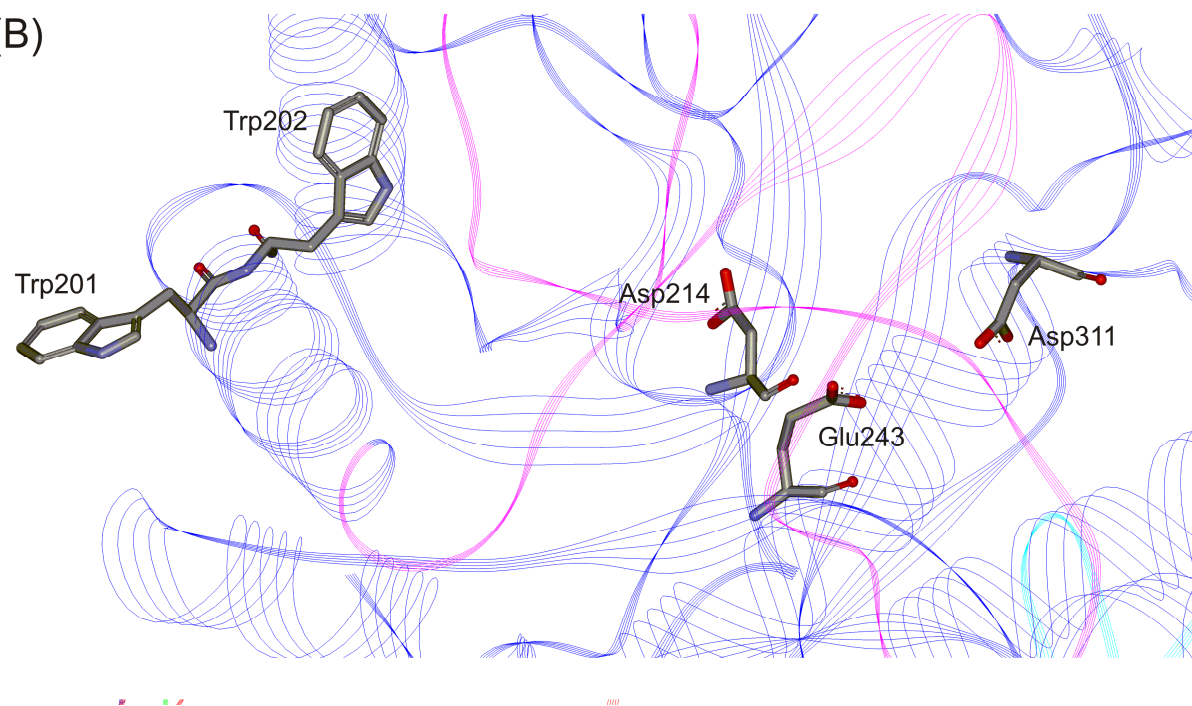


Model Structure of BaqA

(A)



(B)





Source and Identity of NL3 Isolate

***Bacillus megaterium* NL3**

Sea anemon

Entacmaea medusivora





Comparison of Amino Acid Sequences of Four Conserved Regions

Enzyme	Region I		Region II		Region III		Region IV		Acc. No
BmaN1			197	GYVVKDIDQ	220	GEING	288	FLDDVH	This study
BMQ_0659			197	GYVVKDIDQ	220	GEING	288	FLDDVH	YP_003561127
BMAMY	135	DLVVNH	228	GFRLDAALH	272	GEVWD	334	FLTNDH	X07261
BSG1_21175	102	DFIVNH	175	GYRLDTRH	208	GEVFD	271	FIDNHD	ZP_01861961
BmaN3	99	DIVMNH	228	GYRLDAIKH	262	GEFVN	324	FVDNHD	This study
BMQ_5241	99	DIVMNH	228	GYRLDAIKH	262	GEFVN	324	FVDNHD	NC_0104019
BMWSH_0036	99	DIVMNH	228	GYRLDAIKH	262	GEFVN	324	FVDNHD	AEN86920
AmyAc_bac	99	DLVMNH	228	GFRLDAIKH	262	GEFVN	324	FVDNHD	CAA43194





My Team in The Lab





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Thank You

