

A New sphingomyelinase (SMase) from *Streptomyces* & its application in processing phospholipids

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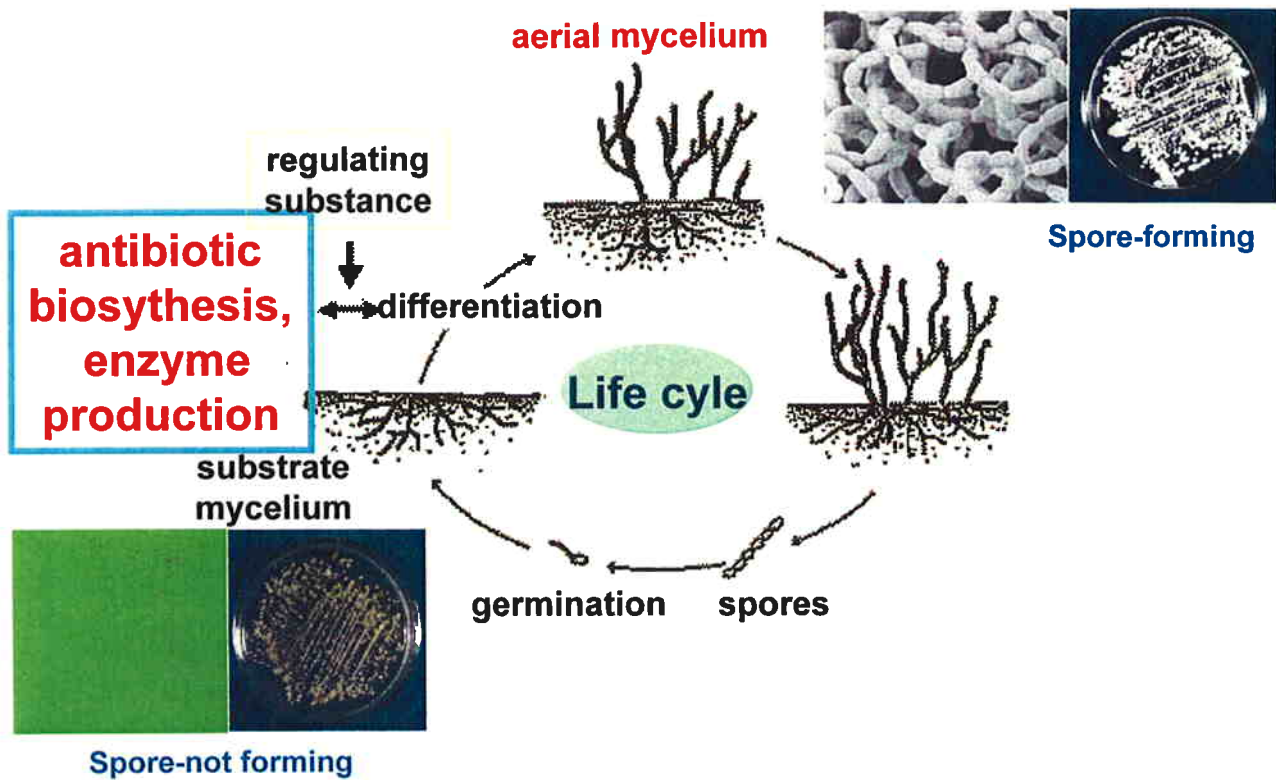
 - Streptomyces* system

 - Phospholipase and SMase

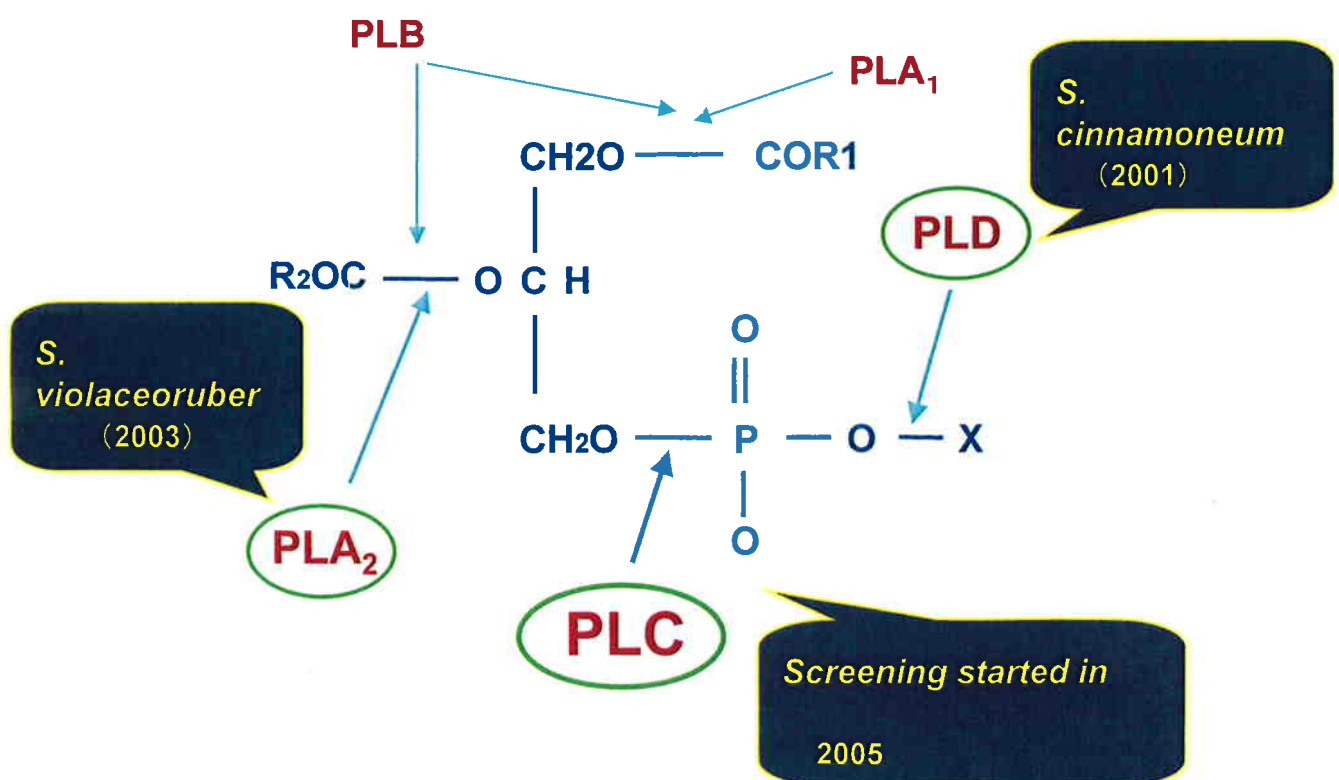
- Production of SMase

- Application of SMase

Streptomyces



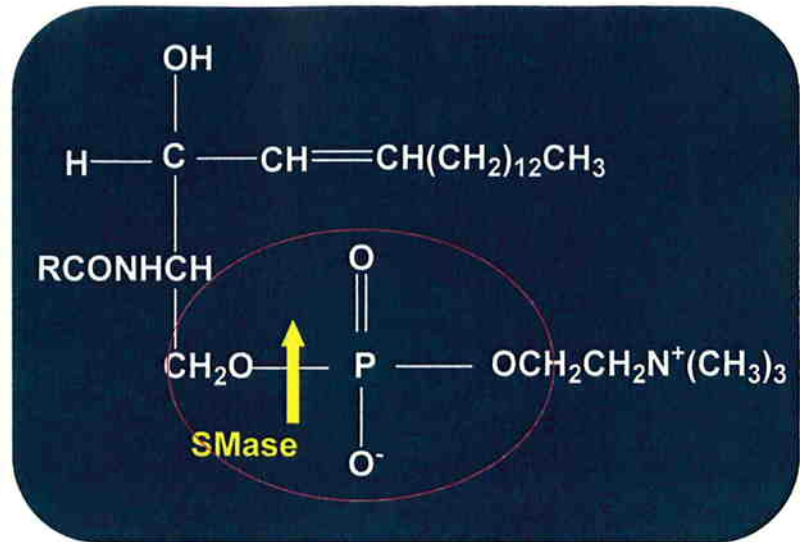
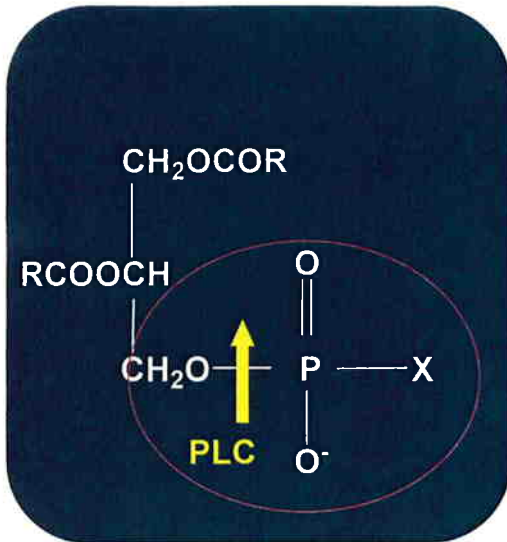
Work on Phospholipases



PLC and SMase

glycerophospholipids
(PA, PC, PE, PG, PI, PS)

sphingomyelin
(SM)



Screening for production strain

Screening: Substrate 6 kinds, test for diacylglycerol

Strain		Specificity					
		PA	PC	PE	PI	PS	SM
<i>S.griseus</i>	NBRC 13350		○	○	○		○
<i>S.avermitilis</i>	NBRC 14893	○	○	○	○	○	○
<i>S.hachijoensis</i>	NBRC 12782	○	○		○	○	
<i>S.netropsis</i>	NBRC 12769	○	○	○	○	○	○
<i>S.mobaraensis</i>	NBRC 13819	○	○	○	○	○	

PA : Phosphatidic acid

PE : Phosphatidylethanolamine

PS : Phosphatidylserine

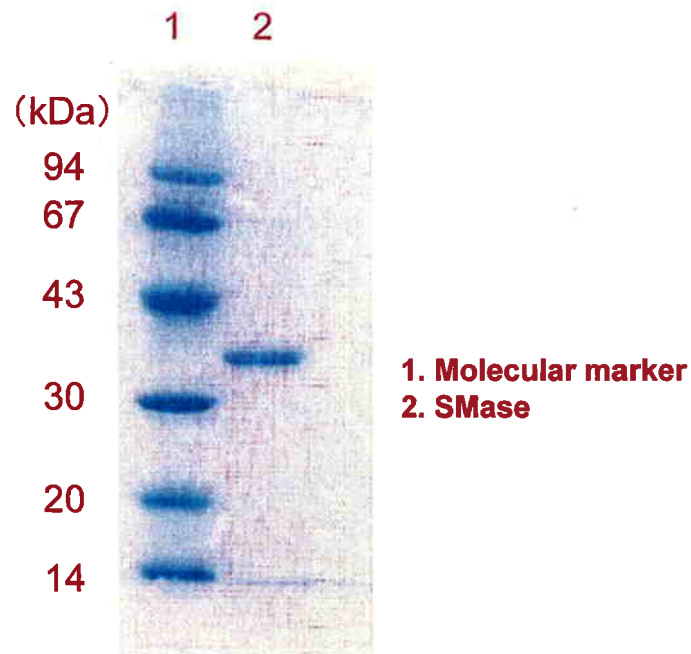
PC : Phosphatidylcholine

PI: Phosphatidylinositol

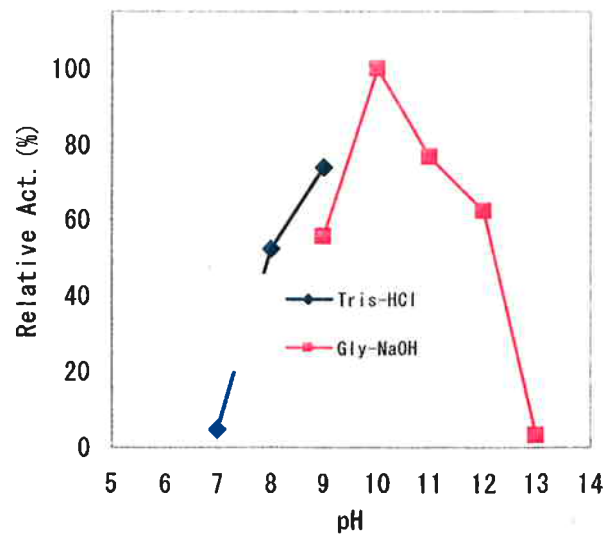
SM : Sphingomyelin

PLC or SMase

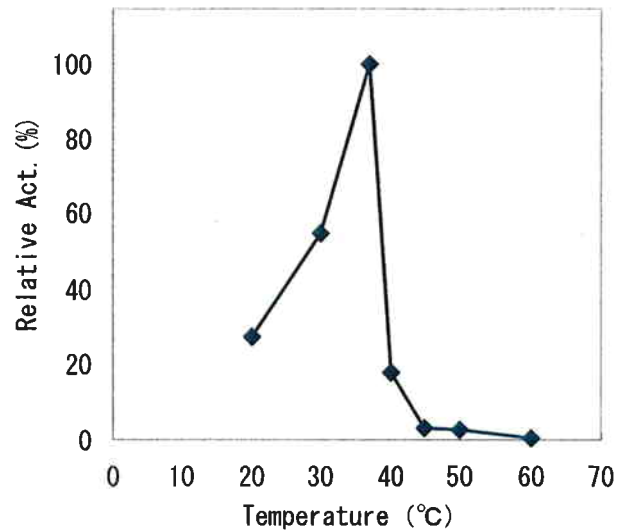
Purification from Streptomyces hachijoensis



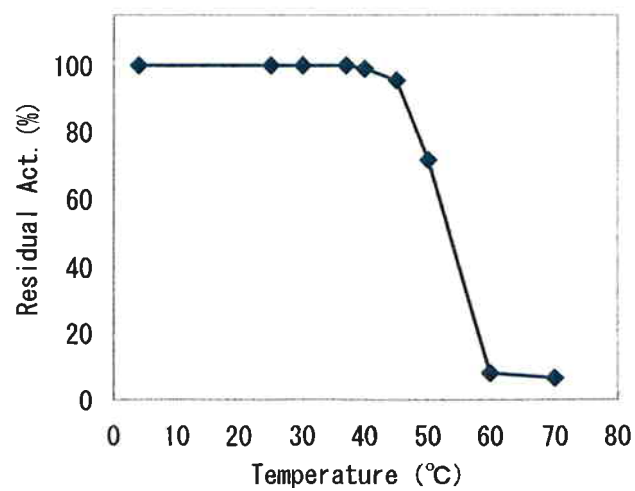
Optimum pH



Optimum Temperature



Thermostability



Specific Activity

Substrate (2mg/ml)	Relative Act. (%)
Sphingomyelin	100
Phosphatidylcholine	2
Phosphatidylinositol	0
Phosphatidic Acid	0
Phosphatidylethanolamine	0
Phosphatidylglycerol	0
Phosphatidylserine	0

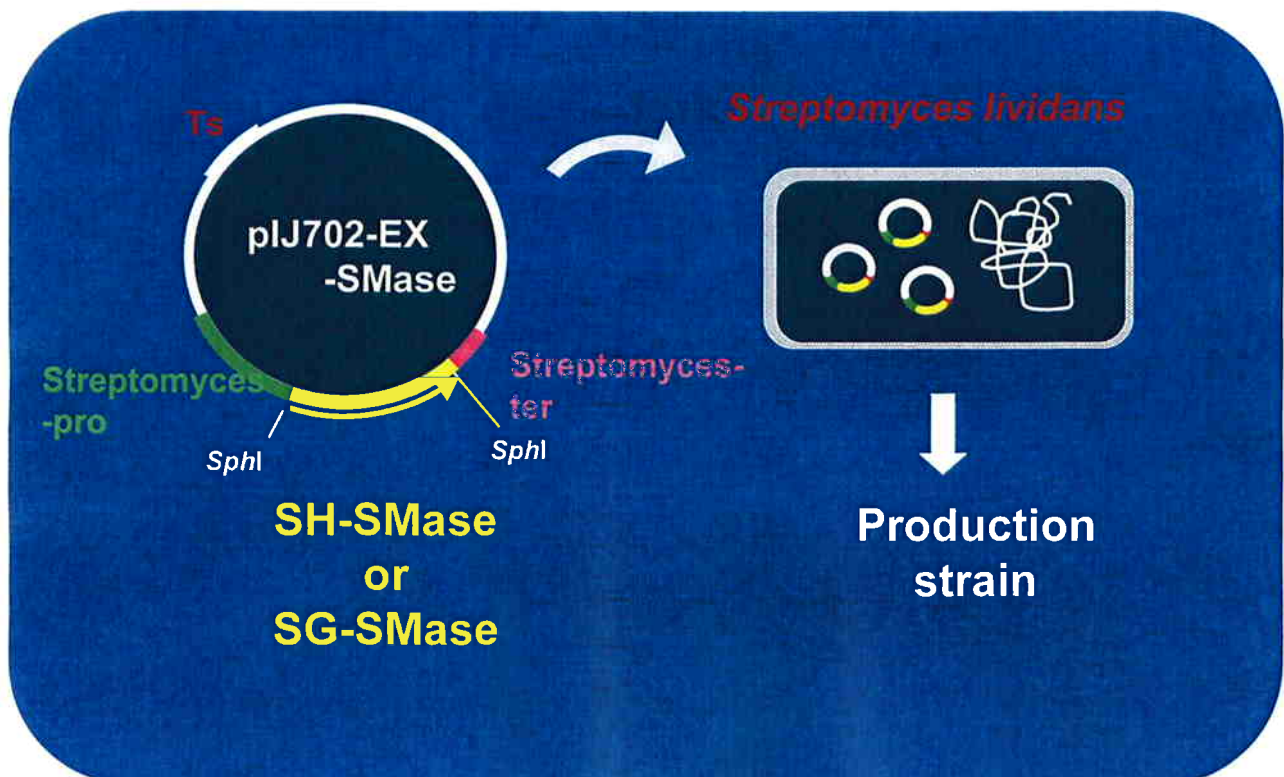
Sequence of SH-SMase

```
1' AGCACCAATC TCTATCCGAA CTGGGGCCAG GAGCACCGGG CCAAGGAGAT AGCGGCCGCC
61' GGCTTCTTCC AGGGCAACGA CGTGGTGGTC CTCCAGGAGG CGTTCGACAA CGCGACCACG
121' GACGCGCTCA AGTCCGCCGC CGCCGACCGG TACCCGTACC AGACGCCGGT CGTCGGGCGG
181' GGCAAGGACG GCTGGGACGC CACCGGCGGG GCCTACTCCA CCACCACGCC GGAGGACGGC
241' GGTGTGACGG TCCTCAGCAA GTGGCCGGTC GTGCGCAAGG AACAGGTGAT CTTCAACGAC
301' GCGTGC GGCG CCGACTGGTG GTCCAACAAG GGCTTCGCCT ACGTCGTGCT GAACGTGGGC
361' GGCGCCAAGG TGCACGTCGT GGGGACGCAC CTGCAGTCGA CGGACAGCGG CTGCAAGGCC
421' GGCGAGGCCG CCGCGGACCG CTCCAAGCAG CTGAAGCAGA TCGACGCGTT CCTCGACGCC
481' AAGAACATCC CGGCGAACGA GCAGGTCATG ATCGCCGGTG ATCTGAACGT GGACTCCCGC
541' AGCGCCGAGT ACCGATCCAT GCTCGCCGAC GGCGACCTCG CCCC GGCGGA CGCCCGCACC
601' GGGCACCCGT ACTCCTTCGA CACCAAGGAG AACTCGATCG CGGCCTACCG CTACCCACC
661' GACGCCCCGC AGGACCTCGA CTACGTCCTC CACCGCAACG GCCACGCCCG GCCCCGCGGC
721' TGGCGGAACA CCGTGATCGC GGAGCGGTCC GCGCCGTGGA AGGTCTCCAG CTGGGGCAAG
781' GAGTACACCT ACACCAACCT C
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SH-SMase & SG-SMase

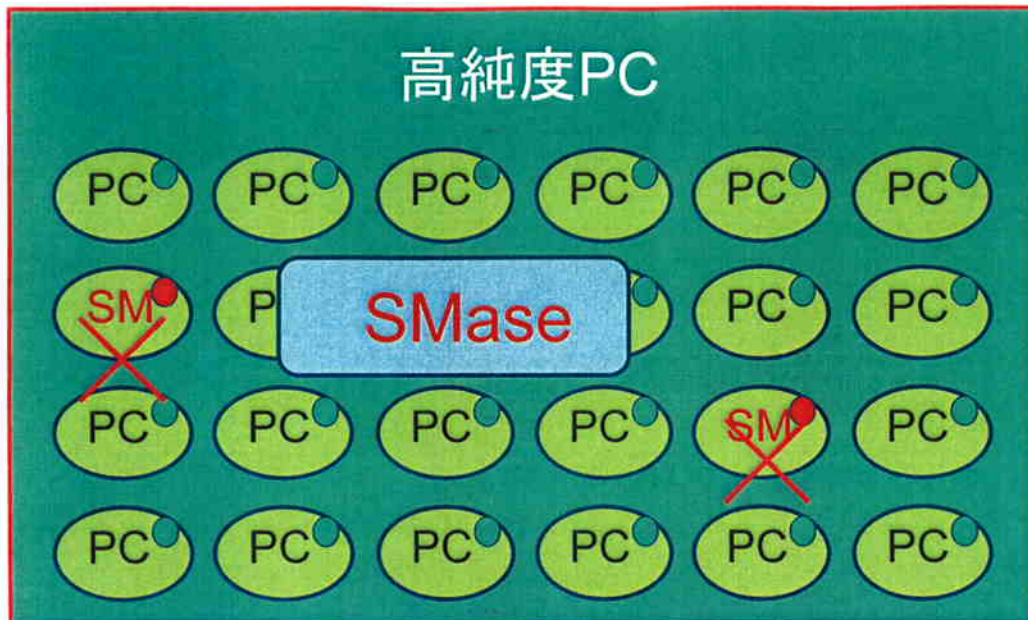
	SH-SMase	SG-SMase
Opt. Temp.	37°C	37°C
Opt. pH	10.0	11.5
Thermo-Stability	95% at 45°C, 30min	95% at 37°C, 30min.
metal ion Activator	Mg, Mn, Co, Cu	Mg, Mn, Co, Cu, Ca
Substrate Spec.	subs. PC: 5.9 (subs. SM: 100)	subs. PC: 0.8 (subs. SM: 100)

Production Strain

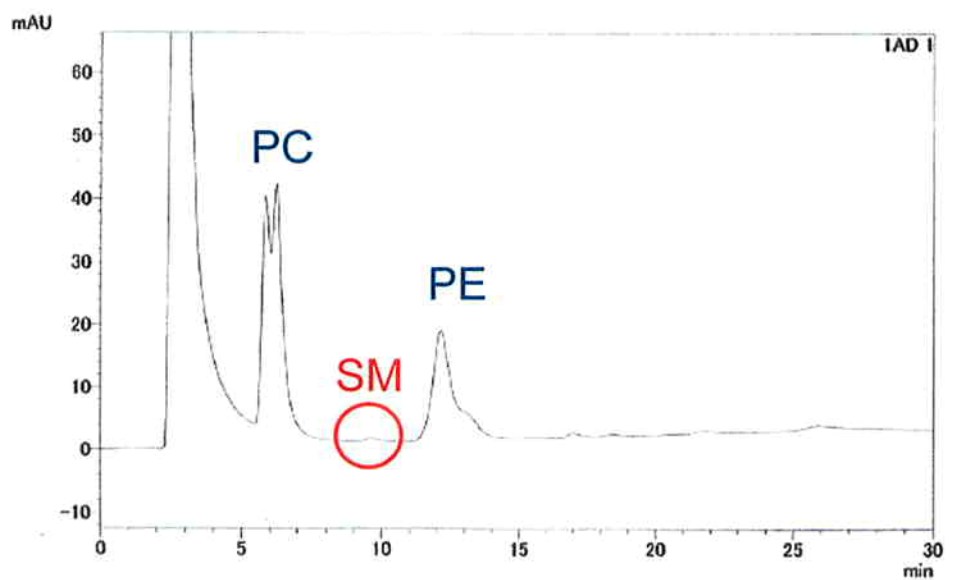


Application 1:

Removing SM from egg lecithin

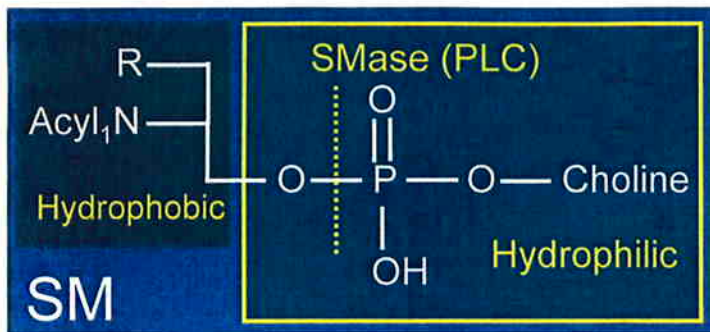
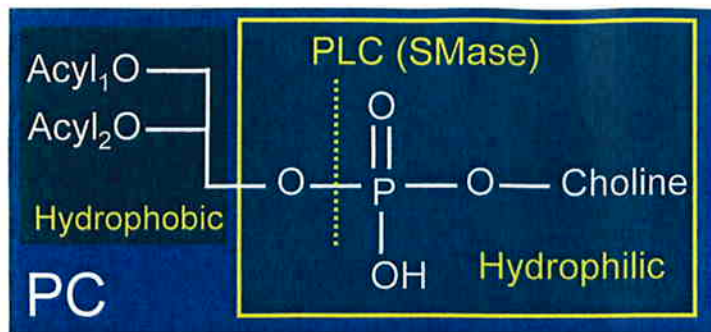


HPLC Analysis (PC30%)

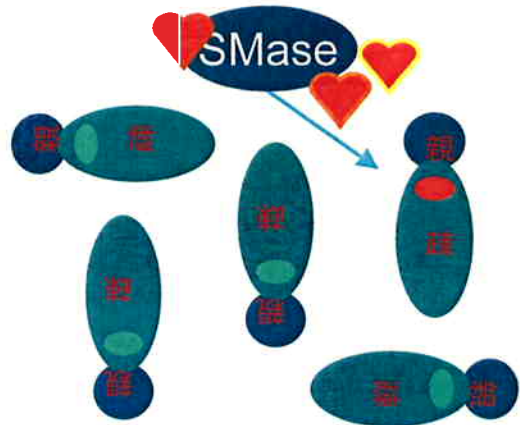
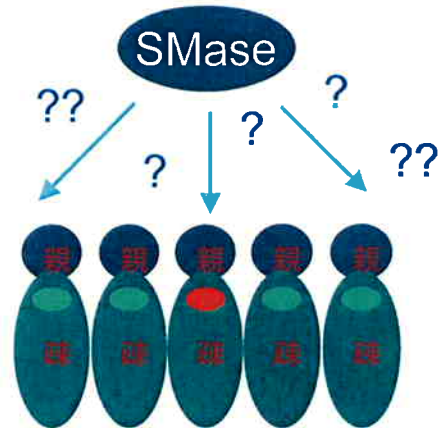


Commercial products: PC 30-98%、SM 0.5-2%

Increased Spec. for SM

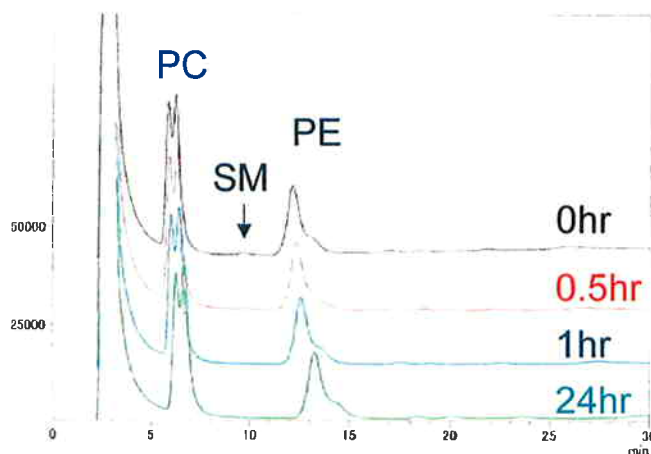


Add ethanol into the system

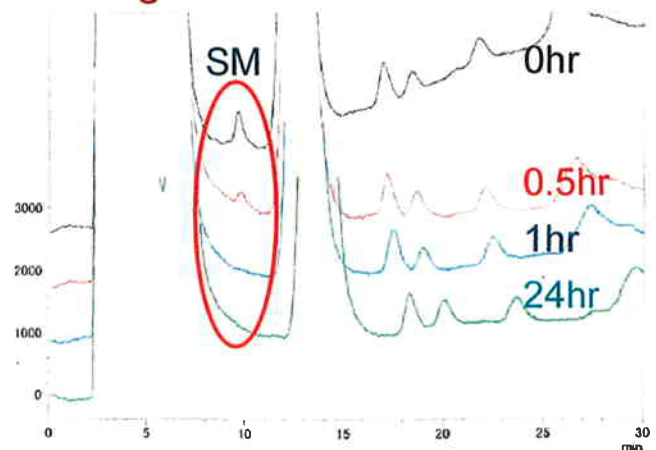


SM Hydrolysis (PC30%)

HPLC



Enlargement



SM amount: <0.1%

Summary & Future Work

- A new sphingomyelinase from *Streptomyces* is useful for production of egg phosphatidylcholine of pharmaceutical grade
- Potential application involves ceramide production as cosmetic material