Identification of squalene-hopene cyclase (SHC) gene from *Alicyclobacillus* spp.

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ABSTRACT

The *Alicyclobacillus* spp. are spore-forming Gram positive bacteria that are prevalently implicated in fruit juice spoilage. This is due to the ability of the *Alicyclobacillus* spores to survive in the acidic fruit juice environment even when they are exposed to pasteurization temperatures during production. Therefore, a rapid, specific and sensitive detection method to detect the presence of *Alicyclobacillus* spp. in both raw materials and final products are desired for industrial quality control. A TaqMan® real time PCR assay targeting at the squalene-hopene cyclase (SHC) gene has the potential to address the issue. The SHC is a key enzyme in the biosynthesis of hopanoid which is believed to confer thermal resistance to *Alicyclobacillus* spp. To date, only SHC gene sequences from *A. acidocaldarius* and *A. acidoterrestris* are available on GenBank. The objective of this study was to identify SHC gene sequences from other alicyclobacilli with spoilage potential. These include *A. herbarius*, *A. hesperidum*, *A. acidiphilus* and *A. acidocaldarius subsp. rittmannii.* Whole genome sequencing on *Alicyclobacillus* spp. was performed using the Ion PGM™ System. The sequencing data were analyzed and SHC gene sequences were identified bioinformatically.

INTRODUCTION

The *Alicyclobacillus* spp. are identified as one of the predominant microorganisms that causes beverage spoilage. The *Alicyclobacillus* spp. are commonly implicated in fruit juice spoilage because of their ability to survive in highly acidic environments (pH 3-6) even though they are exposed to pasteurization during production. The spoilage of the fruit juice is due to the production of guaiacol, which causes off-flavors.

Conventional industrial practice to detect *Alicyclobacillus* spp include agar plate counting or bacterial media enrichment which is followed by biochemical analysis. However, these methods can take days to weeks to complete. Therefore, a rapid, specific and sensitive detection method to detect the presence of *Alicyclobacillus* spp. in both raw materials and final products are desired for industrial quality control. A TaqMan® real time PCR assay targeting at the squalene-hopene cyclase (SHC) region has the potential to address the issue. The SHC is a key enzyme in the biosynthesis of hopanoids which confers membrane stabilizing functions. Study has shown that there is an increase in hopanoid concentration in membranes of *A. acidocaldarius* at high temperature and in acidic environment. This makes the SHC a suitable target for PCR-based detection.

To date, only SHC gene sequences from *A. acidocaldarius* and *A. acidoterrestris* are available on GenBank. The objective of this study was to identify SHC gene sequences from other alicyclobacilli with spoilage potential. These include *A. herbarius*, *A. hesperidum*, *A. acidiphilus* and *A. acidocaldarius subsp. rittmannii.* Whole genome sequencing on *Alicyclobacillus* spp. was performed using the Ion Torrent PGM™ System. The sequencing data were analyzed and SHC gene sequences were identified bioinformatically.

MATERIALS AND METHODS

Bacteria collection and gDNA extraction

A. herbarius (DSM 13609), *A. hesperidum* (DSM 12489), *A. acidiphilus* (DSM 14558) and *A. acidocaldarius subsp. rittmannii* (DSM 11297) were purchased from DSMZ, Germany. They were grown in BAT media (Schaulab) at 40-55°C, 200rpm in a shaking incubator for 48 hours. Genomic DNA (gDNA) was extracted using PureLink® Genomic DNA Mini Kit (K182002) from Life Technologies.

Sequencing Workflow

1.0µg of gDNA each from *A. herbarius*, *A. hesperidum*, *A. acidiphilus* and *A. acidocaldarius subsp. rittmannii* were fragmented using the Ion Xpress™ Plus gDNA Fragment Library Kit (P/N: 4471989; Life Technologies). Adaptor-ligated-200bp-library was prepared according to the procedure recommended in the kit. The adaptor-ligated-200bp-library was subjected to clonal amplification using Ion One Touch 200 Template v2 DL kit (P/N: 4480285; Life Technologies). Sequencing was performed using the Ion Torrent PGMTM Sequencing 200 Kit v2 (P/N: 4482006; Life Technologies) on 316 chips.

Data analysis

The raw sequencing data from Ion Torrent PGMTM was assembled into contigs using Assembler plugin provided by the Torrent Suite Software. CodonCode Aligner was used to identify the SHC gene.

RESULTS

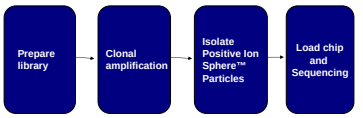


Figure 1. Sequencing workflow with Ion PGM™

Steps involved in Ion PGM™ Sequencing includes:

a) Enzymatic gDNA fragmentation and size-selection using E-gel
b) Clonal amplification of the fragmented size-selected library on ion sphere particles (ISPs) with Ion One Touch
c) Purification of positive ISPs with ES
d) Sequencing using 316 chip on Ion PGM™

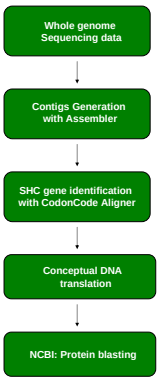


Figure 2. Data analysis: Workflow for identification of SHC gene

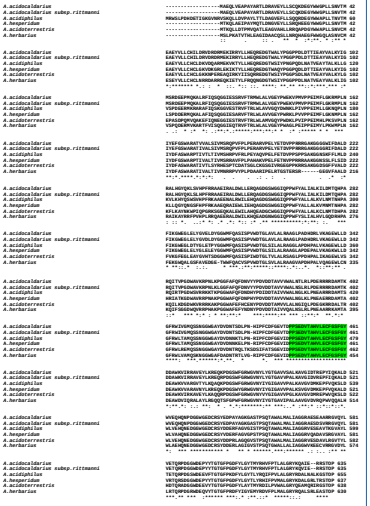
The raw sequencing data from Ion Torrent was assembled into various contigs using the Assembler plugin provided by the Torrent Suite Software. The SHC gene was identified from the contigs with the CodonCode aligner. The identified SHC gene was conceptually translated into protein sequence and subjected to protein blasting using NCBI.

Table 1. Summary of data analysis.

Two sequencing runs were used to identify the SHC gene sequences. If the sequencing data from 2 runs were not 100% homologous, multiple sequencing runs were performed as shown in *A. acidocaldarius subsp. rittmannii.* The length of the SHC gene is defined by the start and stop codons.

No	Species	Run No	SHC gene		
			Full seq	Run consensus	Length (bp)
1	*A. herbarius*	1	Yes	100%	1881
		2	Yes		
2	*A. acidiphilus*	1	Yes	100%	1956
		2	Yes		
3	*A. acidocaldarius subsp. rittmannii*	1	Yes	Extra g	
		2	Partial	No g	
		3	Partial	No g	
		4	Yes	No g	1896
		5	Yes	No g	
4	*A. hesperidum*	1	Yes	100%	1902
		2	Yes		

(A)



(B)

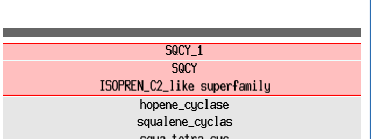


Figure 3. Multiple alignment of SHC

(A) The SHC gene sequences from *Alicyclobacillus spp* were identified using the workflow shown in Figure 2. The SHC gene sequences were conceptually translated into protein sequences and aligned. The boxed region (green) showed a conserved squalene cyclase domain.

(B) The conserved amino acid sequence was subjected to protein blasting using NCBI blast function.

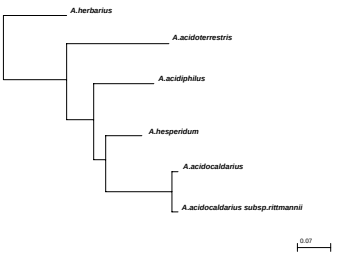


Figure 4. Phylogenetic tree based on SHC gene of *Alicyclobacillus* spp

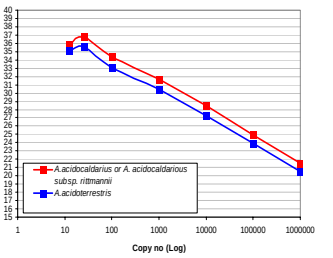


Figure 5. TaqMan® assay for *Alicyclobacillus* spp

Multiplex TaqMan® assay based on the SHC gene was developed to detect the *A.acidocaldarius, A.acidocaldarius subsp rittmannii and A.acidoterrestris.*

DISCUSSION & CONCLUSION

SHC gene sequences were identified from *A. herbarius*, *A. hesperidum*, *A. acidiphilus* and *A. acidocaldarius subsp. rittmannii* using sequencing data obtained from the Ion Torrent PGM™. Bioinformatics analysis indicated that the protein encoded by the SHC gene have a conserved squalene cyclase domain (PPSEDVTAHVLECFGSFGY) which is essential in the biosynthesis of hopanoid.

Development of a TaqMan® assay for the detection of *Alicyclobacillus* spp was done based on the SHC gene sequence. This real time PCR assay provides a sensitive, specific and fast method to detect *Alicyclobacillus* spp contamination during beverage processing and production. In addition, a multiplex TaqMan® assay is currently being developed to allow simultaneous detection and differentiation of multiple *Alicyclobacillus* spp.

REFERENCES

Siedenburg, G and Jendrossek, D. 2011. Squalene-Hopene Cyclases. Applied and Environmental Microbiology, June 2011, p. 3905–3915.

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