Highly sensitive and easy procedure to detect contaminated halophiles in food products containing natural salt by polymerase chain reaction

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Summary

Since the trade of salt has become free competition until 1997 in our country, we can eat many kinds of domestic and imported food products made by natural salts (including itself). Most of natural salts contain contaminated microorganisms, especially halophiles. From ancient, the reddish color of the products has indicated contaminants, because most of halophiles contain carotenoids in the cells. Also, specific membrane-lipids have been utilized to detect the halophiles in food materials by thin-layer-chromatography. These conventional procedures are still reliable, however they also require both relative large amount of the cells in the material and long time-investigation to obtain result. In this study, I tried to establish the highly sensitive and easy procedure to detect contaminated halophiles in food products containing natural salt based on polymerase chain reaction (PCR).

The whole genome sequence of *Halobacterium* sp. NRC-1 was published in 2000 (*PNAS*, **97**, 12176-12181, 2000). The genome of this organism contains unique insertion sequences (insertion sequence of halophile, ISH) in the genome wide. There are twelve ISHs in the genome (ISH1, ISH2, ISH3, ISH4, ISH6, ISH7, ISH8, ISH9, ISH10, ISH11, ISH12 and ISH28). Since the three ones (ISH2, ISH3 and ISH8) of twelve ISHs have been well studied (*Syst. Appl. Microbiol.*, **16**, 560-568, 1994), the three was tried first for PCR. Two different primer sets were used for each ISH amplification with the purified chromosomal DNA of *Halobacterium* sp. NRC-1, *H. salinarum*, *Escherichia coli* K-12 and *Deinococcus radiodurans*. Between the six primer sets, ISH2F1-R1, ISH8F1-R1 and ISH2LC-RC showed specific amplification of only halophiles. Also , ISH1, ISH4, ISH6, ISH10 and ISH11 were amplified from the chromosomal DNA of *Halobacterium* sp. NRC-1. Thus, ISHs seem to be utilized to detect contaminated halophiles specifically. Additionally, I studied to utilize the sequence of archael specific DNA repair enzyme for the detection, and PCR under the high salt condition.

Table 1: Amount of PCR products with each primer set

Primer set	*H.* sp. NRC-1	*H. salinarum*	*E. coli* K-12	*D. radiodurans*
ISH2F1-R1	+++	+++	-	+
ISH3F1-R1	++	+	+	++*
ISH8F1-R1	++	+++	-	+
ISH2LC-RC	+++	+	-	+
ISH3-1C-2C	++	+++	+	++*
ISH8-1C-2C	+	-	-	-

*The PCR products showed different size to that of Halophiles.