アレルギー様食中毒を惹起するヒスチジン脱炭酸酵素における遺伝子解析

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A comparative Study on Histidine Decarboxylase by using Molecular Cloning Analysis

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BACKGROUND

Histamine fish poisoning is a food-borne chemical intoxication caused by the ingestion of histidine-rich scombroid fish such as tuna, bonito, and mackerel where histamine-producing bacteria (HPB) produce a large amount of histamine by the enzymatic activity [1]. It is an illness accompanied by a variety of symptoms, such as rash, nausea, diarrhea, flushing, sweating, and headache. Histamine is very stable even though it is heated. Hence, it is hard to prevent of histamine fish poisoning.

HPB have an enzyme named histidine decarboxylase (HDC) which can convert histidine to histamine[2, 3]. There are few reports on HDC of HPB isolated from the food responsible for histamine poisoning. Therefore, here we isolated HPBs from the fried kamaboko which is a cause of histamine poisoning incident at a nursery school in Nagasaki in Sep. 2008. And, we cloned hdc genes from the isolated HPB.

MATERIALS AND METHODS

1. Bacteria strains:

Morganella morganii JCM 1672 (standard strain); *M. morganii* EC-102 (isolated strain); *Raoultella planticola* ATCC 43176 (standard strain);

R. planticola EC-103(isolated strain).

2. Total DNA isolation:

The histamine producing bacteria (2.0×10^9 cells) was used for total DNA isolation by DNeasy Blood and Tissue Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol.

3 PCR amplification of *hdc* genes:

The primers shown in table 1 were designed based on the hdc genes from *M. morganii* and *R. planticola* (GenBank and NCBI accession number: AB259290.1, M62746.1). Veriti thermal cycler and AmpliTaq Gold (Applied Biosystems, Foster, CA, USA) were used for amplification and the reaction was performed with 1 cycle of 10 min at 95ºC, 35 cycles of 1 min at 94ºC, 1 min at 60ºC, 2 min at 72ºC, and 1 cycle of 7 min at 72ºC. The PCR products separated by agarose gel electrophoresis were extracted from the gel using QIAEX II Gel Extraction kit (QIAGEN) and cloned into pGEM T-Easy Vector (Promega, Madison, WI, USA).

DNA sequencing was carried out by the dideoxy chain termination method using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with 3130 Genetic Analyzer (Applied Biosystems).

RESULTS AND DISCUSSION

1. The *hdc* gene of *M. morganii* isolated strains

The *hdc* genes contained 1,137 bp ORF encoded 378 amino acid residues.

The DNA sequence had 34 base substitutions compared with standard strains, which caused 5 amino acid substitutions.

2. The *hdc* gene of *R. planticola* isolated strains

The *hdc* genes contained 1,137 bp ORF encoded 378 amino acid residues.

The DNA sequence had 4 base substitutions compared with standard strains, without amino acid substitution.

3. Comparison of HDC amino acid sequences of *M. morganii* and *R. planticola*

Amino acid sequences have high identity more than

85% between both strains.

Both of them conserved the substrate binding site (His120), coenzyme binding site (Lys233) and active site (Ser323).

Further study is needed concerning below: the expression of recombinant HDC proteins in *E. coli* is needed for screening of HDC inhibitor.

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