Detection of hdc Gene from Histamine Producing Bacillus subtilis and Serratia marcescens

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Abstract

In the present study, two histamine producing bacteria were used to detect the presence of hdc gene using PCR. The histamine production was confirmed by using Soyabean Casein Digest Agar (Tryptone Soya Agar). The DNA was isolated using Phenol: chloroform method. PCR was carried out using hdc gene and the amplified product was confirmed (657bps and 668 bps respectively). Further, the DNA and the PCR product were transformed into E.coli and the gene transformation was confirmed.

Keywords: Histamine; histidine decarboxylase (hdc); primer; Polymerase Chain Reaction (PCR)

Introduction

Fish are soft-bodied chordates that appeared first and an important source of food for humans since they are rich in protein. They are known for their easy digestibility, high nutritional value and they are highly perishable. As soon as fish dies, spoilage begins. Microbial spoilage of fish produces toxin, histamine in certain fishes. The most commonly reported biogenic amines occurring in foods and beverages are histamine, beta-phenylethylamine, tyramine, tryptamine, putrescine, cadaverine, spermine and spermidine (Doyle et al., 1997).

Histamine intoxication is probably a known sanitary problem of many food-borne diseases associated with eating fish. Also called as scombrotxin poisoning it is a mild illness with a variety of symptoms including rash, urticaria, nausea, vomiting, flushing, tingling, and itching of the skin and diarrhea. The severity of the symptoms depends on the amount of histamine ingested and the individual’s sensitivity to histamine (Lehane and Olley, 2000). Proliferation of many bacterial species such as Bacillus subtilis and Serratia marcescens are known for decarboxylation of amino acid that synthesize histidine decarboxylase (hdc) and convert free histidine to histamine (Taylor et al., 1986). The histamine in fermented products, such as wine, and mustard pickle, is produced by gram-positive lactic acid bacteria and Staphylococcus spp., (Tsai et al., 2007).

Molecular methods for the detection and identification of food-borne pathogens are becoming more widely accepted as an alternative to traditional culture method. PCR is a reliable tool for molecular biology-based diagnosis of a...
variety of infection diseases. PCR techniques mainly targets bacterial histidine decarboxylase gene. Different sets of primers have been developed for detecting gram-positive and gram-negative types (Kung et al., 2012).

Since histamine is the decarboxylation product of histidine catalyzed specifically by the enzyme histidine decarboxylase, it is possible to develop a molecular detection method that detects the gene responsible for the production of this enzyme. Histamine accumulation occurs during the growth of bacteria and results in a rise in pH, which induces a color change, allowing for visualization of positive bacterial colonies. This method is relatively easy to use and inexpensive. However, some researchers have reported loss of histamine production in bacterial strains after cultivation in culture-based media (Landete et al., 2008). False-positive reactions also are frequent, largely because of the formation of other (non histamine) alkaline compounds during microbial metabolism (Lopez et al., 1996).

Materials and Methods

Bacterial Strains

The bacterial strains were obtained from Centre for Bioscience and Nanoscience Research (CBNR), Coimbatore, India in this study. The Bacillus subtilis (KX002210) and Serratia marcescens (KY883376) were inoculated into nutrient broth separately and incubated at 37°C for 24 hours.

Histamine Production in Bacterial Strains

The ability of strains to produce histamine was determined by inoculating the isolates in Soyabean Casein Digest Agar (Tryptone Soya Agar), on which the bacterial growth occurred. (1. monument and 2. X-Gal, IPTG, ampicillin, and 100 µL of the transformed cells were spread with L-Rod and incubated at 37°C for 24 hrs.

DNA Isolation, Primers and PCR procedures (Takahashi et al., 2003)

DNA was isolated from Bacillus subtilis and Serratia marcescens species. The bacteria were taken in microfuge tubes, centrifuged at 5000 rpm for 5 minutes, pellets were collected and 700µL of saline EDTA and lysozyme were added. After mixing the tubes were incubated at 37°C for 30 minutes. 10 % SDS (150µL) was added and incubated in water bath at 65°C for 15 minutes. Then phenol, chloroform and isoamyl alcohol (25:24:1 ratio) were added. After mixing, it was centrifuged at 10000rpm for 10 minutes. The aqueous layer was transferred to another tube with 0.2 volume of sodium acetate and five volume of isopropanol. This was washed with 70% and 90% of ethanol and the DNA was dissolved by adding 50 µL of TE Buffer.

To detect the histidine decarboxylase coding gene (hdc) of gram-positive strains, it has been decided to use the primer 5′-GGATCCGGCGACTTGTTACATCAGGATCC-3′ and 5′-GGATCCTTTTTCGCCGAATGCCAACCTTGGATCC-3′ to generate fragments. Amplification was performed. The PCR mixture contain 10 µL of PCR master mix, Taq buffer of 4 µL, 2 µL of each primer was added and PCR was carried out for 20 cycles (Initial denaturation at 94°C for 2 minutes, Denaturation at 94°C for 30 seconds, Annealing at 58°C for 30 sec, extension at 72°C, then final extension at 72°C for 3 minutes and it was cooled at 4°C). PCR amplified product was run on 1.5% agarose.

Competent Cell Preparation (Transformation) and Plating

The competent cell used here E.coli for transformation. The pellet of the culture was treated with 0.1M CaCl2 (twice). After heat shock method and incubation with Luria Bertani Broth, plasmid DNA and PCR products suspension was added to Bacillus subtilis and Serratia marcescens, White blue screening method were used to identify the transformed colonies. For plating, on plate Luria Bertani Broth with 5 µL of X-Gal, IPTG, ampicillin, and 100 µL of the transformed cells were spread with L-Rod and incubated at 37°C for 24 hrs.

Results and Discussion

Confirmation of Histamine Production in Bacterial Strains

The histamine production mainly occurs in bacteria that possess the enzyme histidine decarboxylase. The histamine production was conformed by using Soyabean Casein Digest Agar (Tryptone Soya Agar), on which the bacterial growth occurs as shown in Fig.1. This finding is in agreement with the result of Lopez-Sabater et al., 1996 and Fletcher et al., 1998, who similarly reported that detection of histamine-producing bacteria using Niven’s agar resulted in 63 and 15 % false-positive rates, respectively.

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DNA Isolation and Agarose Gel Electrophoresis

The DNA was isolated using Phenol chloroform method. Then, 1% agarose gel electrophoresis was performed. As a result, clear bands were obtained for the given DNA sample. Grover et al. (2012) also used phenol chloroform method and extracted DNA.

PCR Amplification of hdc Gene

PCR was carried out using hdc gene and their respective primers hdc-f and hdc-r were used, which amplified DNA fragments of 657 and 668 bps respectively. Le Jeune et al. 1995 is reported that JV16HC/JV17HC primer set was successfully used to detect all histamine-producing lactobacillus 30a and Clostridium perfringens strains and was shown to be suitable for the detection of histamine-producing lactic acid bacteria (Le Jeune et al., 1995). Then the PCR products were made to run on a 1.5% agarose gel, and visualized with a UV transilluminator. Then it was compared with 500 bp ladder as shown in Fig. 2. In this study, the results have suggested that the primers used here were also suitable for the detection of histamine producers.

Similarly, Bjornsdottir, et al., 2009 used the PCR assay targeting the hdc gene, 74 (49%) of the 152 strains screened were positive and 78 (51%) were negative for histamine production.

Transformation and Plating

The DNA and PCR product were made to transform into E.coil and after the plating. The colonies of bacteria are developed in DNA and PCR product plates. Thus, hdc gene transformation was confirmed as shown in Fig. 3.

Fig. 2. Amplified PCR products DNA fragments of 657 and 668 bps respectively for Bacillus subtilis and Serratia marcescens. Lane 2: Control-500 bp ladder and Lane 3: Test sample: 657bp

Fig. 3. The colonies of bacteria are developed in DNA and PCR product plates; the bluish colonies show that the DNA and the PCR products were transformed. Plate 1: Bacterial colony, Plate 2: PCR product and Plate 3: Control plate
Conclusion
In conclusion, the two histamine-producing bacteria were used to detect hdc gene by using PCR. And further, confirmed by using Soyabean Casein Digest Agar (Tryptone Soya Agar). Then DNA isolation and PCR were carried out using hdc primers and amplified products were confirmed as 657 bps and 668 bps respectively. Then PCR product and gene transformation into E.coli was also confirmed. Since the gene shows positive transformation, it will be difficult to eliminate this gene. Hence, it is important to prevent the accumulation of histamine in food products especially fishes.

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