



Research Article

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

C. Aishwarya Laxmi^{1*}, S. Munaliga¹, N.G. Ramesh Babu² and R. Ragunathan³

^{1*}Department of Biotechnology, Adhiyamaan College of Engineering, Hosur-635109, India

²Professor and Head, Department of Adhiyamaan College of Engineering, Hosur-635109, India

³Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore-641021, India

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 scombrototoxin 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

Article may be cited as

C.A. Laxmi *et al.* (2017) Int. J. Appl. Sci. Biotechnol. Vol 5(3): 309-312. DOI: <http://dx.doi.org/10.3126/ijasbt.v5i3.17826>

^{1*}Corresponding author

C. Aishwarya Laxmi,

Department of Biotechnology, Adhiyamaan College of Engineering, Hosur-635109, India

Email: sandeepaishwaryalaxmi@yahoo.com

Peer reviewed under authority of IJASBT

© 2017 International Journal of Applied Sciences and Biotechnology



This is an open access article & it is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>)

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) supplemented with 1 mg L-histamine and incubated at 37°C for 24 hours.

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 (25:24:1ratio) 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'-GGATCCGCGCACTGGTTGTTACATCAGGATCC-3' and 5'-GGATCCTTTTGCCGGAATGCCAACTTGGATCC-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.



Fig. 1. Histamine production was confirmed by using Soyabean Casein Digest Agar (Tryptone Soya Agar), on which the bacterial growth occurred. (1. *Bacillus subtilis* and 2. *Serratia marcescens*)

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.



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

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.

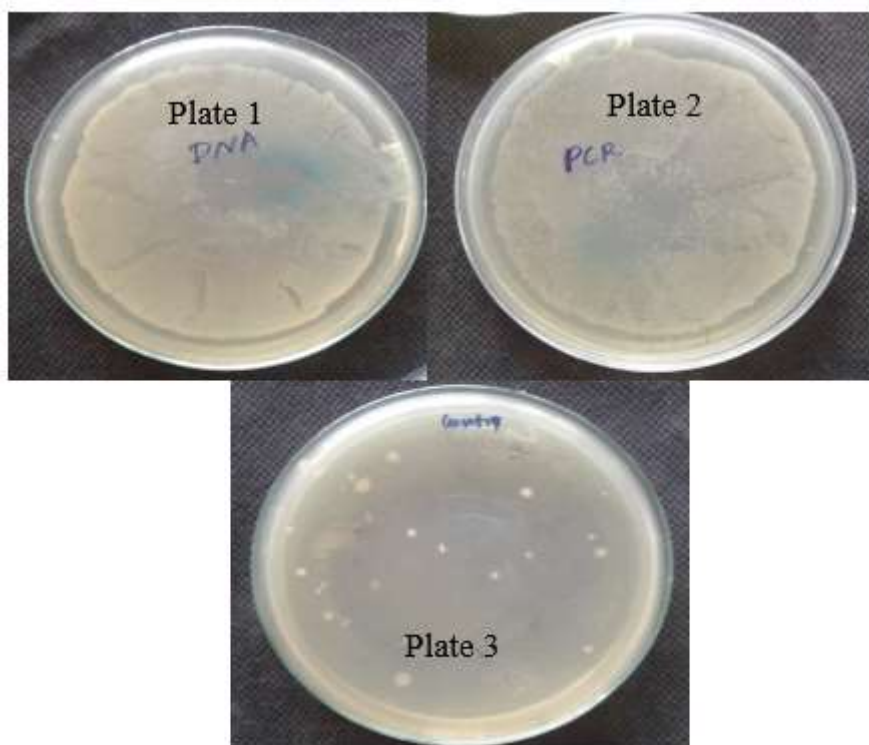


Fig. 3. The colonies of bacteria are developed in DNA and PCR product plates; the bluish colonies show that the DNA and the PCR products were transformed. Plate 1: Bacterial colony, Plate 2: PCR product and Plate 3: Control plate

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.coil* 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.

References

- Baranowski JD, Brust PA, and Frank HA (1985) Growth of *Klebsiella pneumoniae* UH-2 and properties of its histidine-decarboxylase system in resting cells. *J Food Biochem* **9**: 349-360. DOI: [10.1111/j.1745-4514.1985.tb00357.x](https://doi.org/10.1111/j.1745-4514.1985.tb00357.x)
- Bjornsdottir K, Bolton GE, McClellan-Green PD, Jaykus LA and Green DP (2009) Detection of gram-negative histamine – producing bacteria in fish: a comparative study. *J Food Prot* **72**: 1987-1991. DOI: [10.4315/0362-028X-72.9.1987](https://doi.org/10.4315/0362-028X-72.9.1987)
- Doyle MP, Beuchat LR and Montville TJ (1997) *Food Microbiology: Fundamentals and frontiers*. ASM Press, Washington, DC. pp: 872.
- Fletcher GC, Summers G and van Veghel PWC (1998) Levels of histamine and histamine-producing bacteria in smoked fish from New Zealand markets. *J Food Prot* **61**: 1064-1070. DOI: [10.4315/0362-028X-61.8.1064](https://doi.org/10.4315/0362-028X-61.8.1064)
- Grover A, Chakrabarti SK, Azmi W and Khurana SMP (2012) Rapid method for isolation of PCR amplifiable genomic DNA of *Ralstonia solanacearum* infested in potato tubers. *Advances in Microbiology* **2**(4): 441. DOI: [10.4236/aim.2012.24056](https://doi.org/10.4236/aim.2012.24056)
- Kim SH, Field KG, Morrissey MT, Price RJ, Wei CI and An H (2001) Source and identification of Histamine-producing bacteria from fresh and temperature-abused albacore. *J Food Prot* **64**: 1035-1044. DOI: [10.4315/0362-028X-64.7.1035](https://doi.org/10.4315/0362-028X-64.7.1035)
- Kimura B, Konagaya Y and Fujii T (2001) Histamine formation by *Tetragenococcus muriticus*, a halophilic lactic acid bacterium isolated from fish sauce. *Int J Food Microbial* **70**: 71-77. DOI: [10.1016/S0168-1605\(01\)00514-1](https://doi.org/10.1016/S0168-1605(01)00514-1)
- Kung HF, Lee TM, Lin CS, Hong TY and Tsai YH (2012) Polymerase Chain Reaction for the Detection of Histamine-Producing Bacteria Isolated from Taiwanese Foods. *Journal of Food & Drug Analysis* **20**(1).
- Landete JM, De Las Rivas B, Marcobal A and Munoz R (2008) Updated molecular knowledge about histamine biosynthesis by bacteria. *Crit Rev Food Sci Nutr* **48**: 697-714. DOI: [10.1080/10408390701639041](https://doi.org/10.1080/10408390701639041)
- Le Jeune C, Lonvaud-Funel A, ten Brink B, Hofstra H and van der Vossen JM (1995) Development of a detection system for histidine decarboxylating lactic acid bacteria based on DNA probes, PCR and activity test. *J Appl Bacteriol* **78**: 316-326. DOI: [10.1111/j.1365-2672.1995.tb05032.x](https://doi.org/10.1111/j.1365-2672.1995.tb05032.x)
- Lehane L and Olley J (2000) Histamine fish poisoning Revisited. *Int J Food Microbial* **58**: 1-37. DOI: [10.1016/S0168-1605\(00\)00296-8](https://doi.org/10.1016/S0168-1605(00)00296-8)
- Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernandez-Herrero M and Mora-Ventura MT (1996) Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *Int J Food Microbiol* **28**: 411-418. DOI: [10.1016/0168-1605\(94\)00007-7](https://doi.org/10.1016/0168-1605(94)00007-7)
- Stratton JE, Hutkins RW and Taylor SL (1991) Biogenic amines in cheese and other fermented foods: a review. *J Food Prot* **54**: 460-470. DOI: [10.4315/0362-028X-54.6.460](https://doi.org/10.4315/0362-028X-54.6.460)
- Takahashi H, Kimura B, Yoshikawa M and Fujii T (2003) Cloning and sequencing of the histidine decarboxylase genes of gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Appl Environ Microbiol* **69**: 2568-2579. DOI: [10.1128/AEM.69.5.2568-2579.2003](https://doi.org/10.1128/AEM.69.5.2568-2579.2003)
- Tsai YH, Kung HF, Chang SC, Lee TM. and Wei CI (2007) Histamine formation by histamine-forming bacteria in douche, a Chinese traditional fermented soyabean product. *Food Chem* **103**: 1305-1311. DOI: [10.1016/j.foodchem.2006.10.036](https://doi.org/10.1016/j.foodchem.2006.10.036)
- Tsai YH, Kung HF, Lee TM, Lin,G.T. and Hwang,D. F. 2004. Histamine-related hygienic qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. *J. Food Prot.* **67**: 407-412. DOI: [10.4315/0362-028X-67.2.407](https://doi.org/10.4315/0362-028X-67.2.407)