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Research article

MOLECULAR IDENTIFICATION OF BACTERIA ISOLATED FROM MEAT SAMPLE

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ABSTRACT: Contamination of food with bacteria moulds, yeasts, viruses, protozoa or parasites could occur within food premises and result in unfit or unsafe food. Microbial contamination is frequently the result of ignorance, carelessness or negligence by the food operator, or due to lack of space or inappropriate design of food areas. In this work we have identified meat contaminating bacteria through molecular techniques. The bacterium identified through morphological and biochemical tests was *sporosarcina halofila*. This was confirmed after amplification of genomic DNA with suitable primers. Plasmid was isolated and it was identified to be 11kb. PCR amplified product was 100bp and primers were designed accordingly to the suitable organism.

Key words: DNA isolation, Food Contaminants, PCR, *Sporosarcina halofila*

INTRODUCTION

Meat is an important edible postmortem component originating from the live animals that are used as food by human. Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs (Fratamico et al., 2005). Changes in eating habits, mass catering complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors (Hedberg et al., 1992). Contaminated raw meat is one of the main sources of food-borne illness (Bhandare et al., 2007; Podpecan et al., 2007). Meat is the main edible part of domestic mammals; however, recent definition includes species, as well as fish, shellfish, poultry and exotic species such as frogs and allegation (Nakai and Moddler, 2000). Similarly, meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood (Hammer, 1987). Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savory sensation. A number of studies have reported outbreak of infections due to consumption of contaminated food and poor hygiene and in most of the cases, data are loosely based on laboratory isolates which do not reflect the actual ratio of food-borne infections. However, a few community-based reports provide evidence of several outbreak caused by *Salmonella, Shigella, E. coli* and *Listeria* spps in different parts of the world (Zweifer et al.,2008). Bacterial contamination of meat products is an unavoidable consequence of meat processing (Labadie, 1999). Contaminants originate from a variety of processing and animal sources (Boerema et al., 2003; Gill and Landers, 2004; Gill and McGinnis, 2000). Hurdle technologies are commonly used to restrict the subsequent growth of such contaminants. For example, vacuum packaging in combination with chilling produces an environment where only those organisms able to grow at low temperatures in the absence of oxygen can proliferate. Such psychrotrophic organisms include species capable of causing disease and/or early meat spoilage, e.g., Listeria monocytogenes, Brochothrix thermosphacta and Clostridium estertheticum (Broda et al., 2003; Gardner, 1981; Gill and Reichel, 1989; Sim et al., 2002).

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MATERIALS AND METHODS

Material collection

The material was collected from a super market which is a packed mutton which serves for a good microbial activity. The packed food comprises of batch no. 111001- (06J11) and the manufactured on 21/7/2012 with expiry date of 22/07/2013. The sample was taken as 1gm and grind in a pestle into small pieces and further proceeded to serial dilution. After serial dilution technique the sample was introduced into the nutrient broth for growth of culture.

Morphological characterization

Grams staining, End spore staining, Capsule staining and Motility test were carried out for the gram positive or negative, spores or non spores, capsule or noncapusule and motile or non motile respectively. (Table-1).

Culture media biochemical Characterization

Liquid culture medium and Enriched medium were used as basal media. Citrate Agar Medium, LB-agar medium, LB broth medium and Tryptone Broth were prepared for biochemical characterization.

Bio chemical characterization

Catalase, ONPG, Lysine decarboxylase, Ornithine, Urease, Phenyl alanine deamination,Nitrate reduction, H2S production, Citrate utilization, Voges proskaeurs, Methyl red, Indole and Malonate tests were conducted for biochemical studies.(Table-2).

Catalase Test

A bacterial colony was picked up with a toothpick or a platinum loop and mixed with a drop of hydrogen peroxide (10% v/v in water) taken on a glass slide. The effervescence indicates the presence of the catalase enzyme.

Oxidase Test

Pick a well-isolated colony from the culture plate or slant culture and place it on a piece of filter paper. Add one drop of the reagent and results observed within 20 seconds.

TIME the reaction: a positive reaction will occur within 20 seconds. DO NOT READ the reaction after 30 seconds.

Indole test

Inoculate the tryptone broth that was prepared as mentioned in the materials with the test organism and incubate at 37˚C for 24-48h and add 0.5mL of Kovac's reagent and gently agitate. Examine the upper layer of liquid.

MR TEST

Using sterile technique, inoculate each experimental organism into its appropriately labeled tubes of medium by means of an inoculation loop. The last tube will serve as a control. Incubate all cultures for 24 to 48 hrs, at 37˚C. After 24 hours of incubation add 2 to 3 drops of MR reagent.

VP TEST

Using sterile technique, inoculate each experimental organism into its appropriately labeled tubes of medium by means of an inoculation loop. The last tube will serve as a control. Incubate all cultures for 24 to 48 hrs, at 37˚C. After 24 hrs incubation add 1 to 2 drops of Barrett's A reagent and Barrett's reagent and observe for change in color.

TRIPLE SUGAR IRON AGAR

Using sterile technique, inoculate each experimental organism into its appropriately labeled tube by means of a stab-and –streak inoculation. The last tube will serve as control. Incubate for 18 to 24 hrs at 37˚C.

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ONPG Test

Pipette 0.5ml of the saline into a sterile tube. Inoculate with the bacterium and add the ONPG disc in a sterile manner (forceps dipped in alcohol and flamed) to the tube. Incubate at 37C for 4 hours.

Methyl Red Test

Obtain two MR-VP broths from the back shelf. Inoculate one broth using aseptic technique. Leave the other broth uninoculated (this will be a control). Incubate at appropriate temperature (whatever temperature your organism grows well at).Incubate for two to five days. Obtain your broths from the incubator. Add a dropperful of ethyl Red to each broth. Methyl Red is found in small dropper bottles on the back shelf. Observe the color (which should develop within a few minutes).

Malonate Test

Inoculate malonate media with light inoculums from 18 to 24 hours and then incubate 24 to 48 hours at 20-24^{0}C

H_2S Production Test

An inoculum from a pure culture is transferred aseptically to a sterile triple sugar iron agar (TSIA) slant. The inoculated tube is incubated at 35-37^{O}C for 24 hours and the results are determined.

Lysine decarboxylase

An inoculum from a pure culture is transferred aseptically to a sterile tube of lysine decarboxylase brorth. The inoculated tube is incubated at 35-37^{O}C for 24 hours and the preliminary results are determined

Lysine decarboxylase

An inoculum from a pure culture is transferred aseptically to a sterile tube of ornithine decarboxylase broth. The inoculated tube is incubated at 35-37^{O}C for 24 hours and the preliminary results are determined.

Phenyl alanine deamination

An inoculum from a pure culture is transferred aseptically to a sterile tube of phenylalanine agar to streak the slant. The butt of the tube need not be stabbed. The inoculated tube is incubated at 35-37^{O}C for 24 hours. Then, reagents must be added to allow results to be determined.

Urease Test

Inoculate a urea tube with 3 loopfuls of slant culture. Incubate 24 hours, observe for reaction. A pink color formation indicates the breakdown of urea to ammonia and CO_2.

Nitrate Reduction Test

Inoculate a trypticase-nitrate tube with the organism to be tested. Incubate for 48 hours, test for results. Add one ml of sulfanilic acid to each tube, then add one ml of dimethyl 1-naphthylamine solution.

Voges proskaeurs

An inoculum from a pure culture is transferred aseptically to a sterile tube of MRVP broth. The inoculated tube is incubated at 35-37^{O}C for 24 hours.

Biochemical identification test kit

HiMedia provides a range of Biochemical identification test kit (KB001 to KB012) involving single step procedure of inoculation which leads to final identification of test organism being studied. The overnight culture broth was taken for the test. The wells were opened aseptically, add culture of 0.5µl was inoculated into the test well and then incubated for 1-2 min. After the incubation the result was noted.

Molecular detection of food borne pathogens

DNA Isolation

DNA was isolated from overnight grown culture and dissolved in TE buffer (100 mM Tris hydrochloride, 1mM EDTA, PH8.0.DNA concentration was estimated spectrophotometrically at 260 nm. DNA purity was checked by scaning the absorbance of DNA samples between 200 and 400 nm and monitoring the absorbance ratios at 260/280 nm and at 260/230 nm. DNA preparations were also subjected to electrophoresis in 1% agarose gels to check for shearing and degradation. Specific primers are used for the identification of pathogenic organisms which code for the gene sequence of an organism. The sequence of the primers designed is:

Test Primer:

Forward Primer: 5'-GCT TGC GAC AAC TGC TAC AG-3'

Reverse Primer: 5'-TGG ATC CGT CAT TCA TTG TTA T-3'

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*Pl*asmid DNA isolation

Plasmid DNA was isolated from overnight culture, resuspended in the 1x TE buffer and examined by the 1% Agarose gel electrophoresis

PCR Mixture

The PCR mixtures were prepared with H_2O (Mili-Q grade), 2 µl of 20 pmol of both forward and reverse primers, 1µl of 10 mM dNTP, 5 µl of 1U Taq DNA polymerase, 5 µl of 10X PCR buffer, 4.0 µl of 25 mM $MgCl_2$, 1µl DNA Sample. Water was added to adjust the final reaction volume to 50µl. PCR Products were analyzed with 2% agarose gel electrophoresis.

RESULTS AND DISCUSSION

Table-1 : Morphological Characterization

S.No	Property	Sample1	Sample 2
1	Gram staining	Gram-positive	Gram-positive
2	Endospore staining	spores	spores
3	Capsule staining	Non capsulated	Non capsulated
4	Motility test	Motile	Motile

Table-2: Biochemical Characterization

Biochemical Characteristics	Reaction	Results
ONPG	Purple	Positive
Lysine decarboxylase	Purple	Positive
Ornithine	Purple	Positive
Urease	Orangish yellow	Negative
P.D.A	Colourless	Negative
Nitrate reduction	Colourless	Negative
H2S production	Orangish yellow	Negative
Citrate utilization	Blue	Positive
Voges proskaeurs	Colourless	Negative
Methyl red	Methyl red	Negative
Indole	Colourless	Negative
Malonate	Light green	Negative
Esculin	Cream	Negative
Arabinose	Pink	Negative
Xylose	Pink	Negative
Adonitol	Pink	Negative
Rhamnose	Pink	Negative
Cellobiose	Pink	Negative
Mellibiose	Pink	Negative
Saccharose	Pink	Negative
Raffinose	Pink	Negative
Trehalose	Yellow	Positive
Glucose	Yellow	Positive
Lactose	Yellow	Positive

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Molecular Identification of Food Borne Pathogens

Genomic DNA

The genomic DNA was isolated can be purified and used for PCR analysis for the identification of the pathogen.

Plasmid DNA

The plasmid DNA was isolated can be confirmed for running 0.8% agarose gel electrophoresis and has been purified them

PCR Analysis Result

The specific primers TSST and SEA are been bind with the isolated DNA and the bands are observed at 120bp with regarding to the marker and the isolated organism is identified as *sporosarcina halofila*.for the PCR analysis for the identification of pathogen.

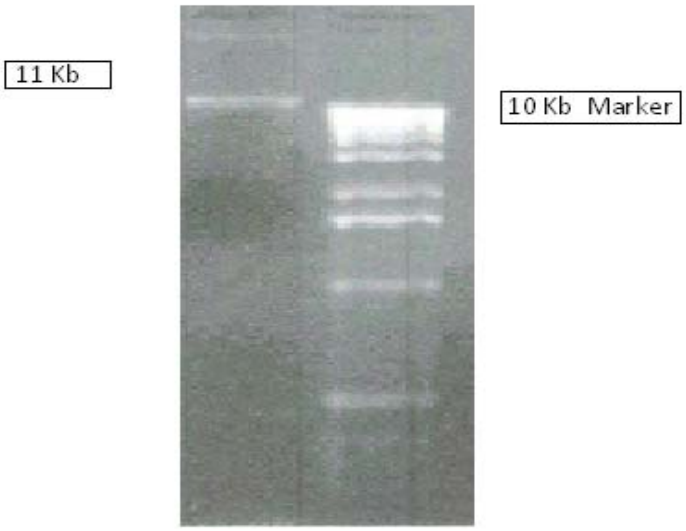

Figure 1: genomic DNA isolation

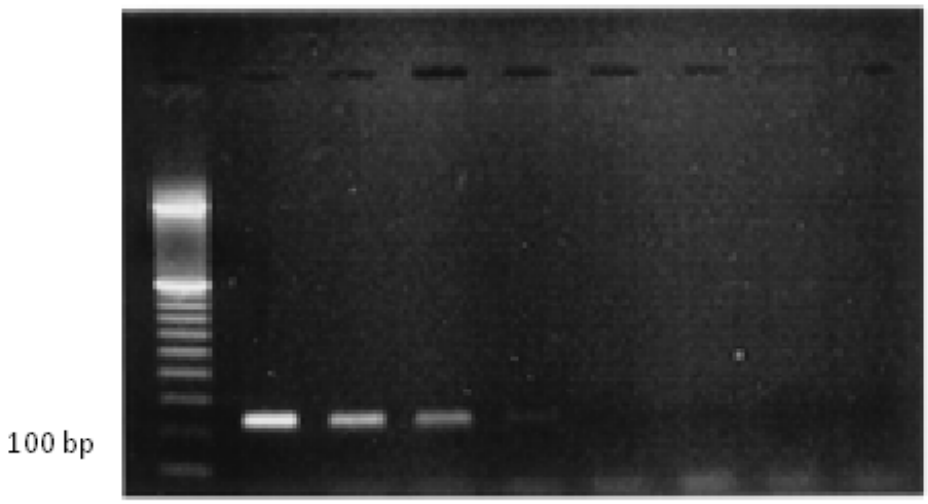

Figure 2: amplification of DNA isolated using primers

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CONCLUSION

From the isolated samples it was identified that that the organism grown in meat sample is sporosarcina halofila. The bacterium is gram positive and sporulated. The biochemical tests have confermed the organism identification. Later the isolated plasmid measures 11kb where as PCR product is of 100bp in length.

REFFERENCES

Bhandare SG, Sherikarv AT, Paturkar AM, Waskar VS, Zende RJ (2007). A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food. Contr., 18: 854-868.

Boerema, J.A., Broda, D.M., Bell, R.G., 2003. Abattoir sources of psychrophilic clostridia causing blown pack spoilage of vacuumpacked chilled meats determined by culture-based and molecular detection procedures. Lett. Appl. Microbiol. 36, 406–411

Broda, D.M., Boerema, J.A., Bell, R.G., 2003. PCR detection of psychrophilic Clostridium spp. causing 'blown pack' spoilage of vacuum-packed chilled meats. J. Appl. Microbiol. 94, 515–522.

Fratamico PM, Bhunia AK, Smith JL (2005). Foodborne pathogens in Microbiology and Molecular Biology, Caister Academic press, Wymondham Norfolk, UK. Pp. 270-275.

Gardner, G.A., 1981. Brochothrix thermosphacta (Microbacterium thermosphactum) in the spoilage of meats: a review. In: Roberts, T.A. (Ed.), Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Academic Press, London, pp. 139–175.

Gill, C.O., Reichel, M.P., 1989. Growth of the cold-tolerant pathogens Yersinia enterocolitica, Aeromonas hydrophila and Listeria monocytogenes on high-pH beef packaged under vacuum or carbon dioxide. Food Microbiol. 6, 223–230.

Gill, C.O., McGinnis, J.C., 2000. Contamination of beef trimmings with Escherichia coli during a carcass breaking process. Food Res. Int. 33, 125–130.

Gill, C.O., Landers, C., 2004. Proximate sources of bacteria on boneless loins prepared from routinely processed and detained carcasses at a pork packing plant. Int. J. Food Microbiol. 97, 171–178

Hammer GF (1997). Meat processing: ripened products Fleischwirtschaft, 67: 71-71.Labadie, J., 1999. Consequences of packaging on bacterial growth. Meat as an ecological niche. Meat Sci. 52, 299–305.

Hedberg CW, Levine WC, White KE, Carlson RH, Winsor DK, Cameron DN, MacDonald KL, Osterholm MT (1992). An international foodborne outbreak of Shigellosis associated with a commercial airline.JAMA 268: 3208-3212.

Nakai S Modler WH (2000). Food protein, 1st ed. Wiley-VCH, Inc., New York, U.S.A. pp. 128-133.

Podpecan B, Pengov A, Vadnjal S (2007). The source of contamination of ground meat for production of meat products with bacteria Staphylococccus aureus. Slov. Vet. Res., 44: 24-30.

Sim, J., Hood, D., Finnie, L., Wilson, M., Graham, C., Brett, M.,Hudson, J.A., 2002. Series of incidents of Listeria monocytogenes noninvasive febrile gastroenteritis involving ready-to-eat meats. Lett.Appl. Microbiol. 35, 409–413.

Zweifer C, Fischer R, Stephan R (2008). Microbiological contamination of pig and cattle carcasses in different small-scale Swiss abattoir, Meat. Sci., 78: 225-231.