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Microbial isolate for the production of quality white pepper (*Piper nigrum* L.)

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Abstract

White pepper, the value added product of black pepper is traditionally produced by decortications of ripe or dried black pepper berries. Excessive time taken for retting imparts off odour to the traditionally prepared white pepper making the process and product less acceptable. An experiment was conducted to standardise a microbial method that could reduce the retting period and off odour. Screening of 25 microbial isolates comprising 21 bacterial and 4 fungi was undertaken. The experiment was replicated four times. Six best isolates (five bacteria and one fungus) which reduced retting period and produced good colour and appearance to the white pepper were selected by visual observation. Selected six isolates were inoculated again on the ripe berries in four replications along with uninoculated control. Three bacterial isolates were found very effective to reduce the retting period by half compared to control. Isolate I_1 could ret the ripe berries with minimum time (4.25 days) and produce white pepper which had superior white colour and appearance and which was on par with uninoculated control as per sensory score. The odour of the white pepper produced by all isolates was significantly better than uninoculated control. Quality parameters, such as essential oil and oleoresin produced by the best isolate (I_1), was on par with the control. Morphological, biochemical and molecular characterisation of the best three isolates was done. The 16S r DNA sequencing (1500bp) revealed that isolate I_1 was a relative of *Bacillus pumilus*. White pepper produced by the isolate of *Bacillus pumilis* was superior as it required reduced retting period with product having less off odour, suggesting that the isolate could be used for commercial production of white pepper. The other two isolates belonged to *Rhizobium* and *Sphingobacterium* genus.

Keywords: *Bacillus pumilus*, Microbial retting, *Piper nigrum* L., White pepper.

Introduction

Black pepper (*Piper nigrum* L.), one of the most widely used spices in the world, originated in the Western Ghats of India and is cultivated in the tropics. White pepper, a value added product of black pepper, is preferred in western countries for products like cream soups, mayonnaise, white pickles, sea food salad, casseroles of chicken, egg and fish where black specks of black pepper pericarp are not desired (Chithra et al., 2011). Vietnam leads in black and white pepper production and export (IPC, 2016).White pepper of commerce is produced by retting, steaming or boiling, and chemical, microbial or enzymatic methods (Aziz et al., 2019). The traditional retting method is still the most widely used technique worldwide. Retting by traditional process requires long retting period resulting in the development of characteristic faecal off odour to the product. Hence the present experiment was conducted with the objective to develop a microbial technique to produce white pepper using new bacterial strains that could reduce retting period and produce quality product with acceptable colour, appearance and odour.

Materials and Methods

The experiments were conducted at the Departments of Plantation Crops and Spices and Agricultural

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Microbiology, College of Agriculture, Vellayani, Kerala Agricultural University, India during 2014-2015. Ripe fresh black pepper berries harvested and kept for one day and dried black pepper berries harvested and dried one year earlier were kept for retting, and twelve bacteria and three fungi were isolated by serial dilution and plate count method. These isolated microorganisms along with ten effective microbial isolates already identified for their efficiency in degradation of waste materials from Department of Agricultural Microbiology were screened for identifying the most effective organism for retting. The experiment was repeated with the six best isolates, and the quality parameters along with retting period, as well as the sensory characters of the product were evaluated. Three most promising isolates obtained after the screening trial were characterized using morphological and biochemical methods, while the best isolate was characterized using molecular methods also.

Isolation of microorganisms

Dried and ripe black pepper berries of variety Panniyur1 were collected from Farming System Research Station, Kottarakkara, Kerala state, India. One hundred grams each of dried black pepper berries were kept in 10 conical flasks with sterilized water (200 ml) and sealed with cotton for 13 days for retting. Similarly, one hundred grams each of fresh ripe black pepper berries of Panniyur -1 were separately kept in conical flasks containing different substrates like cowdung, surface soil, rhizosphere soil collected from muddy and garden soil along with water and kept for 11 days for retting. Bacterial populations were isolated from these samples following serial dilution upto 10^{-8} using sterilised distilled water. An aliquot of 100µL of each dilution was spread plated onto Nutrient agar medium (peptone 0.5 %, sodium chloride 0.5 %, beef extract 0.3% and agar 2 %) with pH 7.0 and incubated at 35- 37^0 C for 48 hrs. Morphologically dissimilar and discrete colonies were picked from different dilution plates and streaked on separate nutrient agar plates and incubated at 35- 37^0 C. Twelve purified bacterial colonies were selected, allotted code numbers and maintained on nutrient agar slants. The isolate slants were stored at 4^0 C and subculturing was done every 21 days. Fungi were isolated in potato dextrose agar medium containing potato (20 %), dextrose (2 %) and agar (2 %). Three fungi were isolated and pure culture maintained for further studies.

Screening of microbial cultures for effectiveness in retting

Twelve bacteria and three fungi isolated in the present study and ten microbial isolates (nine bacteria and one fungus) maintained at Department of Agricultural Microbiology, College of Agriculture, Vellayani which were in use for solid waste decomposition were used for the initial screening trail. Ripe black pepper berries of Panniyur-1 were collected and 50 g of fresh pepper berries after surface sterilization with 95 % ethanol for 1 minute were added to conical flask. One hundred ml of seven day old cultures of each of the twenty five isolates with >1x 10^8cfu/ml viable cells in one litre inoculum of each treatment were poured into one hundred (four replication of each treatment) conical flasks containing berries. An uninoculated control without the culture but with the same quantity of sterile water was also maintained as control. Four days after the application of culture, first replication from each treatment was taken and the percentage retting was observed. This was continued with the second, third and fourth replications at two days interval till 100% fruits were retted. The number of days taken for 100% retting was found out for each treatment. Each replication of uninoculated control was washed from tenth day onwards at two days interval after keeping in water and the process was continued till 100% retting, and the number of days taken for 100 % retting was recorded.

Six isolates (five bacteria and one fungus) which produced 100 % retting within nine days after application were selected for repeating the experiment. Five hundred grams ripe berries with each isolate were taken in conical flasks. After

surface sterilization with 95 % ethanol the berries were inoculated with respective isolates containing > 1 x 10^8cfu/ml viable cells in one litre inoculum. Four replications were maintained. Appropriate control treatment without culture was also maintained. Four days after the application of isolate, berries were washed and the process was continued till 100% were retted except for the uninoculated control. The uninoculated control when washed at fourth and sixth days were found not retted and hence retting was continued. The uninoculated control was then washed from ninth day onwards and the process continued till 100% retting was obtained.

Quality of white pepper

Quality parameters such as essential oil and oleoresin of the white pepper were estimated following standard procedures as per FSSAI (2015). The retted white pepper berries were washed, oven dried at 70^0C, pulverized and subjected to hydrodistillation in Clevenger apparatus for obtaining essential oil, while the oleoresin of white pepper was extracted by Soxhlet apparatus using ethanol as solvent. The white pepper produced by the microbial isolates were scored by semi trained sensory panelists for colour, odour and appearance using a 9 point hedonic scale ranked from 'dislike extremely' to 'like extremely' (Meilgaard et al., 2006).The different scores were evaluated by Kruskall- Wallis test to get mean rank values for all the treatments.

Morphological and biochemical characterisation of microorganisms

Morphological and biochemical characterisation of the three best and effective isolates were carried out as described by Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984) to arrive at a tentative genus level identification of the isolates. The isolates were inoculated in yeast extract pectate plates and colony characters like colony appearance, shape elevation, margin, opacity and consistency were recorded after 24 hours of growth. Gram staining was also done.

Biochemical characteristics including indole, methyl red, vogesproskauer, citrate utilisation, triple sugar iron, carbohydrate fermentation, maninitol motility, gelatin hydrolysis and presence of catalase were assessed (Fawole and Oso, 2004) and it was confirmed using Himedia kits (HiCarbo ™, KB009, Hi 25 ™ Enterobacteriaceae identification Kit KB003). The inoculation in the Himedia kits were done according to the protocol given in kit and colour changes were recorded after 72 hours of incubation.

Molecular characterization of the effective isolate

After phenotypic and biochemical characterisation, species status of the best isolate was identified at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala through molecular characterization by 16S rDNA cataloging. The steps involved in molecular characterization were:

Isolation of genomic DNA

Pure genomic DNA was isolated following the method of Ausubel et al. (2002). The isolates were grown in nutrient broth (3ml) with shaking. Then 1.5 ml of the culture was centrifuged at 12,000 *g* for 10 min and the pellet obtained was resuspended in 567 µL 1× TE buffer (10 mM Tris pH 8.0, 1 mM EDTA). Proteinase K (100 µg/ml) and SDS (0.5 %) were added and incubated at 37ºC for 1 h. After incubation, NaCl (5 M) and CTAB/NaCl (10% w/v cetyl trimethyl ammonium bromide in 0.7 M NaCl) were added and incubated at 65ºC for 10 min. The mixture was extracted once each with an equal volume of chloroform-isoamyl alcohol (24:1) and phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated from the aqueous phase using 0.6 volumes of isopropanol and washed once with 70% ethanol. The DNA pellet obtained after final centrifugation was vacuum dried and dissolved in 50 µL 1× TE buffer. DNA quantification was done using UV-1800 spectrophotometer (Schimadzu Corporation).

PCR amplification of bacterial 16S rDNA

The 16S rDNA of the best isolate, I_1 was PCR

amplified (Veriti, Applied Biosystems) using the universal primer combination (Lane, 1991). 27 forward - 5' AGAGTTTCCTGGCTCAG 3' and 1492 reverse 5' ACGGCTACCTTGTTACGATT 3' (Sigma- Aldrich).

PCR was performed in 20µL reaction mixture containing 2.0µL of 10X assay buffer, 1.0µL dNTP mix of 2.5 mM, 0.5µL of $MgCl_2$, 1.0µL each of forward and reverse primers (5pmol), 0.5µL of Taq polymerase, 1.0µL of template DNA and 13 µL of double distilled water. The PCR program involved initial denaturation at 95^0C for 4 min followed by 38 cycles of denaturation, annealing and extension done at 94^0C for 1 min, 59.9^0C for 2 min and 72^0C for 2 min followed by final extension at 72^0 C for 20 min. PCR product was electrophoresed on 0.5% agarose gel and prepared in 0.5 % TBE buffer containing 0.5µg/ml ethidium bromide. The molecular ladder used was 2 log DNA ladder (NEB). The gel was visualised in UV transilluminator (Genei) and the image was captured in Gel documentation system (Bio rad). Exo SAP- IT (USB) treatment was done to remove unwanted primers and dNTPs from the PCR product. Five µl of PCR product was mixed with 2µL of Exo SAP-IT and incubated at 37^0 C for 15 minutes followed by enzyme inactivation at 80^0 C for 15 minutes.

DNA sequencing of the PCR amplified 16S rDNA

Sequencing reaction was carried out using Big Dye terminator v 3 in Gene Amp PCR system 9700 thermal cycler and the sequencing was done in ABI 3730 DNA Analyser (Applied Biosystems).

The quality 16S rDNA sequence obtained was checked using Sequence Software v.1. (Applied Biosystems). Sequence alignment and editing was carried out using Geneious Pro .v.5.6.

The 16S rDNA sequences obtained were analysed by BLASTN analysis and sequence identity *vis- a vis* the bacterial identity was established by closest match. The partial 16S rDNA sequences derived in this study of isolate, I_1 has been deposited in GenBank.

Results and Discussion

Isolation of microorganisms

Twelve bacteria and three fungi were altogether isolated from dried black pepper and ripe berries with different substrates such as cowdung, surface soil, rhizosphere soil collected from muddy and garden soil.

Screening of isolates based on retting period, quality and sensory parameters

Surface sterilized ripe black pepper berries were inoculated with 25 isolates of microorganisms (designated as T_1 to T_{25} in Fig 1) and six were identified as better in reducing the retting period. All microbial isolates took less time for retting compared to the uninoculated control. The isolates which reduced the retting period below 60 % compared to control were selected for further confirmation. Six isolates which reduced the retting period below 9 days (Fig. 1) were T_6 (5 days), T_{20} (5.25 days), T_{23} (6 days), T_{10} (7 days), T_{15} (7.75 days) and T_{13} (8.25 days), while the control took 14.5 days. When these six isolates (named I_1 to I_6)were screened in the second round, isolates I_1 (T_6) and I_2 (T_{20}) reduced the retting period to less than half the time required for uninoculated control. The isolates $I_1$and I_2 took only 4.25 and 4.75 days respectively for 100 per cent retting and were on par. Isolate, I_6 (T_{23}) was also efficient in reducing the retting period (5.25 days). The control, which was the uninoculated treatment, took 10.25 days for one hundred per cent retting.

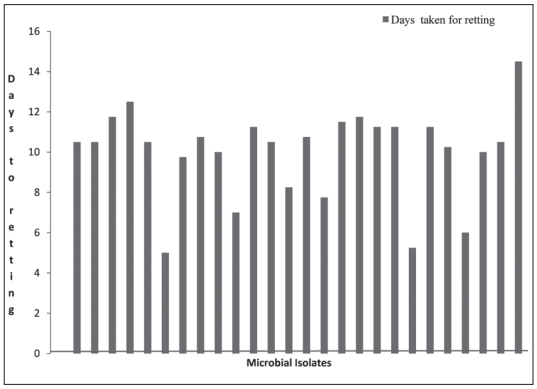
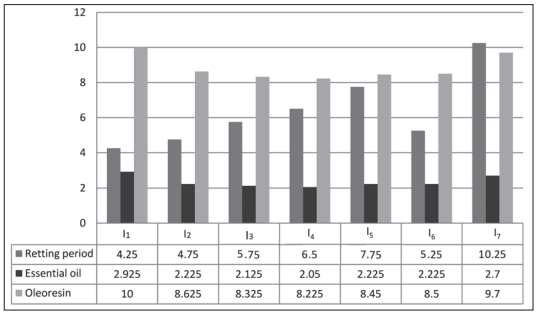


Figure 1. Duration of retting by different microbial isolates

White pepper is an important product of *Piper nigrum* which is traded world over. White pepper is produced by pericarp degradation of either ripe black pepper or dried pepper. The traditional retting of pericarp is a natural fermentation process involving several microorganisms. Pericarp is made of polysaccharides like cellulose, hemicellulose and pectin. The key enzymes attributed for the decortication of pericarp were cellulase (Thankamony et al., 1999) and pectinase (Gopinathan and Manilal, 2004). Many microorganisms produce enzymes capable of degrading these polysaccharides. The main enzymes that hydrolyze cellulose are endoglucanases, exoglucanases, and â-glucosidases. Enzymes hydrolysing pectins are broadly known as pectinases and include polygalacturonases, pectin esterases, pectin lyases and pectatelyases (Alkorta et al., 1998). Thankamony et al. (1999) reported that cellulase producing bacteria can be used for the production of white pepper. Carbohydrate-cleaving enzyme preparations, i.e., cellulases and pectinases, can hydrolyze the glycosides which are in conjugated forms with phenols thus releasing the phenolic aglycones (McCue and Shetty, 2003; Mandalari et al., 2006). Bacterial isolates such as *Bacillus subtilis* (IISR WP 33, 34, 38), *Bacillus licheniformis* (IISR WP 43), *Acinetobacter baumanii* (IISR WP 35), *Klebsiella pneumonia* (IISR WP 19) and *Microbacterium barkeri* (IISR WP25) were found to decorticate black pepper (>60%) and fresh pepper berries (98–100%) into white pepper within 5 days of immersion in bacterial suspension and were found to secrete multiple hydrolytic enzymes such as cellulase, pectinase, amylase, protease and xylanase (Vinod et al., 2014). In the present study the enzymes cellulose and pectinase produced by the isolates might have been responsible for the reduction in time of retting period.

Essential oil content as well as the oleoresin of white pepper produced by control as well as by isolate I_1 were on par, indicating that isolate I_1 produced quality white pepper. The essential oil and oleoresin contents of white pepper produced by isolate I_1 were



	I_1	I_2	I_3	I_4	I_5	I_6	I_7
Retting period	4.25	4.75	5.75	6.5	7.75	5.25	10.25
Essential oil	2.925	2.225	2.125	2.05	2.225	2.225	2.7
Oleoresin	10	8.625	8.325	8.225	8.45	8.5	9.7

Figure 2. Effect of isolates on retting period and quality parameters of white pepper

2.9 and 10.0 % respectively compared to 2.7 % and 9.7% respectively in control treatment (Fig 2). The quality of white pepper is constituted by the essential oil and the pungency principles. The essential oil of pepper is a mixture of a large number of volatile chemical compounds while pepper oleoresin consists of the essential oil and resinous matter of the spice including the pungent alkaloid piperine (CBI, 2018). Pepper oleoresin has a relatively full flavour profile characteristic of pepper as compared to pepper oil. Apart from colour, quality also determines the market of the product.

The sensory parameter odour of white pepper produced by isolates was ranked higher over those of control (Fig. 3). White colour and general appearance for white pepper produced by I_1 and control were statistically on par. The off odour was very less for the white pepper produced by all the isolates compared to control. The white pepper

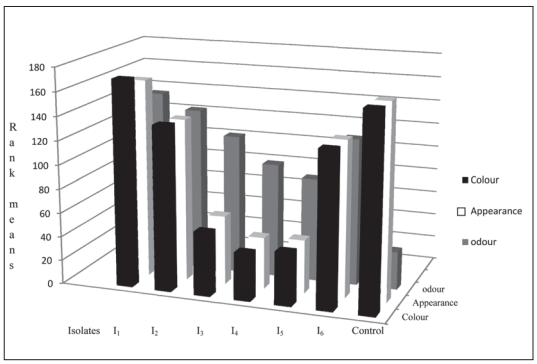


Figure 3. Organoleptic evaluation of colour, appearance and odour of white pepper produced by different isolates

Table 1. Morphological characterization of effective isolates

Sl. No.	Bacteria	Gram staining	Cell shape	Motility	Colour	Colony Shape	Margin
1.	Isolate I_1	+	Rod	+	Transparant	circular	Entire
2.	Isolate I_2	-	Rod	-	Yellow	Round	Entire
3.	Isolate I_6	-	Rod	+	Yellow	circular	Wavy

produced by isolate 1_1, I_2 and I_6 had superior general appearance and less off odour. On the contrary, the white pepper produced by traditional method of retting often has faecal off odour which is not preferred by consumers. The longer fermentation time coupled with anaerobic fermentation resulted in the faecal odour. Short fermentation time in water along with the aerobic nature of the bacteria might have resulted in less off odour of the product produced by the isolates I_1 and I_2. According to Steinhaus and Schieberle (2005), the white pepper samples directly taken from a retting plant in Thailand revealed the presence of 3 methyl indole, 4 methyl phenol, 3 methyl phenol and butanoic acid as the main source of faecal off odour. The odour arose due to anaerobic degradation. Chemicals such as 3-methylindole (faecal, swine-manure-like), 4-methylphenol (faecal, horse-like), and butanoic acid (cheese-like) were biochemically formed during retting. Thus keeping black pepper berries under water for long time may result in accumulation of the above chemicals resulting in off odour. Since fermentation is the crucial step in retting, the formation of these chemicals cannot be prevented. Use of aerobic organisms as well as reducing time under water for retting can reduce off odour. The present isolate, I_1 was an aerobic microorganism and the time taken for retting was less, resulting in less off odour.

Morphological, biochemical and molecular characterization of best isolates

The morphological characterization of the three best isolates I_1, I_2 and I_6 are presented in Table 1. The isolate 1_1 was gram-positive, aerobic, rod-shaped endospore-forming bacteria forming white circular colony and flat colony elevation. The colony margin was transparent with entire margin. The isolate I_2 was gram negative rod shaped, non spore formers, forming round colony with convex colony shape, entire and yellow. The isolate I_6 was gram negative, aerobic, rod shaped spore forming bacteria, producing white colony with circular and wavy margin. Colony formation was wrinkled and irregular and opaque on nutrient agar media.

The isolates I_1, I_2 and I_6 could utilize sucrose, glucose, arabinose and trehalose (Table 2). Catalase test was also positive for the three isolates thereby generating oxygen when treated with H_2O_2. Regarding citrate utilization, ONPG and Voges-Proskauer test, I_1 showed positive reaction.

The morphological and biochemical characterization of the three best isolates I_1, I_2 and I_6 revealed that they belonged to the genera *Bacillus, Rhizobium* and *Sphingobacterium* respectively. Of these the best isolate I_1 was characterized molecularly and it was found to have similarity with ribosomal sequences of *Bacillus pumilus*. In a study conducted to isolate efficient pectinolytic microbial inocula which could improve fibre quality, retting time and evade environmental pollution due to retting in jute, eight efficient microorganisms were obtained, four of which belonged to *Bacillus pumilus* having higher pectinolytic activity (Das et al., 2012).

Table 2. Biochemical characteristics of effective isolates

Tests	Malonate	Voges Proskauer's	Citrate Utilisation	ONPG	Nitrate reduction	Catalase	Arginine	Sucrose	Mannitol	Glucose	Arabinose	Trehalose
Isolate I_1	-	+	+	+	-	+	-	+	+	+	+	+
Isolate I_2	+	+	-	-	+	+	-	+	+	+	+	+
Isolate I_6	-	-	+	+	-	+	+	+	_	+	+	+

A mutant of *B. pumilus* (BpCRI) has been reported to produce promising quantity of cellulase. This has several advantages such as high activity in presence of substrates like CMC and cellobiose and enhanced production of cellulase under catabolite repression (Kotchoni et al., 2003)**.** *B. pumilu*s is widely used in industrial processes, such as the production of several traditional fermented foods, the treatment of waste water and the degradation of environmental pollutants (Saranya et al., 2014). Thus the present technology utilizing *Bacillus pumilus* for white pepper production can be effectively exploited for the commercial production of white pepper.

Nucleotide sequence accession number

The sequence of 16S rDNA of isolate I_1, (*Bacillus pumilus)* derived in this study has been deposited in GenBank under the accession number MN309903.

The traditional retting of black pepper to produce white pepper takes 10-18 days with off odour products. In the present experiment ripe berries were retted faster, producing superior quality white pepper with the isolate I_1 which has been identified to be close to *Bacillus pumilis*. Thus the microbial technology of retting using the culture of *Bacillus pumilus* could be exploited for commercial production of white pepper.

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References

Alkorta, I., Garbisu, G., Llama, M.J., and Serra, J.L. 1998. Industrial applications of pectic enzymes: A review. Process Biochem., 33:21–28.

Ausubel, F.M., Brent, R., Kingston, R.E., and Moore, D.D. 2002. Short Protocols in Molecular Biology (5th Ed.). John Wiley and Sons Publications, New York.

Aziz, N.S., Seng, N.S.S., Razali, N.S.M., Lim, S.J., and Mustapha,W.A.W. 2019. A review on conventional and biotechnological approaches in white pepper production. J. Sci. Food Agric., 99: 2665-2676.

CBI [Centre for the Promotion of Imports from developing countries]. 2018. Exporting oleoresins for food to Europe. Netherlands Enterprise Agency, Netherlands Ministry of Foreign Affairs [on-line]. Available:https://www.cbi.eu/market-information/natural-food-additives/oleoresins[2 August 2019].

Chithra, G., Mathew,S.M., and Deepthi, C. 2011. Performance of a power operated decorticator for producing white pepper from black pepper. J. Food Process Eng., 34(1): 1-10.

Das, B., Tripathi, S., Chakraborty, A., Ghosh, S., Majumdar, B., and Chakrabarti, K. 2012. Screening and molecular characterization of pectinolytic bacterial strains from jute retting water bodies. Res. J. Microbiol., 7(1): 50-58.

Fawole, M.O. and Oso, B.A. 2004. Characterization of Bacteria: Laboratory Manual of Microbiology (4th Ed.). Spectrum Book Ltd., Ibadan, pp. 24-33.

FSSAI [Food Safety and Standards Authority of India]. 2015. Lab Manual of Methods of Analysis of Foods,Spices and Condiments. Food Safety and Standards Authority of India, New Delhi, 50p.

Gopinathan, K.M. and Manilal, V.B. 2004. Pectinolytic decortication of pepper (*Piper nigrum* L.). J. Food Sci. Technol., 41: 74–77.

IPC [International Pepper Community]. 2016. Available: http://www.ipcnet.org/index_n.php

Kotchoni, O.S., Shonukan, O.O., and Gachomo, W.E.2003. *Bacillus pumilus* BpCRI 6, a promising candidate for cellulase production under conditions of catabolite repression. Afr. J. Biotechnol., 2(6): 140–146.

Krieg, N.R. and Holt, J.G. 1984. Bergey's Manual of Systematic Bacteriology, Vol.1, Williams and Wilkins, Baltimore, London, 964p.

Lane, D.J. 1991. 16S/23S rRNAsequencing. In:Stackebrandt, E. and Goodfellow, M. (eds), Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons, New York, pp. 115–175.

Mandalari, G., Bennett, R.N., Kirby, A.R., LoCurto, R.B., Bisignano, G., Waldron, K.W., and Faulds, C.B. 2006. Enzymatic hydrolysis of flavonoids and pectic oligosaccharides from bergamot (*Citrus bergamia* Risso) peel. J. Agric. Food Chem., 54:8307-8313.

McCue, P. and Shetty, K. 2003. Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with

Rhizopus oligosporus. Food Biotechnol., 17: 27-37.

Meilgaard, M., Civile, G.V. and Carr, B.T. 2006. Sensory Evaluation Techniques (4th Ed.). Boca Raton, FL: CRC Press, New York, 441p.

Saranya, P., Kumari, H.S., Rao, B.P.,and Sekaran, G. 2014. Lipase production from a novel thermo-tolerant and extreme acidophile *Bacillus pumilus* using palm oil as the substrate and treatment of palm oil-containing wastewater. Environ. Sci. Pollut. R., 21: 3907–3919.

Steinhaus, M. and Schieberle, P. 2005. Role of the fermentation process in off-odorant formation in white pepper: On-site trial in Thailand. J. Agric. Food Chem., 53(15):6056-6060.

Thankamony, A., Menon, N., Omanakuttyamma, M., Sreedharan, V.P., and Narayanan, C.S. 1999. Bacterial removal of skin from pepper. Spice Ind., 12: 10-11.

Vinod, V., Kumar, A., and Zachariah, T.J. 2014. Isolation, characterization and identification of pericarp degrading bacteria for the production of off odour free white pepper from fresh berries of *Piper nigrum* L. J. Appl. Microbiol., 116: 890-902.