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KEY WORDS: Bacillus Cereus, Food Poisoning, Boiled Rice.

ISOLATION AND IDENTIFICATION OF DEGRADING BACTERIA FROM RICE

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ABSTRACT

Security of nourishment is a fundamental necessity of sustenance quality. A total of two different Rice samples randomly taken from 2 different places i.e. the samples were brought from Guntur, Andhra Pradesh to college laboratory for bacteriological analysis for the isolation of degrading bacteria by using Streak plate technique. Sequential dilutions of the samples were used after 1 ml from each tube was pipette into a supplement nutrient agar plates and incubated for 24 hours at 37°C. The plates were inspected for the development of the bacteria. The Sub-refined of settlements from the development was done on bacteriological agar. All screened tests had levels of bacterial development going from 1.0 X 10^5 to 3.0 X 10^6 cfu/ml.90% of the examples had bacterial tallies over the worthy limits (10^4 cfu/ml).Two pathogenic micro-organisms were confined from sullied rice. Pursued by biochemical tests and confined that among 10 isolates 8 belongs to *Bacillus cereus,* 2 *belongs to Escherichia coli*.The isolated bacteria species were identified as *Bacillus cereus*. This investigation uncovered that road nourishments are potential vehicles for transmitting nourishment borne diseases in and aroun areas in Guntur.

INTRODUCTION:

Food is a substance that is devoured to keep up life and devel opment of the body.Food contains the nutrition that people and animals need to be healthy. Food can make people sick if it is contaminated by microorganisms, bad metals, or chemicals. Spoilage may occur if there is improper handling, cooking, cooling, using unhygienic utensils, and unnecessary time lag between the preparation and consu mption [4, 9]. Bacterial food spoilage is any sensory change (tactile, visual, olfactory or flavor) which makes the food to be unacceptable for consumption. Symptoms of food borne illne sses include: diarrhea, vomiting, abdominal cramp and nausea which are caused by*Staphylococcus aureus, Salmonella spp, Clostridium perfringens, Clostridium botulinum, Campylobacter, Vibrio parahaemolyticus, Bacillus cereus and* Entropathogenic *Escherichia coli.* This work investigated for the most common bacterial species present and causative for the spoilage of the food from selected food samples. Isolated and identified bacterial species associated with food contamination and their microbial loads were determined and pursued by biochemical tests and confined that among 10 isolates 8 belongs to *Bacillus cereus,* 2 *belongs to Escherichia coli*. The isolated bacteria species were identified as Bacillus cereus.These are the two pathogenic micro-organisms were confined from sullied rice.

Bacillus cereus is a Gram-positive, rod-shaped, aerobic, facu ltative anaerobic, motile, beta haemolytic bacterium com monly found in soil and food[1].Some strains are harmful to humans and causes foodborne illness, while other strains can be beneficial as probiotics for animals. It is the cause of "fried rice syndrome", as the bacteria are classically contracted from fried rice dishes that have been sitting at room temperature for hours[12]. B.cereus bacteria are facultative anaerobes, and like other members of the genus Bacillus, can produce protective endospores. It composes of seven closely related species: B. cereus sensustricto (referred to herein as *B. cereus*), *B. anthracis, B. thuringiensis, B. mycoides, B. pseudomycoides, B. weihenstephanensis, and B. cytotoxicus*. Its virulence factors include cereolysin and phospholipase C. Genomic sequencing data has shown thatB. anthracis, B. cereus and B. thuringiensis to be very closely related [1] with their 16S rRNA gene sequence sharing more than 99% similarity [2].

In Guntur selling of street foods in markets is common but most consumers and vendors have little or no knowledge about food safety. Consumption of street food has grown over the years due to rapid population growth, unemployment, poverty and availability of relatively low cost foods. Food safety depends on conditions necessary during the production, processing, storage, and preparation of food to ensure that it is safe, sound, wholesome, and fit for human consumption (FAO/WHO, 1990).This work investigated for the most commonbacterial species present and causative for the spoilage of the food from selected food samples.Isolated and identified bacterial species associated with food contamination and their microbial loads were determined.

The public health implications of consumption of such foods were established.

METHODS AND MATERIALS:

COLLETION AND SCREENING OF RICE DEGRADING BACTERIA:

The Rice samples containing the bacteria were collected from different areas of Guntur, Guntur District Andhra Pradesh. The rice sample collected was dispensed in the sterile bags and sealed and the sample was brought to the Malineni Pharmaceutical Biotechnology laboratory. 10g portion of each food sample was homogenized and serial dilution made and examined using pour plate method (*Fig-1& Fig-2*). Tenfold serial dilution of the samples was made in distilled water and 0.1ml of 10-5 to 10-9 dilutions were plated on different media (*Fig-3*). The plates were incubated at 37°C for 24 hours and observed for clearance of surrounding the colonies after incubation. The isolated colonies were streaked on Nutrient agar slants for growth and maintenance. Stock cultures were sub cultured monthly and stored at 4°C (*Fig-4*).

Fig-1 Samples of contaminated Rice

Fig-2 Samples of homogenized mixture.

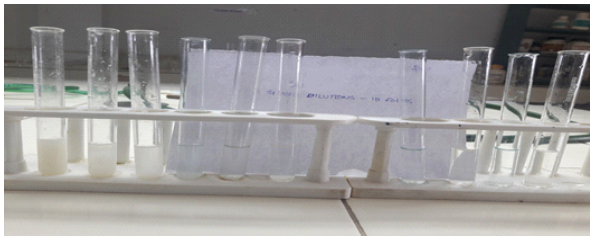

Fig-3sample of serial dilutions of homogenized mixture

Fig-4 sample ofculture of Bacillus cereus

IDENTIFICATION TESTS OF THE BACTERIA

The isolated bacteria were identified using different staining, biochemical and molecular techniques following standard protocols described by [3].

I. BIOCHEMICAL TESTS:

- Indole production test
- Methyl red and Voges Proskauer tests
- Gelatin Hydrolysis (Production of Gelatinase) Test
- Starch Hydrolysis test
- Oxidase Production Test
- Catalase Activity Test

II. BACTERIAL GROWTH CURVE DETERMINATION:

- Effect of temperature on Test Bacteria
- Growth observation of Test Bacteria at different pH levels
- Growth observation of Test Bacteria at different salt concentrations

RESULTS& DISCUSSION:

A. IDENTIFICATION OF RICE DEGRADING BACTERIA

In the present study, a total of 10 isolates, which belongs to one species of bacteria were isolated from Rice sample collected from Guntur, Guntur (Dist) Andhra Pradesh. The isolated bacteria species were identified as *Bacillus cereus*. Among 10 isolates 8 belongs to *Bacillus cereus*, 2 *belongs to Escherichia coli*. The morphological and biochemical characters exhibited by bacteria are presented in Table-1.

Table 1: morphological and biochemical tests of Bacillus cereus.

S.No	Test	*Bacillus cereus*
1	Simple staining	Rod Shaped
2	Gram staining	Gram positive Bacilli
3	Motility Test	Motile
4	Indole production Test	Negative
5	Methyl Red test	Positive
6	Voges-Proskauer Test	Positive
7	Citrate utilization Test	Positive
8	Starch Hydrolysis Test	Positive
9	Gelatin Hydrolysis Test	Negative
10	Catalase Test	Positive
11	Caesin Hydrolysis	Negative
12	Oxidase Test	Negative

B. MORPHOLOGICAL TESTS

BACILLUS CEREUS:

From morphological examination shows it is found that *Bacillus cereus* is a rod shaped gram positive bacterium with square ends and it is motile. All biochemical characters are identified through various tests which gave positive results to starch hydrolysis test, vogesproskauer, catalase test, citrate utilization and negative results to indole production test, methyl red test, gelatin hydrolysis test, casein hydrolysis and oxidase test andcatalase negative. (*Fig-5*).

Fig-5: microscopic examination of Bacillus cereus

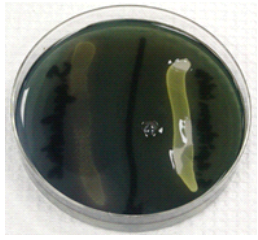

Fig-6:Starch hydrolysis of Bacillus cereus

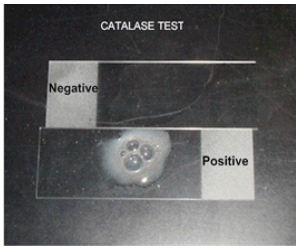

Fig-7: Catalase test of Bacillus cereus

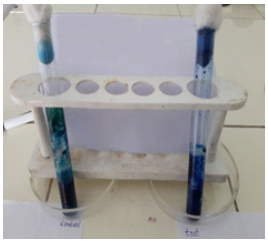

Fig-8:Voges-proskauer test of Bacillus cereus

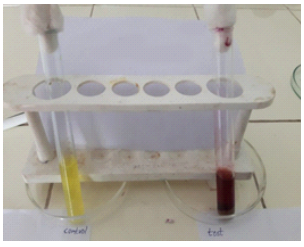

Fig- 9:Showing Citrate utilization test of Bacillus cereus

C. BACTERIAL GROWTH CURVE DETERMINATION

In the present study, bacterial growth curve was determined at different intervels. bacterial growth curve studies indicates that *Bacillus cereus* (0.95), were shows maximum growth between 2 to 4 hrs. (Table -2& Fig-10).

Table-2 Showing the O.D (600nm) Values at different time interval of the growth of Bacillus cereus,

Sample	No. of Time interval	O.D at 600 nm
Bacillus cereus	0	0.14
	1	0.32
	2	0.89
	3	0.94
	4	0.95
	5	0.96
	6	0.87
	7	0.74
	8	0.63

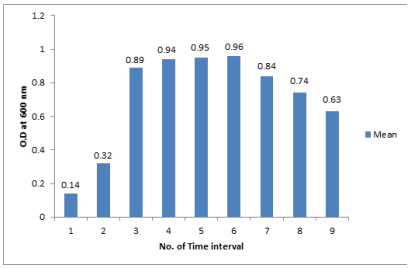

Fig-10 Showing O.D values at different time intervals of the growth of Bacillus cereus

D. EFFECT OF DIFFERENT PHYSICO - CHEMICAL PARAMETERS ON THE GROWTH OF BACTERIAL SPECIES

Effect of various physico-chemical parameters such as temperature, P^H and NaCl were studied on the growth of degrading bacteria involved was carried out during the present investigation. The results obtained indicates the effect of physico-chemical parameters on the growth of the four bacteria involved in degrading lead and mercury, namely, *Bacillus cereus.*

D.1 EFFECT OF TEMPERATURE

The bacteria *Bacillus cereus* of the present study when cultured on growth medium, have shown good growth at temperature ranging between 5 to 40 degrees C. (Table – 3).

D.2 EFFECT OF P^H

The bacteria *Bacillus cereus* are susceptible to the pH ranging between 5.0. (Table – 3)

D.3 EFFECT OF NACL

Good growths of *Bacillus cereus* were obtained in 0 to 0.5% of NaCl solution. Growth was not observed in 2.5 and above concentration of NaCl solution. (Table – 3)

Table 3: Studies on the effect of Physico-chemical parameters (Temperature, pH and NaCl) on the growth of degrading bacteria

Factor	Varied Volume	Rice
		Bacillus cereus
Temperature	**25**	**+**
	28	**+**
	30	**+**
	35	**+**
	37	**+**
	40	**+**
	45	**_**
	55	**_**
P^H	**2.5**	**_**
	3.5	**_**
	4.5	**_**
	5.5	**+**
	6.5	**++**
	7	**+**
	7.5	**+**
	8.5	**+**
	9.5	**_**
NaCl (%)	**0**	**+**
	0.5	**+**
	2.5	**_**
	5	**_**
	7.5	**_**
	10	**_**

Bacillus cereus is common in soil and on vegetation and has been isolated in several countries from a wide variety of routine samples of food [6]. For example, reported an isolation rate of 47-8 % after examination of 3,888 samples of food and food ingredients in Sweden. In food poisoning outbreaks in this country the most likely source of B. cereus is the uncooked rice. The heat resistance of B. cereus during the boiling, frying or reheating of rice is important and the data presented show that organisms, presumably spores, survive cooking and are capable of germination and outgrowth. The D 1000 values of the nine spore suspensions of B. cereus studied were in the range 1-2-7-5 min., similar to those reported by [7] 0-8-14-2 min., 5-5 min. and reported by [8] 8-0 min., for spores heated in aqueous suspension or phosphate buffer.

The experiments described in this paper were designed to simulate the times and methods of cooking and the storage conditions used by some Chinese restaurateurs [4]. It appears to be the practice in many Chinese restaurants and 'take-away' shops to save portions of boiled rice from bulk cooking until required for frying. The boiled rice is allowed to 'dry off' at room temperature for varying periods of time from a few hours to about 3 days, but usually overnight. The rice is then either reheated or more usually fried for a very short time with beaten egg and a small amount of oil before serving: the beaten egg is not always freshly prepared and may itself be highly contaminated with a variety of bacteria [10]. The Chinese are reluctant to store boiled rice in a refrigerator because they say the rice grains stick together and it becomes difficult to 'toss them' in beaten egg during frying. In some instances the fried rice is stored at room temperature and 'flash' fried again before serving.

The situation is made worse by the preparation of large bulks of boiled rice which take several hours to cool down, and there are reports [5] of the practice of adding fresh batches of boiled rice to the remains of old, which are sometimes left over from the previous day. Whether the boiled rice is allowed to dry off for varying periods of time at kitchen temperature or is left in or near a warm oven, conditions may be ideal for the germination and outgrowth of spores which have survived the boiling process.

CONCLUSION:

In the present study variations in sporulation medium and incubation conditions had little effect on the heat resistance of the spores produced and none of the spore suspensions showed any evidence of exceptional resistance to heat [11]. Results from growth experiments in boiled rice inoculated with spore suspensions of B. cereus (BC 2, 9 or 25) showed that the optimum temperature for vegetative cell growth was between 300 and 370 C. The minimum temperature for vegetative growth was between 100 and 150 C. [9] reported that B. cereus would grow in yeast extract phosphate broth when stored at 120 C. for a few days but not at 80 C. when held as long as 4 months. Until the methods described in this paper for the preparation and in particular the storage of cooked

rice is discontinued, outbreaks of food poisoning will occur. Boiled or fried rice must not be stored under warm conditions and never at a temperature between 150 and 500 C. Under no circumstances, therefore, should cooked rice be stored at kitchen temperature for more than 2 hrs. The boiled rice is allowed to 'dry off' at room temperature for varying periods of time from a few hours to about 3 days, but usually overnight. The rice is then either reheated or more usually fried for a very short time with beaten egg and a small amount of oil before serving: the beaten egg is not always freshly prepared and may it be highly contaminated with a variety of bacteria. This investigation uncovered that road nourishments are potential vehicles for transmitting nourishment borne diseases in and nearby surrounding areas in Guntur.

REFERENCES

1. Rajkowski KT, Bennett RW Bacillus cereus. Ch 3 In: Miliotis MD, Bier JW (eds) International Handbook of Foodborne Pathogens. Marcel Dekker, New York, (2003);p. 27–39
2. Ash C, Farrow JAE, Wallbanks S & Collins MD (1991) Phylogenetic heterogeneity of the genus Bacillus revealed by comparative analysis of small subunit - ribosomal RNA sequences. Lett. Appl. Microbiol. 13:202-206.
3. Buchanan, R. E. & Gibbons, N. E., eds. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams & Wilkins Co., Baltimore, Md. 21202. xxvi + 1246 pp.
4. Food and Drug Administration (FDA), 2004. Food-borne pathogenic organisms and natural toxins handbook: Department of Health and Human Services Publishers, U.S.A. Pp. 20 22.
5. Parry, J.M., Gilbert, R.J., 1980. Studies on the heat resistance of Bacillus cereus spores and growth of the organism in boiled rice. Journal of Hygiene 84, 77 – 82.
6. MORTIMER, P. R. & MCCANN, G. Food poisoning episodes associated with Bacillus cereus in fried rice. Lancet I, (1974);1043.
7. Rasko, D. A., Ravel, J., Okstad, O. A., Helgason, E., Cer, R. Z., Jiang, L., Shores, K. A., Fouts, D. E., Tourasse, N. J., Angiuoli, S. V., et al. (2004) Nucleic Acids Res. 32 , 977-988.pmid:14960714.
8. Murrell, W.G., &A.DWarth. 1965. Composition of heat resistance of bacterial spores, p. 1-24 In L,L, Campbell and H2O2, Halvorson (ed), spores III, American society for Microbiology, Washington, DC.
9. Munide, O.K., Kuria, E. 2005. Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. Afr. J. Food Agric. Nutr. Dev., 5: 1 13.
10. MOLIN, N. & SNYGG, B. G. Effect of lipid materials on heat resistance of bacterial spores. Applied Microbiology (1967);15, 1422.
11. MOL, J. H. H. The temperature characteristics of spore germination and growth of Bacillus cereus. Journal of Applied Bacteriology (1957);20, 454.
12. Arnesen SLP, Fagerlund A, Granum PE From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiology Reviews (2008);32:579–606