

**Studies on
The Bacterial Flora of Sausage
and Bacon from Government Bacon
Factory, Ranchi**

Thesis
SUBMITTED TO THE
Rajendra Agricultural University, Bihar
in Partial Fulfilment of the Requirements
FOR THE DEGREE OF
Master of Science
(VETERINARY)

By
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Public Health & Food Hygiene, Bihar Veterinary College, Patna

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P A T N A

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P A T N A

Dated, the 1st June, 1975.

This is to certify that the work embodied in this
Thesis entitled " STUDIES ON THE BACTERIAL FLORA OF
SAUSAGE AND BACON FROM GOVERNMENT BACON FACTORY, RANCHI".
is the bonafide work of Sri Laliteshwar Prasad Yadava
and was carried out under my guidance and supervision.

L. N. Mandal
(L. N. MANDAL) 1.6.75

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30.10.75
EXTERNAL EXAMINER

C E R T I F I C A T E

Certified that the research work
incorporated in this Thesis have not been
published in part or in full in any other
journal.

Pd.Yadav.
(Laliteshwar Pd.Yadava)

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(LALITESHWAR PRASAD YADAVA)

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1915-1916

The present day Indian population are confronted with the great challenge of meeting the huge demand of animal products for the consumption of the enormously growing human population of the world. In our country, this problem has become all the more acute because of the rapid increase in the population of the Indian people, but it is the rapid increase in the demand for animal products that is the main cause of the problem. This demand is being met by the production of animal products in the country. The demand for animal products is being met by the production of animal products in the country. The demand for animal products is being met by the production of animal products in the country.

INTRODUCTION

With the fast changing food habits of our people and rapid industrialization of our country, the "ready to eat" and "ready to cook" type of meat products are gaining great popularity day-by-day. Thus the supply of prepared sausage and bacon can be regarded as a step forward in the right direction. Of late, the role of pig as a source of meat of high nutritive value for the fast growing human population of our country has been realized. Till now in India, pig was a cheap source of animal protein, particularly for the people of lower social and economic strata. But in recent years, it is being treated as a good source of meat and meat products. The demand for animal products is being met by the production of animal products in the country. The demand for animal products is being met by the production of animal products in the country. The demand for animal products is being met by the production of animal products in the country.

I N T R O D U C T I O N



The present day Veterinarians are confronted with the great challenge of meeting the huge demands of animal protein for the consumption of the enormously growing human population of the world. In our country, this problem has become all the more gruesome because of the socio-economic and religious stand points of the Indian people. But in the recent years, even some of the orthodox Indians have been seen converting nonvegetarians. This favourable trend in the change of the food habits of the Indians has considerably eased the problem of animal protein shortage. Today meat and meat products form an important part of human food.

With the fast changing food habits of our people and rapid industrialization of our country, the "ready to eat" and "ready to cook" type of meat products are gaining much popularity day-by-day. Thus the supply of prepared sausage and bacon can be regarded as a step forward in the right direction. Of late, the role of pig as a source of meat of high nutritive value for the fast growing human population of our country has been realised. Till now in India, pigs were a cheap source of animal protein, particularly for the people of lower social and economic strata. But in recent years, it seems that the pig, as a good source of meat and meat products of better quality has caught our attentions. In India itself many swine meat industries have come-up and have started producing bacon and other swine meat products.

According to the F.A.O. estimate of 1972 there are 4.78 million pigs in India. The total number of pigs slaughtered during the same period was 1.6 million. The annual production of pork according to "the times of India Directory and year book including W.H.O.'s 1973" was estimated to be 0.02 million tons during the year 1972.

Bihar is next only to U.P. so far as the total population of pig is concerned. The total number of pig population in Bihar according to 1972 livestock census is 0.88 million. Annual production of pork in Bihar was 391.2 tons during the year 1966-67 according to the report of Directory of Marketing and Inspection Ministry of Food and Agriculture, New Delhi.

In Bihar, this industry has immense potentiality for its development, specially in its tribal belt. The Government of Bihar has established a bacon factory at Ranchi in the year 1967, which is producing 60 thousand kg of bacon and other meat products annually. These meat products have good market, specially in the industrial areas. These days preparations like sausage and bacon are commonly seen on the dinning table instead of the other conventional swine meat products. So it should be the endeavour of the Veterinary Public Health worker to supply clean sausage and bacon to the consumers. The consumers today are also becoming equally conscious about the microbial contamination of the meat and meat products.

There are a score of human ailments on record, which have been attributed to the swine meat. The first meat borne faecal streptococcal food poisoning outbreak among the school

children has been reported in U.S.A. in the year 1931 after the consumption of canned sausage. Streptococcal food poisoning after consumption of a special dish made up of sausage has also been reported by Allison et al. (1949).

The Public Health Laboratory Service Report for 1964 has revealed that meat was responsible for 110 outbreaks of food poisoning in England and Wales, 84% being from made up or processed meat; precooked cold or reheated meat or meat pies made with precooked meat at home or in canteens are commonly implicated. Surkiewicz et al. (1972) has isolated *Salmonella* organisms from 28% of the 560 fresh sausage samples.

With the wider recognition of meat as potent source of disease disseminator, increasing surveillance on the production process has become essential. The meat - a necessary item of food passes through several channels before it finally reaches the consumers' kitchen. Thus it gets unending opportunities for its microbial contamination which may adversely affect its organoleptic quality and it may also prove to be a potent source of public health hazard.

Now it is the sole responsibility of the Veterinary Public Health Workers to guarantee the supply of clean, disease-free meat and meat products to the consumers.

With this objective in mind the present project was undertaken to study the bacterial microflora of the sausage and bacon prepared at Bacon Factory, Ranchi. The present study may throw light on the standard of production process and help in evolving suitable control measures needed in the supply of clean meat to the consumers.

REVIEW OF LITERATURE

MICROBIOLOGY OF MEAT:

(A) Total viable count:

Ayers (1955) reported presence of 10^4 - 10^5 bacteria per sq. c.m. on superficial surface of the various organs of slaughtered pigs. The author observed higher counts to the extent of 1.5×10^{18} and 2×10^{18} aerobes per sq. inch on the skin of the neck and emphasised that a larger number of micro-organisms might be observed on outer and inner portions of tissue and in cases of faulty handling and insanitary conditions prevailing in the abattoir and butcher's shops.

Weiser (1962) pointed out that the viable counts might range from 100 to 100,000 per gramme of beef and 5,000 to 10,00,000 per gramme of pork and ham. He further examined chopped, smoked and dried meat samples and enumerated a wide variation in counts, ranging from 50,00000 to 1,00,00,000 per gramme of chopped meat whereas counts in smoked and dried meat appeared to be significantly low.

Strokes and Redmond (1966) after examining various foods of animal origin, reported that 35 to 93% bacterial population of these products were psychrophilic and 7.83% mesophilic. A survey of hamburger, beef stew, pork sausage, pork chops, beef livers, chilled chicken and frozen fish revealed 54×10^6 , 6.53×10^7 , 13×10^1 , 14×10^4 , 36×10^4 , 8×10^6 , 22×10^4 and 77×10^2 psychrophilic bacteria per gramme and 19×10^6 , 6×10^5 , 24×10^6 , 1×10^4 , 5×10^4 , 8×10^6 , 11×10^4 and

37×10^4 of mesophilic bacteria per gramme of meat respectively.

Milev et al. (1970) examined 90 samples of frozen beef and pork obtained under hygienic conditions from healthy slaughtered animals and observed that the total number of aerobic micro-organisms ranged from 2,000 - 14,55,000 while the average number did not exceed 5,00,000 micro-organisms per gramme. They recommended that the total bacterial count should not be surpassed 5,00,000 micro-organisms per gramme of meat.

Walton et al. (1971) examined 25 samples each of fresh meat (Minced beef and sausage) and cooked meat (boiled ham and roast pork) and observed that all samples fresh and cooked were heavily contaminated with different types of bacteria to the extent of 10^6 bacteria per gramme of boiled ham and 10^5 bacteria per gramme of roast pork.

Surkiewicz et al. (1972) during a bacteriological survey of fresh pork sausage collected in 44 plants observed the aerobic plate counts in the range of 5,00,000 or fewer per gramme.

(B). Types of Microflora:

Prokhorov (1941) carried out extensive studies on the bacterial flora of 16,438 meat samples from various species of animals slaughtered in emergency during the period 1938-1939 in U.S.S.R. Out of 7,650 pork samples examined, the incidence of Salmonella was 16%, Bact.coli 22.8%, Cocci 16.6%, Proteus 1.3% and other pathogenic bacteria (including Erysipelothrix

rhusiopathiae) 5.9%.

Jepsen (1957) studied the bacterial flora on the freshly slaughtered pork and established following genera and species of micro organisms; Micrococcus, Sarcina, Staphylococcus, Achromobacter, Flavobacterium, Proteus, Pseudomonas, Escherichia, Aerobacter, Alkaligenes, Bacillus, Clostridium sporogenes, Cl. bifermentans, Cl. butyricum, Cl. oedematiens, Cl. septicum, Cl. multif fermentans, Cl. cochlerium.

Baylet (1962) reported isolation of Salmonella from mesenteric lymphnodes but not from liver or muscle of pig. Escherichia, paracoli, Aerobacter, Proteus and occasionally Pseudomonas were recovered from liver, lymph nodes and muscle of cattle and pigs; Staphylococcus aureus and albus were isolated from muscles and Clostridium welchii from liver of cattle.

Frazier (1967) isolated following genera of bacteria from the surface of meat samples. Pseudomonas, Achromobacter, Micrococcus, Staphylococcus, Sarcina, Leuconostoc, Lactobacillus, Proteus, Flavobacterium, Bacillus, Clostridium and Escherichia.

Vanderzant and Nickelson (1969) established following genera and species of micro-organisms from the muscle tissue of beef, pork and lamb carcasses. These being Staphylococcus, Micrococcus, Sarcina, Streptococcus, Coryneforms, Bacillus, Clostridium, Flavobacterium, Pseudomonas, Moraxella, Alkaligenes, Acinetobacter, Anitratum (Hexella).

Milev et al. (1970) carried out investigation on the microflora of minced meat. Escherichia coli and other enterococci were found in 90 samples of frozen beef and pork obtained under hygienic conditions from healthy slaughtered animals. Proteus

occurred in 53.3%, Clostridium perfringens in 33.3%, Staphylococcus aureus in 25.5% and Bacillus cereus in 13.3% of the samples.

Riha et al. (1970) studied the microflora of fresh pork sausage casings. Samples comprised of salt packed as well as wet packed casings. Isolates from the salt packed casings revealed 60.5 % Bacillus, 7.9 % Pseudomonas, 15.8% Clostridium, 7.5 % Micrococcus, 5.6 % Proteus, 1.9 % Lactobacillus and 5.7 % unidentified.

MICROFLORA AND PUBLIC HEALTH:

(a) Streptococcus:-

The first meat borne outbreak of faecal streptococcal food poisoning were reported in 1931 from U.S.A., where canned sausages were served at an institution supper to 182 boys of whom 75 suffered with gastrointestinal disturbances.

Buchbinder et al. (1948) observed that Streptococci were implicated as the etiological agent in a number of food poisoning outbreak.

Buttiaux (1959) emphasised the importance of enterococci (faecal streptococci) as bacterial indicators of faecal pollution of foods. Niven (1963) stressed that they may play a distinctive role in indicating poor significance owing to their relatively higher resistance to drying, high temperature, detergents or disinfectants.

Diebel and Silliker (1963) observed that the significance

of Str.faecalis as a food poisoning agent appeared to be controversial.

Iris and Koburger (1970) surveyed 109 samples of different food products for the incidence of Betahaemolytic streptococci and isolates 87 strains of these organisms from 18 to 53 meat and fish products. Beta haemolytic streptococci could not be isolated from the rest of the samples which included vegetables, dairy products, dehydrated foods and miscellaneous food items.

Sedova (1970) carried out experiments on 20 healthy volunteers to know the enteropathogenic properties of enterococci. He reported 30 strains of enterococci comprising of Streptococcus faecalis, Str.faecalis var liquefaciens, Str.faecalis var zymogenes, Str.faecium, and Str.durans. These strains were inoculated on meat and milk. After 24 hours incubation at 37°C, the inoculated meat and milk were given as a food to volunteers. He conducted 511 such tests and showed that all strains of enterococci had enteropathogenic properties except Str.faecium and Str.durans.

Sterile filtrates of Str.faecalis var liquefaciens were given to 19 volunteers but they could not cause any food poisoning.

(b) Staphylococcus:

Allison et al. (1949) reported a wide spread outbreak of staphylococcal food poisoning (441 reported cases) which occurred after consumption of a special dish made up of sausage meat. Staphylococci of the same phage and serological types were isolated from the samples of vomit and stools of the victims of

the outbreak and from 102 samples of the sausage meat recovered from different shops and homes of the area involved.

Jay (1961) examined 40 frozen meat samples and isolated 37 coagulase positive staphylococci from them. He also isolated haemolytic staphylococci from hamburger, pork, beef, liver and round steak.

Moy (1962) recovered characteristic coagulase positive strains of Staphylococcus from a variety of fresh meat cuts such as chicken, pork, liver, fish, spiced ham, round beef steak, hamburger, beef liver, pork chops, veal steak and lamb chops.

Jay (1964) further examined 209 samples of non-frozen meats from 34 retail-grocery stores and reported the incidence of Staph.aureus from 38.7% of 175 samples obtained from 27 stores. No coagulase positive Staph.aureus was isolated from 36 samples obtained from 7 of the stores. The meats from which Staph.aureus was recovered in order of frequency of percentage recovery was as follows: chicken, pork, liver, fish, spiced ham, round beef steak, hamburger, beef liver, pork chops, veal steak and lamb chops.

Hobbs(1965) suggested that raw meat was frequently contaminated with staphylococci of human origin, but most outbreaks of food poisoning of Staphylococcal origin arise from cooked foods especially salt cooked meats contaminated with food handlers.

Staphylococcal food poisoning of meat has not been reported in India, however, it has been reported in case of

other foods. D'Souza et al. (1965) reported an outbreak of acute bacterial food poisoning involving 37 adults and 27 children. Coagulase positive staphylococci was traced out as etiological agent.

Grabovsky et al. (1966) investigated food poisoning in 81 patients, due to consumption of boiled smoked pork, which was contaminated with pathogenic staphylococci of 7/53/54/70/75/80/ phase types and by proteus.

Sinell and Kusch (1969) isolated 217 strains of Staphylococcus from chopped meat. The human being is considered the main source of Staphylococcus contamination of chopped meat. Angelottie (1970) reported that all the strains of coagulase positive staphylococci were potentially pathogenic. These organisms are ubiquitous in man's environment and may be isolated from air, food, water, milk, dust, faeces and sewage. The organisms are carried on the skin and mucous membranes of the nasopharynx. Since they are prevalent in the Environment and exist as "normal flora" of the skin and nasopharynx. So it is no wonder that Staphylococci are present in foods, particularly those that come in intimate contact with food handlers during processing and preparation.

Sinha et al. (1970) reported that 80 % of coagulase positive staphylococcal strain isolated from intestinal content, mesenteric lymphnode and liver of pigs were pathogenic to mice.

Milling (1971) reported four outbreaks of food poisoning associated with commercially prepared barbecued chicken, hot turkey-sandwiches and ham. Large numbers of coagulase positive staphylococci were isolated from the vomit and stool of the

cereus was 5 of 23 cream cakes, 5 of 16 rice and meat balls, 6 of 34 dehydrated soups, 6 of 23 milk powdered and none of 16 tins of tunny in oil.

(e) Escherichia:

Baylet (1962) carried out bacteriological survey of slaughtered animals and isolated Escherichia, Paracoli, Aerobacter and Proteus from liver, lymph nodes and muscles of cattle and pigs. Pseudomonas was also isolated occasionally. Staphylococcus aureus and albus were isolated from muscles and Clostridium welchii from liver of cattle.

Hobbs et al. (1962) studied 25 samples of meat, fish, fruit, and sweets for coliforms and other pathogens.

Walton (1971) examined 25 samples each of fresh meat (minced beef and sausage) and cooked meat boiled ham and roast pork) for contamination by antibiotic resistant faecal coliform bacteria. Out of these meat samples, 11 sausage, 3 minced beef and all of the boiled ham and roast pork were found free from the contamination by the said organism.

Tamura et al. (1971) isolated enteropathogenic E.coli strains from 11 of the 118 frozen broiler samples and 3 of the 15 pork samples examined but could not isolate the organism from 25 minced meat and 23 beef samples.

(f) Salmonella:

Innumerable literatures are available on the role of Salmonella as a pathogen. It is not possible to cover all of

patients as well as from all foods implicated.

(c) Micrococcus:

The genus micrococcus comprises of only non-pathogenic species which can be isolated from the skin and from the skin gland secretions of the animal and man. The food becomes contaminated by these organisms during handling (Breed et al., 1957; Cruick-Shank, 1965; Marchant and Parker, 1967). Thus the bacterial load of the food and meat is increased and its keeping quality is reduced.

Breed et al. (1957) reported that Micrococcus are never truly pathogenic. They are found on dust particles, milk and dairy products and are saprophytic, facultatively parasitic or parasitic in nature.

(d) Bacillus:

Ionescu et al. (1965) investigated the presence of Bacillus cereus in 77 samples of meat products and observed it in 23 % of minced pork, 11 % of hamburger and 68 % of salami. Jantea et al. (1965) examined 200 samples of 11 different types of prepared dishes including salad, meat and vegetables and reported the incidence of Bacillus cereus which ranged from zero to 36.3% in the samples tested.

Deriu et al. (1971) examined several food stuffs like cream cakes, rice and meat balls, tinned tunny in oil, dehydrated table soups, meat extract cubes and powdered milk for the incidence of Bacillus cereus. The incidence of Bacillus

them in the present review. However, a few related to meat borne outbreaks are given below.

Cherry et al. (1943) collected various meat products from retail market and examined them for the presence of Salmonella. Out of 250 samples examined, 13 (5.2%) were found to be positive for Salmonella. He has reported greater incidence of Salmonella in pork and pork products than in beef and beef products.

Hauser et al. (1945) reported an outbreak of food poisoning involving 14 persons due to consumption of pork sausage meat. Salmonella berta was detected as the causative organism responsible for the outbreak.

Jones and Symones (1948) have described an outbreak of gastroenteritis caused by Salm. dublin in sausage sold by two manufacturers.

Clarenburb et al. (1956) reported an outbreak of food poisoning caused by Salm. typhimurium in pork which had been boiled on the previous day and eaten without cooking.

Baylet (1962) reported isolation of Salmonella from mesenteric lymph nodes but not from liver and muscles of pig. Escherichia, paracoli, Aerobacter, Proteus and occasionally Pseudomonas were recovered from liver, lymph nodes and muscle of cattle and pig. Staphylococcus aureus and albus were isolated from muscles and Clostridium welchii from liver of cattle.

Alosi and Iannuzzi (1966) examined faeces, mesenteric

lymphnodes, bile and muscle from 250 pigs and 450 cattle and isolated different salmonellae e.g. S.dublin, S.typhimurium, S.enteritidis, S.choleraesuis, S.marscilla, S.reading, S.manchester, S.typhisuis and S.choleraesuis var kunzendorf from five cattle and six pigs.

Fessel (1968) isolated salmonella from meats of cattle, pig and sheep during bacteriological meat inspection. The commonest species was S.choleraesuis (68%) followed by S.dublin (27%) and 16 other serotypes.

Takaas et al. (1969) reported that in 1947-67 an outbreak of salmonella infection occurred in vast majority of pork and sausage meat. He established S.anatum, S.choleraesuis, var kunzendorf, S.typhimurium and S.derby.

(g) Pseudomonas:

Merchant and Packer (1969) reported that Pseudomonas aeruginosa may be responsible for number of pathological conditions in human being such as otitis, pericarditis, meningitis, septicaemia, bronchopneumonia, infantile diarrhoea and wound infections.

Adamcis et al. (1970) were of opinion that the pigmented variety of Pseudomonas are more proteolytic among the common psychrotolerant types of bacteria.

(h) Aeromonas:

Vongravenitz and Mensch (1968) reported Aeromonas strains as causative agent of several pathological conditions

in human beings. These include gastroenteritis, urinary tract infections, multiple metastatic necrosis of muscles and septicaemia.

(i) Proteus:

Tomasoffova and Novaka (1965) reported an outbreak of proteus food poisoning due to consumption of jelled pork left overnight in the kitchen after preparation. The stools, vomit and also the jelled meat were examined and isolated Proteus mirabilis serotype 8a, Ia, Ib, Ic.

Scharner et al. (1968) studied various types of sausage for the presence of the proteus group of bacteria and reported Proteus morganii, Proteus vulgaris and Proteus rettgeri. 37 of the 48 strains were characterised P.mirabilis. The remaining 111 strains were P.morganii, P.vulgaris and P.rettgeri.

MATERIALS AND METHODS

I. SOURCES OF MEAT SAMPLES:

The materials in the present study constituted the products of Bacon Factory, Ranchi (Bihar) established in the year 1966 by the Government of Bihar. The factory produces pork and various other products of swine meat.

II. COLLECTION OF SAMPLES:

For the present study, samples of Hot Dog sausage and smoked sliced bacon were obtained from the following retail stores.

- (i) Roshan Brothers, Patna,
- (ii) Government Pork shop at Veterinary Hospital, Sabjibagh, Patna.

The products are stored in refrigerator by the retailers. Samples packed in cellophane bags were purchased from the sale counter of the retailer shops and were brought to the laboratory in a big thermos-flask. The material from the retailers shops were processed within 1-2 hours after the procurement.

III. BACTERIOLOGICAL EXAMINATION:

The seal of the packed meat samples were opened near the flames and a small part of the meat was taken with the help of sterile forceps and scalpal. One gramme of meat sample was weighed in a sterile watch glass and transferred to a sterile pestle and mortar. The meat samples were triturated and mixed in 10 cc. of normal saline solution (0.85%) as suggested by American Public Health Association for the examination of

food materials. One ml. of the triturated solution was transferred to a test tube containing 9 ml. of N.S.S. and was thoroughly mixed making 10^2 dilution of the samples. In this way serial dilution upto 10^6 was prepared. Clean and sterile pipettes and test tubes were used for making the serial dilution.

IV. TOTAL COUNT:

Standard plate counts (S.P.C.) technique was employed in the present study.

One ml. of homogenous suspension from each dilution was transferred with the help of sterile pipette to different sterile petridishes. The molten Nutrient agar medium (Approx. 20 c.c.) was poured in each petridish and they were rotated in such a manner which facilitated uniform distribution of the dilution in the molten medium. The medium was allowed to solidify and the plates were incubated at 37°C for 24 hours. The plates containing between 30 to 300 colonies of bacteria were selected for counting. The colonies of the micro-organisms were examined. The number of bacteria per gramme of samples was calculated by multiplying the number of colonies counted with the dilution factor of the samples.

V. PROCEDURE FOR ISOLATION AND IDENTIFICATION OF THE ORGANISMS:

From the original 10% homogenous suspension of the samples were also inoculated on the following enrichment and selective media for isolation of the different types of organisms.

1. MacConkey agar:-

This medium was used for the isolation of lactose

fermenting and non-lactose fermenting organisms.

2. Staphylococcus no.110:

This medium was used for the isolation of staphylococci. High salt concentration of this medium suppresses the growth of several undesirable organisms.

3. Blood agar:

This medium was used to isolate various gram positive and gram negative organisms. It was further used for studying haemolytic properties of different organisms such as B.cereus, Streptococci and Staphylococci.

4. Kauffmann-Muller's tetra-thionate broth:

Approximately one gramme of the meat samples was directly inoculated in Kauffmann-Muller's tetra-thionate broth and incubated at 37°C for 24 hours for the isolation of Salmonella organisms. One loopful from T.T.Broth was streaked over the surface of MacConkey agar plate and incubated over night at 37°C for isolation of Salmonella sp.

In addition to the above mentioned media, the following other media were also used for identification and characterisation of different organisms.

- (1) Aesculin agar (2) Christensen's α urea medium
 (3) Gelatine agar (4) Hugh and Leifson's (O.F.) medium (5)
 Peptone water (6) King's agar A.& B. (7) MacConkey broth (8)
 Nitrate broth (9) Peptone water sugars such as Glucose, Lactose,
 Sucrose, Maltose, Mannitol, Arabinose, Dulcitol, Sorbitol,
 Trehalose (used for carbohydrate study) (10) Simmon's citrate
 (11) Triple sugar iron agar (T.S.I.) (12) Clark and Lubbs

medium (MR-VP medium) (13) Glucose phosphate broth (14) Kauffmann-Muller's tetra-thionate broth.

Isolates from various media were obtained in Pure-culture by using conventional method of transferring isolates from solid to liquid and from liquid to solid medium.

For identification of different organisms, the detailed plan of schedules as described by Cowan & Steel (1970) were followed.

The following primary and secondary tests were carried out during the course of identification and characterisation of various organisms isolated under this study.

Primary tests:

(i) Morphology and staining characters:

Shape, arrangement, presence or absence of spores and staining character of the organisms were studied by using Gram's method of staining.

(ii) Motility:

Broth cultures of 18 hours growth were examined microscopically in "hanging drop" preparations.

(iii) Catalage activity:

A loopful of the culture from the Nutrient agar medium was transferred on a clean slide and a drop of 3% H_2O_2 was added over it. Emergence of gas bubbles indicated a positive reaction.

(iv) Oxidase activity (Kovack, 1956):

On a piece of filter paper in a petri dish, 2-3 drops of freshly prepared oxidase reagent (1% tetramethyl-p-phenylenediamine aq. solution) was placed. A loopful of culture from the

solid medium was smeared on this impregnated paper. Appearance of a dark purple colour on the smear line within 10 seconds indicated a positive result.

(v) Carbohydrate studies:- Different peptone water sugars (described earlier) were inoculated with the cultures and incubated at 37°C. Tubes were examined daily upto 7 days for acid or acid and gas production. Gas production was observed in the previously inverted Durham's tube .

(vi) Oxidation or fermentation of glucose (Hugh and Leifson, 1953):- Duplicate tubes of O.F. medium were inoculated by stabbing with a straight wire. In one of the tubes, a layer of melted soft paraffin was added to a depth of 1 cm. The inoculated tubes were incubated and examined for several days, to know if the culture under question is oxidative, fermentative or negative. This medium also provided informations regarding gas production and motility of organisms.

SECONDARY TESTS:

(i) Citrate utilisation: - Slant of Simmon's citrate was inoculated with a single streak of the culture and was examined upto 7 days for growth and colour change. When a change in colour from original green to blue and growth on streak line were observed a positive result was indicated.

(ii) Coagulase test (Gillespie, 1943):- 0.1 ml. of a 18-24 hours broth culture was added to a tube containing 0.5 ml. 1/10 dilution of rabbit plasma in saline. The tube was incubated at 37°C followed by examination after 1, 3 and 6 hours for a coagulum. Negative tubes were left at room temperature overnight and then were re-examined.

(iii) Gelatin liquefaction:- Gelatin agar plates were

inoculated with the culture and incubated at 37°C for 3 days. After due incubation, the plates were flooded with 5-10 ml. of acid mercuric chloride solution. Clear zone, around the streak lines indicated areas of gelatin hydrolysis.

(iv) Hydrogen sulphide production:- Two types of methods were followed - (a) Tubes of triple sugar iron agar (TSI) were inoculated by stabbing the butt and streaking the slant. Incubated tubes were examined upto 7 days for the ~~appearance~~ of blackening due to H_2S production. (b) Organisms were grown in nutrient broth and a lead acetate paper was inserted between the cotton plug and the tube. Tubes were examined upto 7 days for blackening of the inserted paper indicating H_2S production.

(v) Indole production:- One ml. xylol was added to a 48 hour broth culture and shaken well. 0.5 ml. Ehrlich's reagent was run through the side of the tubes. A pink or red colour at the juncture of ^{the} two fluids indicated the presence of indole.

(vi) M.R. (Methyl red) and V.P. (Voges Proskauer) reaction:-

(a) Methyl red:- Glucose phosphate broth was inoculated with the culture and incubated at 37°C for 2 days. Two drops of methyl red solution was added, shaken and examined. Red colour indicated a positive result and yellow colour, a negative result. The tube was further kept for V-P- test.

(b) Voges-Proskauer (Barritt, 1936):- After completion of the methyl red test, 0.6 ml. α -naphthol solution and 0.2 ml. 40% KOH aq. solution were added to the same tube. After mixing the contents by shaking, the tubes were placed in a slanting position. Examination was followed after 15 minutes and 1 hour. A positive reaction was indicated by a strong red colour.

(vii) Nitrate reduction:- Nitrate broth was inoculated with the culture and incubated at 37°C for upto 5 days. One ml. nitrite reagent A was added followed by one ml. reagent B. Red colour developing within a few minutes indicated the presence of nitrite. In the tubes, which did not show red colour, powdered zinc was added 0.5 mg/ml. of the culture and the tube was allowed to stand. Red colour indicated presence of nitrate in the medium whereas absence of red colour indicated absence of nitrate in the medium.

(viii) Pigment production:- Staphylococcal and Micrococcal pigments were studied on nutrient agar plates.

(ix) Ureas activity:- Slants of Christensen's urea medium were heavily inoculated with the cultures, and incubated at 37°C. Tubes were examined daily upto 5 days. Presence of purple pink colour indicated that the organism was able to hydrolyse urea. Negative tubes did not show any change in the normal colour of the medium.

Organisms were identified and characterised according to Cruickshank (1957) and Cowan and Steel (1970)

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EXPERIMENTAL

The purpose of this experiment was to determine the effect of temperature on the rate of reaction between hydrogen peroxide and potassium iodide. The reaction was carried out at four different temperatures: 10°C, 20°C, 30°C, and 40°C. The rate of reaction was measured by the volume of oxygen gas evolved over a period of five minutes.

Materials and Apparatus

The materials used were hydrogen peroxide solution (3%), potassium iodide solution (10%), and dilute sulfuric acid. The apparatus consisted of a conical flask, a delivery tube, and a gas syringe. The reaction was carried out in a water bath maintained at the required temperature.

Procedure

A fixed volume of hydrogen peroxide solution was added to a fixed volume of potassium iodide solution in a conical flask. A small amount of dilute sulfuric acid was added as a catalyst. The flask was then placed in a water bath at the required temperature. The delivery tube was inserted into a gas syringe, and the volume of oxygen gas evolved was measured over a period of five minutes.

Results

The results of the experiment are shown in the following table. The rate of reaction was measured as the volume of oxygen gas evolved per unit time.

Table 1: Rate of reaction between hydrogen peroxide and potassium iodide at different temperatures.

The results show that the rate of reaction increases with increasing temperature. This is because the molecules have more kinetic energy and are therefore more likely to collide with sufficient energy to overcome the activation energy barrier.

OBSERVATIONS

In the present study one hundred samples of prepared food meat comprising of Hot Dog sausage and smoked sliced Bacon from different sale counters of local market were subjected to Bacteriological examination for assessing microbial load (total viable count) and types of microflora present in them.

TOTAL VIABLE COUNT:

The total viable count of Hot Dog sausage and smoked sliced bacon samples have been presented in table I. Microbial load of various meat samples was found to be varying from one sample to other as given below:-

Hot Dog Sausage samples:-

Total viable count of Hot Dog sausage samples ranged from 7.5×10^3 to 2.05×10^5 per gramme with an average of 5.08×10^4 /gm.

Smoked sliced Bacon:-

Examination of smoked sliced bacon samples revealed total viable count of 3.5×10^3 to 1.82×10^5 /gm. with an average of 3.75×10^4 /gm.

AEROBIC MICROFLORA OF HOT DOG SAUSAGE AND SMOKED SLICED BACON SAMPLES:

Incidence and distribution of various micro-organisms, isolated from Hot Dog Sausage and smoked sliced Bacon samples have been presented in table II. Detailed observation on microflora of various meat samples are as follows:

Table- I.

Table showing microbial load (Total viable counts)/gm.of samples by standard plate-count (SPC) method (37°C incubation for 24 hrs. on Nutrient agar medium).

Source of samples.	Samples number.	Hot Dog sausage	Smoked sliced Bacon.
1	2	3	4
Roshan Brothers Patna.	1	156×10^2	203×10^2
-do-	2	200×10^2	103×10^3
-do-	3	105×10^3	137×10^3
-do-	4	195×10^2	130×10^2
-do-	5	205×10^3	35×10^2
-do-	6	98×10^3	67×10^2
-do-	7	105×10^3	46×10^2
-do-	8	120×10^3	48×10^2
-do-	9	123×10^3	87×10^2
-do-	10	102×10^2	80×10^2
-do-	11	85×10^2	76×10^2
-do-	12	75×10^2	138×10^2
-do-	13	109×10^2	145×10^2
-do-	14	110×10^2	75×10^2
-do-	15	54×10^3	58×10^2
-do-	16	72×10^3	50×10^2
-do-	17	150×10^2	230×10^2
-do-	18	142×10^2	60×10^3
-do-	19	194×10^2	45×10^2
-do-	20	32×10^3	38×10^3
-do-	21	50×10^3	54×10^2
-do-	22	75×10^3	138×10^2
-do-	23	128×10^2	45×10^3
-do-	24	58×10^3	52×10^2
-do-	25	182×10^3	82×10^2

Table- I (continued)

1	2	3	4
Government Pork shop at Veterinary Hospital Sabjibagh, Patna.	26	138×10^2	103×10^2
	27	102×10^2	104×10^3
	28	92×10^3	135×10^2
-do-	29	51×10^3	82×10^2
-do-	30	148×10^2	102×10^3
-do-	31	112×10^2	144×10^3
-do-	32	104×10^2	154×10^2
-do-	33	162×10^2	95×10^3
-do-	34	52×10^3	82×10^2
-do-	35	54×10^3	102×10^3
-do-	36	143×10^2	144×10^3
-do-	37	58×10^3	154×10^2
-do-	38	149×10^2	95×10^3
-do-	39	132×10^3	182×10^3
-do-	40	41×10^3	65×10^2
-do-	41	108×10^3	58×10^2
-do-	42	146×10^2	45×10^2
-do-	43	47×10^3	38×10^2
-do-	44	102×10^3	65×10^2
-do-	45	172×10^2	109×10^2
-do-	46	131×10^2	45×10^2
-do-	47	138×10^3	38×10^2
-do-	48	43×10^3	105×10^3
-do-	49	149×10^2	102×10^2
-do-	50	155×10^2	96×10^2

Total samples from both the sources.	Average	(50894)	(37540)
	or	5.08×10^4	or 3.75×10^4
	Maximum	2.05×10^5 (205000)	1.82×10^5 (182000)
	Minimum	7.5×10^3 (7500)	3.5×10^3 (3500)

Table - II

Table showing relative frequency and percentage incidence of different types of micro-organisms isolated from 50 samples each of Hot Dog sausage and smoked sliced Bacon.

Sl. no.	Types of micro-organisms isolated	<u>Hot Dog sausage</u>		<u>Smoked sliced Bacon</u>	
		Number of samples positive.	% incidence	No. of samples positive	% incidence.
1.	<u>Micrococcus:</u>	(5)	(10)	(11)	(22)
	<u>Micrococcus sp.</u>	3	6	8	16
	<u>Micrococcus luteus</u>	2	4	3	6
2.	<u>Staphylococcus :</u>	(33)	(66)	(23)	(46)
	<u>Staphylococcus epidermidis</u>	25	50	18	36
	<u>Staphylococcus aureus</u>	8	16	5	10
3.	<u>Bacillus:</u>	(44)	(88)	(40)	(80)
	<u>Bacillus subtilis</u>	18	36	3	6
	<u>Bacillus laterosporus</u>	4	8	4	8
	<u>Bacillus macerans</u>	6	12	20	40
	<u>Bacillus polymyxa</u>	15	30	8	16
	<u>Bacillus alvei</u>	1	2	5	10
4.	<u>Citrobacter :</u>				
	<u>Citrobacter freundii</u> (1)		(2)	-	-
5.	<u>Enterobacter :</u>				
	<u>Enterobacter liquefaciens</u>	(1)	(2)	-	-

N.B.(1) Numerical figures under bracket represent percentage of incidence of various organisms upto generic level.

(11) Numerical figures without bracket represent percentage of incidence of various organisms upto species level.

Micrococcus:

In all 16 strains of micrococci were isolated from sausage and Bacon samples.

Micrococcus sp.: were recovered from three (6%) sausage and eight (16%) Bacon samples.

Micrococcus luteus: was isolated from two (4%) sausage and three (6%) Bacon samples.

Micrococcus sp.: were positive for catalase activity. They were negative for glucose fermentation, V-P and Nitrate reduction. They proved oxidative in Hugh and Lefson's (O-F) medium.

Micrococcus luteus:- strains were catalase positive. They proved negative for glucose fermentation, O-F test, V-P and nitrate reduction. On nutrient agar these strains gave yellow colonies. The important characters of Micrococcus strains isolated under the present study have been presented in table III.

Staphylococcus:

In the present study, the coagulase production was considered as the property of Staphylococcus aureus strains whereas the coagulase negative strains were treated as Staphylococcus epidermidis irrespective of their mannitol fermentation, gelatin hydrolysis and M-R and V-P reactions.

Staph. aureus was recorded in eight (16%) sausage and five (10%) Bacon samples.

Staph. epidermidis was isolated from 25 (50%) sausage and 18 (36%) Bacon samples.

All the 56 strains of Staphylococci isolated from sausage and Bacon samples were positive for catalase activity and negative

Table - III

Table showing important physiological and Biochemical characters of Micrococcus species isolated from Hot Dog sausage and smoked sliced Bacon samples.

Tests and reaction	<u>Micrococcus</u> <u>sp.</u>	<u>Micrococcus</u> <u>luteus</u>
Catalase	+	+
Oxidase	-	-
Glucose	-	-
O-F	O	-
V-P	-	-
Nitrate	-	-
Yellow pigment	-	+

+ = Positive for the test.

- = Negative for the test or no.
fermentation.

O = Oxidative.

for oxidase. They produced acid from glucose and attacked glucose fermentatively in Hugh and Lefson's (O-F) medium. Out of 13 strains of Staph.aureus, four strains (30.7%) produced Beta haemolysis on sheep blood agar. Nine strains (69.2%) of Staph.aureus fermented mannitol, 10 strains (76.9%) of them liquified gelatin and 10 strains (76.9%) were positive for V-P reaction. All strains of Staph.aureus were positive for M-R reaction.

Out of 43 strains of Staph.epidermidis, six strains (13.9%) fermented mannito, 35 strains (81.3%) liquified gelatin, 25 strains (58.1%) gave positive M-R reaction, whereas all of them (100%) proved V-P- negative. None of Staph.epidermidis strains produced haemolysis. The important characters of Staphylococcus isolated under the present investigation have been presented in table - IV.

Bacillus (Aerobic spore bearers):

In all 84 strains of Bacillus were isolated from sausage and Bacon samples.

Bacillus subtilis was recovered from 18 (36%) sausage and three (6%) Bacon samples.

B.Laterosporus: was recovered from four (8%) sausage and four (8%) Bacon samples.

B.macerans was recovered from six (12%) sausage and 20 (40%) Bacon samples.

B.polymyxa was recovered 15 (30%) sausage and eight (16%) Bacon samples.

B.alvei was recovered from one (2%) sausage and five (10%) Bacon samples.

Table - IV

Table showing important biochemical characters of Staphylococcus sp. isolated from Hot Dog sausage and smoked sliced Bacon samples of pig meat.

Sl. no.	Tests	Total strains of Staphylococci isolated: 56			
		<u>Staphylococcus aureus</u>		<u>Staphylococcus epidermidis</u>	
		No. of strains: 13		No. of strains: 43	
		(23.2%)		(76.8%)	
		No. of strains	%	No. of strains	%
		positive		positive	
1.	Coagulase	13	100	0	0
2.	Mannitol(acid)	9	69.2	6	13.9
3.	M-R reaction	13	100	25	58.1
4.	V-P reaction	10	76.9	-	-
5.	Gelatin liquifaction	10	76.9	35	81.3
6.	Haemolysis	4	30.7	-	-

Remarks:- The differentiation of Staph. aureus and Staph. epidermidis were done on the basis of coagulase activity. Coagulase positive strains were treated as Staph. aureus, irrespective of other characteristics observed (Breed et al., 1957).

These strains of *Bacillus* exhibited typical gram positive, spore bearing rods in stained preparation. They were all motile, catalase positive and oxidase negative. In general they fermented glucose, but attacked it variably in Hugh and Leifson's (O-F) medium.

Bacillus subtilis strains fermented glucose, mannitol and arabinose but they did not ferment lactose. They were also indole negative and V-P-positive. Further, they also utilised citrate, reduced nitrate to nitrite, hydrolysed gelatin and were positive for urease activity.

B. laterosporus strains were positive for glucose, mannitol, and negative ^{for} indole, V-P, urease, citrate utilisation and positive for nitrate and gelatin hydrolysis.

B. macerans was positive for nitrate, gelatin hydrolysis and negative for urease activity. Glucose, mannitol and arabinose were fermented while lactose was not fermented. They were negative for indole, V-P and citrate utilisation.

B. polymyxa strains fermented glucose, mannitol, arabinose but did not ferment lactose. They were also indole negative and V-P positive. Further, they also hydrolysed gelatin, nitrate to nitrite, but were negative for citrate utilisation and urease activity.

B. alvei strains fermented glucose only. They did not ferment lactose, mannitol or arabinose. They proved positive for indole, V-P, nitrate reduction and gelatin hydrolysis. They were negative for citrate utilisation and urease activity. The important characters of *Bacillus* strains isolated under the present study have been presented in table V.

Table- V

Table showing the important physiological and Biochemical characters of Bacillus species isolated from Hot Dog sausage and smoked sliced Bacon samples.

Tests and reactions.	<u>Bacillus subtilis</u>	<u>Bacillus latero-sporus.</u>	<u>Bacillus macerans</u>	<u>Bacillus polymyxa</u>	<u>Bacillus alvei</u>
Gram reaction	+	+	+	+	+
Spore	+	+	+	+	+
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
O-F	F	F	F	F	F
Glucose	+	+	+	+	+
Lactose	-	-	-	-	-
Mannitol	+	+	+	+	-
Arabinose	+	-	+	+	-
Indole	-	-	-	-	+
V-P	+	-	-	+	+
Citrate utilization	+	-	-	-	-
Nitrate	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+
Urease	+	-	-	-	-

+ = Positive for the test.

- = Negative for the test or no fermentation.

F = Fermentative.

Table - VI

Table showing important physiological and biochemical characters of Citrobacter and Enterobacter species isolated from Hot Dog sausage and smoked sliced Bacon samples of pig meat.

Tests and reaction	<u>Citrobacter</u> <u>freundii</u>	<u>Enterobacter</u> <u>liquefaciens</u>
Catalase	+	+
Oxidase	-	-
O-F	F	F
Glucose	Ag	Ag
Lactose	Ag	-
Sucrose	A	A
Mannitol	A	A
Arabinose	A	A
Dulcitol	A	-
Indole	-	-
M-R	+	+
V-P	-	-
Citrate utilisation	+	+
H ₂ S (in T.S.I.)	+	-
Gelatin hydrolysis	-	+
Urease	-	-

+ = Positive for the test.

- = Negative for the test or no fermentation.

A = Acid.

Ag = Acid and gas.

N.B.- Attempts to study gas production were done only in case of glucose and lactose.

Citrobacter:

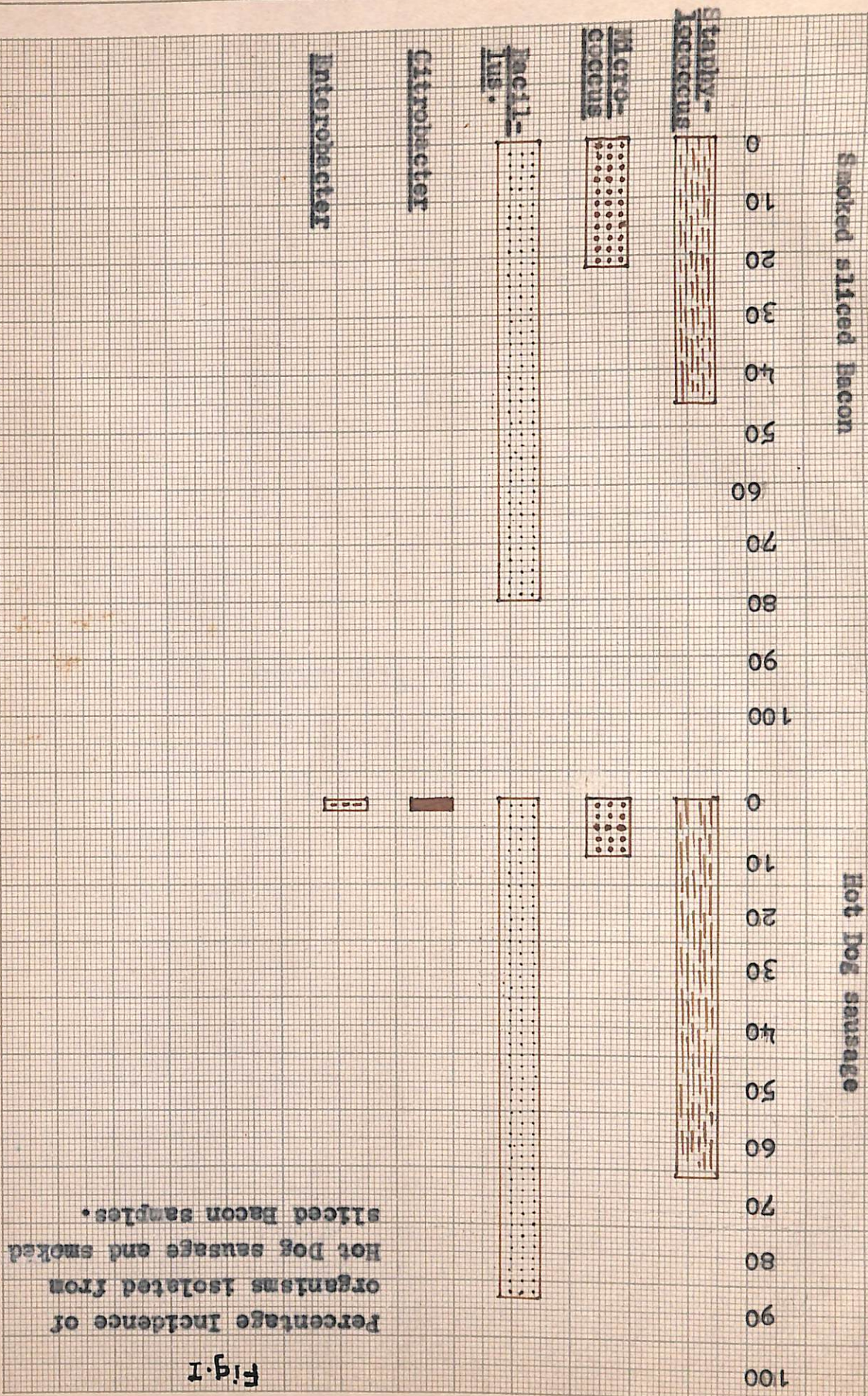
Citrobacter freundii was isolated from one (2%) sausage sample. It was gram negative motile rod. It was positive for catalase and negative for oxidase activity. It attacked glucose fermentatively in Hugh and Leifson's (O-F) medium. In addition to glucose, it fermented lactose, sucrose, mannitol, arabinose, and dulcitol. It was positive for M-R, citrate utilisation and H_2S production. It proved negative for indol, V-P, gelatin hydrolysis and was positive for urease activity. The important characters of Citrobacter freundii strain isolated under the present investigation have been presented in table VI.

Enterobacter:

In all only one strain (2%) of Enterobacter was isolated from sausage sample.

E. liquefaciens strain fermented glucose, sucrose, mannitol and arabinose. It did not ferment lactose and dulcitol. It was positive for M-R, citrate utilisation and gelatin hydrolysis. It proved negative for indole, V-P, H_2S production and urease activity. The important characters of Enterobacter strain isolated under the present study have been presented in table VI.

The percentage incidence of the different organisms isolated from Hot Dog sausage and smoked sliced Bacon samples has been represented in fig.no.1.



Percentage Incidence of
organisms isolated from
Hot Dog sausage and smoked
sliced Bacon samples.

Fig. I

D I S C U S S I O N

D I S C U S S I O N

DISCUSSION

The meat products such as Hot Dog sausage and smoked sliced Bacon pass through several channels of processing before they finally reach to the consumer's table. As a result they get various opportunities of microbial contamination which may adversely affect their quality and the contaminated meat products may also prove to be a potent source of public health hazards.

TOTAL VIABLE COUNT (TVC):

It is evident from table-I that the average TVC of Hot Dog sausage and smoked sliced Bacon were 5.08×10^4 (50894) and 3.75×10^4 (37540) per gramme respectively. The maximum count of these two samples were 2.05×10^5 (205000) and 1.82×10^5 (182000) and the minimum count were 7.5×10^3 (7500) and 3.5×10^3 (3500) per gramme respectively.

Weiser (1962) observed that the viable count of pork and ham might range from 5000 to 10,00,000 per gramme. Milev et al. (1970) observed that the total number of aerobic microorganisms ranged from 2000 - 14,55,000 while the average number did not exceed 5,00,000 micro-organisms per gramme. They recommended that the total bacterial count should not be surpassed 5,00,000 micro-organisms per gramme of meat. Surkiewicz et al. (1972) during a bacteriological survey of fresh pork sausage collected from 44 plants, observed the aerobic plate counts in the range of 5,00,000 or fewer per gramme.

In the present study the TVC of sausage and Bacon were found to be much below the maximum limit of 5,00,000 micro-organisms per gramme of meat as recommended by Milev et al. (1970) and Surkiewicz et al. (1972). The significantly, low count of the smoked sliced bacon observed under the present investigation, is also in agreement with the reports of Weiser (1962).

On the basis of above mentioned findings it can be safely concluded that the samples (Hot Dog sausage and smoked sliced Bacon) marketed by the Bacon factory, Ranchi come within the standards prescribed by the various workers abroad for such type of meat products.

TYPES OF MICROFLORA IN HOT DOG SAUSAGE AND SMOKED SLICED BACON SAMPLES:

The different types of microflora isolated from Hot Dog sausage and smoked sliced Bacon have been shown in table-II.

Microflora isolated comprised of Micrococcus, Staphylococcus, Bacillus, Citrobacter and Enterobacter.

Various workers such as Prokhorov (1941), Ayers (1955), Jepsen (1957), Baylet (1962), Frazier (1967), Vanderzant and Nickleson (1969), Milev et al. (1970) and Riha et al. (1970) have studied the microflora of various meat samples such as beef, veal, pork sausage and other meat products.

MICROSCOCOCCUS:

The incidence of Micrococcus sp. was 6% in Hot Dog sausage and 16% smoked sliced Bacon samples. Micrococcus luteus was isolated from 4% of sausage and 6% Bacon samples.

Jepsen (1957), Frazier (1967), Vanderzant and Nickelson

(1969) and Riha et al. (1970) have also found the presence of Micrococcus in pork and pork sausage^{casing}. The present findings of Micrococcus in sausage and Bacon are in agreement with the findings of above mentioned workers.

The strain of Micrococcus isolated under the present study may not be pathogenic but according to Breed et al. (1957), Cruickshank (1965), Merchant and Packer (1969) these organisms increase the bacterial load of the meat and reduce its keeping quality.

STAPHYLOCOCCUS:

In the present study Staphylococci were isolated from 33 (66%) and 23 (46%) of Hot Dog sausage and smoked sliced Bacon samples respectively. Out of the total 56 strains of Staphylococci isolated from sausage and Bacon samples, 13 strains proved Staph. aureus whereas 43 strains were Staph. epidermidis on the basis of coagulase production.

In the present study 13 strains of Staph. aureus were isolated from the different samples of Hot Dog sausage and smoked sliced Bacon and were thought to be pathogenic on the basis of coagulase positive strain. The author agrees with the findings of Moy (1962) and Angellotic (1970) who reported that all strains of coagulase positive Staphylococci were pathogenic. Sinha et al. (1970) also observed that 80% of coagulase positive Staphylococci from pig viscera were pathogenic to mice.

During this study four (30.7%) strains of Staph. aureus produced Beta haemolysis on sheep blood agar. Thus the observation

in the present study is in agreement with the findings of Behrend (1972).

Outbreaks of meat borne food poisoning due to Staphylococci have been reported by various workers from time to time. Allison et al. (1970), reported a wide spread outbreak of Staphylococcal food poisoning which occurred after consumption of sausage meat. Grabovsky et al. (1966) investigated food poisoning in 81 patients due to consumption of boiled smoked pork, which was contaminated with pathogenic Staphylococci and Proteus.

During the present investigation no outbreak of food poisoning has been reported. But due to the presence of pathogenic strain of Staph.aureus in sausage and Bacon samples, One should always take care for wholesome products of meat.

BACILLUS:(Aerobic spore formers):

The aerobic spore formers encountered in the Hot Dog sausage and smoked sliced Bacon samples of present investigation were Bacillus subtilis, B.laterosporus, B.marcerans, B.polymyxa and B. alvei.

Recovery of Bacillus from a variety of meat and meat products such as beef, pork, lamb, minced meat and sausage casings have been reported by various workers (Frazier, 1967) Vanderzant and Nickelson, 1969; Milev et al., 1970 and Riha et al., 1970).

All the Bacillus species isolated during present study are non-pathogenic to man.

From the available literature, it seems that these five species of Bacillus have been reported for the first time from sausage and Bacon samples in the present study.

Presence of Bacillus sp. in sausage and Bacon samples under the present study may be related to their wide range of distribution in soil, water, dust and other spoilage products from where they get easy access to meat samples during various slaughtering and processing operation.

Their presence may be regarded as an indicator of sub-standard sanitary condition at any stage of slaughtering, processing or packing operations in the manufacture of these products.

CITROBACTER:

Citrobacter freundii was isolated from (2%) sausage sample. On perusal of available literature it appears that recovery of Citrobacter has not been reported from pig meat. They are widely distributed in nature and are normally found in soil, water, intestinal canal of man and other animals (Breed et al., 1957). Its presence in meat indicate the external contamination either during processing or transportation.

ENTEROBACTER:

Enterobacter liquefaciens was recovered from only one (2%) sample of Hot Dog sausage. Lepovetsky et al. (1953), Jepsen (1957), Baylet (1962) have also reported the presence of Enterobacter in various meat samples viz. muscle, lymph nodes, marrow samples of cattle and pigs.

Enterobacter sp. are widely distributed in nature. They are usually found on grains and plants, water, milk, and alimentary tract of man and other animals (Breed et al., 1957). Their recovery from sausage samples may be attributed to faecal contamination during dressing and processing operations.

From the observation made under the present study it can be safely concluded that the products "Hot Dog sausage and smoked sliced Bacon" produced and marketed by the Government Bacon Factory, Ranchi are of standard quality as far as their bacteriological quality is concerned. However, presence of coagulase positive Staphylococci in 8 (16%) sausage samples and 5 (10%) bacon samples, warrants attention. As contamination with Staphylococci occur chiefly from food handlers during various processing operations, it would be advisable that a care should be exercised while processing these products.

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S U M M A R Y

The objective of the present study was to assess the bacterial load and to investigate the aerobic microflora associated with Hot Dog sausage and smoked sliced Bacon prepared at Ranchi Bacon Factory and to evolve suitable control measures for the production of clean and wholesome products for consumers.

In all, fifty Hot Dog sausage and fifty smoked sliced Bacon samples prepared by Ranchi Bacon Factory were collected from the sale counters of local market "Roshan Brothers, Patna" and "Government Pork shop" Veterinary Hospital, Sabjibagh, Patna.

The total viable counts (TVC) were determined by standard plate count technique and the organisms were identified mainly on the basis of cultural, morphological and biochemical characters.

The total counts of (TVC) of Hot Dog sausage and smoked sliced Bacon ranged respectively from 7.5×10^3 to 2.05×10^5 and 3.5×10^3 to 1.82×10^5 per gramme of meat with the average of 5.08×10^4 per gramme of Hot Dog sausage and 3.75×10^4 per gramme of smoked sliced Bacon.

Bacteriological study of sausage resulted in the recovery of Micrococcus sp., Micrococcus luteus, Staphylococcus aureus; Staphylococcus epidermidis, Bacillus subtilis, Bacillus laterosporus, Bacillus macerans, Bacillus polymyxa, Bacillus alvei, Citrobacter freundii and Enterobacter liquefaciens.

From the smoked sliced Bacon samples, Micrococcus sp., Micrococcus luteus, Staphylococcus aureus, Staph. epidermidis, Bacillus subtilis, Bacillus laterosporus, Bacillus macerans, Bacillus polymyxa, Bacillus alvei, were isolated.

Bacillus subtilis, Bacillus laterosporus, Bacillus macerans, Bacillus polymyxa, Bacillus alvei and Citrobacter freundii were recorded from the pig meat for the first time.

The importance of microbial load and types of microflora isolated from Hot Dog sausage and smoked sliced Bacon samples in the present study has been discussed in relation to public health and economy of meat industry.

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