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Radiation Response (Low Dose Gamma Radiation) of Members of the *Enterobacteriaceae* in Dry Fruiting Bodies of *Pleurotus ostreatus* and Their Identification Using API 20E System

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Abstract

The effectiveness of using low dose gamma radiation (1, 2, 3, 4 and 5 kiloGray) was tested on dry fruit bodies of *Pleurotus ostreatus* stored in polythene and polypropylene and stored at 28-30 ^{o}C up to 12 months for identification of members of Enterobacteriaceae with API 20E test kit (Biomerieux ®, France). Encountered microorganisms of the Control samples (0 kGy) of both packages recorded included *Klebsiella pneumoniae, Citrobacter freundii, Pseudomonas aeroginosa Proteus mirabilis, Serratia marscesens* and *Enterobacter spp*. There was an observed decrease in bacteria species with increasing dose. However, *Klebsiella pneumonia* persisted after the application 5 kGy dose in both packaging materials. Health and immuno compromised persons stand a greater health risk after interaction with some of these bacteria spp. Case in point, infection of patients with cystic fibrosis by *Pseudomonas aeruginosa* is known to be lethal over a long period of time. Information on Enterobacteria in the food industry is essential since they are potential food poisoning agents and thus their control is imperative.

1 Introduction

Members of the Enterobacteriaceae are generally facultative anaerobes, gram negative, non-spore formers. Most of these organisms are pathogenic, while others produce toxins responsible for food intoxication[1]. Their presence in food usually indicates faecal contamination or insanitary conditions. Even though these microorganisms were not of much public concern, they have been involved in a number of health problems since they are responsible for nonsocomial infections such as respiratory and urinary tract infections, several diarrhoeal and pulmonary infections, meningitis and septicemia in young children as well as neonates[2,3].

In the quality concept of a food product, its microbiological quality is a significant factor to consider. More often than not, the constitution of the microflora of vegetables, fruits and mushrooms are non pathogenic and epiphytic. Treatment of soil with organic fertilizers such as composted manure, sewage sludge and from irrigation water may be the outcome of contamination. Occurrence of certain food borne illnesses, has a direct correlation with the consumption of raw vegetables, fruits and mushrooms due to improper handling from the farm gate to the plate. These have often occurred in developing countries and have become more frequent in developed countries in the last decade [4,5,6]. It is noteworthy that under the right set of circumstances, everyone is prone to illness resulting from contaminated foods. Nonetheless, the risks and dangers connected with foodborne illness are much greater for the vulnerable group; the elderly, infants, pregnant women and immuno- compromised persons.

According to Food Safety & Hygeine[7], although eliminating the risks is difficult; it is possible to manage them based on the identification and manipulating the dynamics in preventing

contamination or suppressing the growth of these pathogenic microorganisms.

Perusal of pertinent literature by some researchers [8,9,10,11] confirms the possibility of application of ionizing radiations to control spoilage and contaminations by pathogenic microorganisms to improve hygienic quality, increase shelf life and reduce or substitute the use of decontaminating chemicals, which are hazardous to human health.

The objective of this study was to investigate the efficacy of low dose gamma radiation on enterobacteria isolated from dried mushroom fruit bodies stored up to 12 months in polythene and polypropylene packs.

2 Materials and Methods

2.1 *Materials*

2.1.1 *Mushroom Samples*

Growth and harvesting of matured *P. ostreatus* mushrooms samples were done on composted sawdust according to method outlined by [12] which were carried out at the Council for Scientific and Industrial Research- Food Research Institute, Mycology Unit, Accra, between the periods of

Accra, between the periods of February to May 2014.

2.1.2 Processing

2.1.3 Drying of mushroom samples

Drying was carried out in a solar dryer at a temperature of 50-60^{o}C to reduce moisture content to a range of about 11- 13% for an average period of 12 days.

2.1.4 Irradiation of mushroom materials

Gamma radiation doses of 0, 1, 2, 3, 4 and 5 kGy at a dose rate of 1.7 kGy per hour in air from a ^{60}Co source (SLL 515, Hungary) batch irradiator were applied to forty (40) grams each of dried oyster mushrooms (*Pleurotus ostreatus)* packaged in containers (polythene and polypropylene). Fricke's dosimetry system was used to confirm absorbed doses. It is a reference chemical dosimeter that produces ferric ions in aqueous sulphuric acid solution from the chemical oxidation of ferrous ions (Fe^{2+}) obtained by ionizing radiation at the Radiation Technology Centre of the Ghana Atomic Energy, Kwabenya, Accra.

2.1.5 Determination of moisture content

Gravimetric method of [13] was used to determine the moisture content.

2.1.6 Determination of Characteristics of isolates

Purification of colonies was carried out by picking a bacteria colony which appeared to be constituted of one cell type with an inoculating loop. Streaking was done onto the selective agar plates which were then inoculated at 37^{o}C for a period of 19-24hrs. Restreaking of the isolated colony was carried out after incubation. To obtain identical colonies, the process was repeated two times. Gram staining was carried out to obtain pure bacteria colonies as a confirmation procedure. There was an observed dark purple for Gram positive bacteria and a pink colour for Gram negative.

2.1.7 Biochemical Characterization of Isolated Colonies

Random selection was done for pure colonies from the mushroom samples for identification using the Analytical Profile Index (API 20E) system[14].

2.1.8 Preparation of the Strip

Five milliliters (5ml) of sterile distilled water was carefully distributed into the honey comb wells of the tray after an API incubation box (tray with lid) was prepared. The essence was to create a humid atmosphere. The API strip was then placed in it.

2.1.9 Preparation of Inoculum

Inoculation was done using fresh isolates (19 - 24 hours old). Removal of a single well isolated colony from the isolation plate was done with an inoculation pin. Emulsification was carefully done to obtain a homogeneous bacterial suspension in a tube containing 5ml of sterile distilled water.

2.2. Inoculation of the Strip

Miniature microtube reaction chambers are used by the API system. A microtube consists of a tube and a cupule section. Both the cupule of the test citrate (CIT), Voges Proskauer (VP) and gelatinase (GEL) and the tube, were filled with the bacterial suspension using a pipette.

The bacterial suspensions were used to fill to the tube level (not the cupule) for the rest of the tests. By adding mineral oil to the cupule level of the microtube, anaerobic conditions were formed in the tests arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decaboxylase (ODC), hydrogen sulphide (H_2S) production as well as urease.

Incubation of the box was done at 35°C - 37°C for 19 - 24 hrs after lid was closed.

2.2.1 Reading of Strip

Examinations of the strips were done by comparing to the reference, and all spontaneous reactions (+/−) were recorded on a result sheet after incubation for 19 - 24 hrs. Extra reagents were added to some tests such as Indole (IND) test for Indole production of which just a drop of James reagent[14] (BioMerieux®, France), was added. Tryptophane deaminase (TDA) of which one drop was added to the bacterial suspension in both the tube and the cupule. An observed dark brown colouration indicated a positive reaction was recorded. Development of a pink colouration in the whole cupule indicated a positive reaction VP test: for the reaction to take place, one

drop each of VP1 as well as VP2 reagents were added to the tube and which was then left for 10 mins for reaction to occur. Pink/red indicated a positive reaction.

2.2.2 Interpretation and identification

By the Analytical Profile Index (BioMerieux®, France), patterns of the reactions were coded numerically. The tests were separated into different groups on the result sheet. Groups of 3 and a value 1, 2 or 4 as designed by the manufacturer were indicated for each. A seven-digit value was obtained for the 20 tests of the API 20E strip (BioMerieux®1998, France), by adding the values corresponding to positive reactions within each group.

2.2.3 Statistical Analysis

Mean values of the microorganisms were carried out with Microsoft Excel (Windows version 7)

3 Results

Increasing doses of gamma radiation (0-5 kGy) caused commensurate decrease in species diversity. For example, bacterial species isolated from non-irradiated (0 kGy) mushroom fruit bodies stored in polypropylene packs were *Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis, Serratia marscesens* and *Enterobacter spp*. After applying 1 kGy of gamma irradiation only *Klebsiella pneumoniae* remained and was also isolated from samples exposed to 5 kGy of gamma radiation (Table 1). The same trend was found for samples stored in polythene packs (Table 2). In the dried mushrooms samples stored in either polypropylene or polythene pouches. Only *Klebsiella pneumoniae* was persistent and was isolated in the bags treated with 5 kGy gamma irradiation.

Table 1: List of members of the Enterobacteriaceae isolated from dry mushroom fruit bodies treated with 0-5 kGy of gamma irradiation and stored in polypropylene packaging material

Package	Applied dose (kGy)	Microorganism (s)
Polypropylene	0	*Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis, Serratia marscesens*
	1	*Klebsiella pneumoniae, Citrobacter freundii*
	2	*Klebsiella pneumoniae*
	3	*Klebsiella pneumoniae, Enterobacter spp*
	4	*Klebsiella pneumoniae*
	5	*Klebsiella pneumoniae*

Table 2: List of members of the Enterobacteriaceae isolated from dry mushroom fruit bodies treated with 0-5 kGy of gamma irradiation and stored in polythene packaging material

Package	Applied dose (kGy)	Microorganism (s)
Polythene	**0**	*Pseudomonas aeroginosa, Klebsiella pneumoniae, Enterobacter spp.*
	1	*Klebsiella pneumoniae, Pseudomonas aeroginosa*
	2	*Klebsiella pneumoniae*
	3	*Klebsiella pneumoniae*
	4	*Klebsiella pneumoniae*
	5	*Klebsiella pneumoniae*

4 Discussions

Enterobacteriaceae comprise a large group of genetically and biochemically related bacteria[1]. The Enterobacteriaceae family is generally facultatively anaerobic, gram negative non spore formers and range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length[15,16] .

Most of these organisms are pathogenic while others produce toxins responsible for food intoxication. With certain foods Enterobacteriaceae can also provide a measure of food quality

and spoilage potential. Some Enterobacteriaceae are commonly found in the gastrointestinal tract of animals, including humans. *Klebsiella pneumoniae* is part of the normal flora in the nose, mouth and intestines. It may cause lesions in almost every part of the body, pneumonia, chronic lung absecess, upper respiratory tract infections, sinusitis, endocarditis, septicemia, meningitis, gastroenteritis, peritonitis, liver and bilary tract disease, wound infections, salpingitis and skin and uterine tract infections[17]. The bacterial spp. is also known to cause high fever, chills as well as flu-like symptoms in humans[18].

The observed persistence of *Klebsiella pneumoniae* demonstrates its resistance to low gamma radiation doses. Belonging to the family Enterobacteriaceae, they are encapsulated rod shaped, non motile and gram negative bacteria. Amako *et al*[19] described the composition of its encapsulated wall to consist of a heavily packed accumulation of fine fibers, which represented a polymer of capsular polysaccharide with approximate layer thickness of 160 nm. Sridha-Rao[20] showed that *K. pneumoniae* has a very large capsule which is impregnated with water which might have contributed to its radiation resistance. According to Ali *et al*,[21] some bacteria can repair the damage of DNA and resist the effect of irradiation. The effectiveness of the process depends on the organism's sensitivity to irradiation, the rate at which it can repair damaged DNA, and especially on the amount of DNA in the target organism. Molins and Ricardo[22] stated that the survival of a microorganism may also depend on the dose as well as the nature of radiation used in the process. Furthermore, radiosensitivity of bacteria varies depending on the packaging atmosphere used. Bacteria are very sensitive to irradiation in the presence of oxygen[10]. The presence of oxygen under the packaging conditions might have enhanced the lethal effect of radiation, due to oxygen radical and ozone formation during the treatment[10]. During irradiation, various oxygen species are produced from the formation of free radicals which causes distress in the DNA thereby the multiplication of bacteria. In general, the most common free radicals created following irradiation treatment stem from oxygen and water.

Generally, the decrease in population of *K. pneumoniae* in this study was probably due to the effect of energy produced from increasing doses of irradiation, which might have broken the bonds in the DNA molecules, leading to inability of microorganisms to replicate and reproduce resulting in bacterial death[23]. The overall decline and death of microorganisms is largely dependent on two factors namely unfavourable environmental conditions such as pH, moisture, temperature, access to nutrients and water activity content (Aw). Results obtained in this present study agreed with that of similar previous research by Nyoagbe[2] who reported a decrease in Enterobacteriaceae population on snails after treatment with gamma radiation up to 6 kGy. Moini *et al*,[24] also reported a success in reduction of Enterobacteria on refrigerated rainbow trout (*Onchorhynchus mykiss*) fillets after irradiation with 1-5 kGy.

5 Conclusion

Enterobacteria sp. populations on dehydrated oyster mushrooms (*Pleurotus ostreatus*) stored in polythene and polypropylene packs decreased satisfactorily to achieve acceptable levels with the application of low dose gamma radiation. Consumers, in particular, those with health challenges or compromised immune system need to be cautious by ensuring that mushrooms are washed and properly cooked before consumption.

Further studies are recommended in this field of study to investigate the influence of ionizing radiations on bacterial spores. Additionally, mechanisms involved in bacterial resistance to radiation exposure need to be investigated thoroughly.

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7 Conflicts of Interests

The authors hereby declare that there are no conflicts of interests

8 Author's contributions

NKK, GTO, MO and MWK carried out data analysis/interpretation and manuscript preparation. NKK and GTO worked on the final document approval. NKK and MWK carried out the research conception/design and data acquisition.

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