*FUDMA Journal of Sciences (FJS)*
*ISSN online: 2616-1370*
*ISSN print: 2645 - 2944*
*Vol. 4 No. 1, March, 2020, pp 473 – 479*

# OCCURRENCE AND IDENTIFICATION OF *Bacillus* SPECIES FROM DIFFERENT SOURCES OF HONEY

**[*1]Ndukwe, N. N. and [2]Agbagwa, E. O.**

[1]Federal University of Kashere, Faculty of Sciences, Gombe state
[2]University of Port Harcourt, Faculty of Sciences, Rivers State

***Corresponding Author's Email: n.n.ndukwe@fukashere.edu.ng, nelson_ndukwe@yahoo.com**

**ABSTRACT**

Honey is a sweet viscous liquid produced by honey bee Apis mellifera from the nectar of plants. This study was carried out to determine the occurrence and identification of *Bacillus* species in honey samples from apiaries, locally harvested, commercial and packaged honey samples marketed in some states of Nigeria. Twenty seven honey samples were obtained from: Abia, Enugu, and Benue and Rivers state. Honey samples were diluted with sterile distilled water and pour plated onto Hi-Chrome Bacillus agar. The isolates were identified using chromogenic media, phenotypic and Analytical Profile Index (API) kits (AP 50CHB). Nine species of *Bacillus* species identified include: *Bacillus* badius, B. cereus, B. circulans, B. laterosporus, B. licheniformis, B. megaterium, B. mycoides, B. polymyx and B. subtilis. The Bacilli count for honey samples were in the range 3.48 -2.96 log cfu/ml (Abia), 3.44 - 3.20 log cfu/ml (Benue), 3.36 - 3.15 log cfu/ml (Enugu) and 2.91-3.40 log cfu/ml (Rivers state) respectively. The occurrences of Bacillus species were significantly higher ($p<0.05$) in samples from commercial traders compared to Apiaries, locally harvested and the branded products. There was no significant difference ($p>0.05$) between the branded product samples and those from apiaries. This study has demonstrated that Bacillus species are prevalent in different sources of honey.

**Keywords**: *Apiaries, Honey, Prevalence, Bacillus, Hi-Chrome Media, Characterization*

## INTRODUCTION

Honey is a sweet substance produced by bees through sucking nectars of flowering plants. The type of honey manufacture by honey bees (Apis melifera) are the type harvested by majority of the local farmers and beekeepers for the purpose of consumption (François et al., 2018). Insects such as bumblebees, stingless bees produce honey comprising of different properties from Apis melifera. Honey sweetness is derived from the simple carbohydrate sugars (fructose and glucose), and has almost the same taste as table sugar (Bryant et al., 2001). Honey contains glucose oxidase, catalase, ascorbic acid, phenolic acids, polyphenols, organic acids, amino acids, and proteins (Bogdanov et al., 2008). Mechanism involved in honey production includes process of regurgitation and evaporation (Afroz et al., 2016). They store their product in wax honey combs inside the beehive and utilizes it as primary source of food. Honey is universally accepted, and its uses cut across religious, ethnicity, cultural beliefs and civilization.

Apitherapy is an available possibility in branch medicine that involves the treatment of ailment with honey and products from bee (Bogdanov et al., 2008). Most common medical use for honey deals with specific application on wounds, burns, allergies, sore throats (pharyngitis), and skin diseases, more so honey has become a possible replacement for table sugar in food and beverages as sweetener.

Honey also have been proven to contains many nutritional component that provides biological benefits, such as antioxidant, anti-inflamatory, antiviral and immunosuppressive activities, thus making it a valuable food supplement and additive to diets. The benefits of honey do not just end with the aforementioned biological benefit as it is taken as alternative to industrial glucose used mainly by athletes to regain energy. Studies had also revealed that honey inhibit the growth of bacteria and Fungi such as Rubella, Leishmania and Echinococcus spp because of its low water activity, high sugar concentration and antioxidant activities (Iurlina, 2005; Amy et al., 2011).

However not totally free from microorganism as honey in most cases contains viable spores as contaminant from bee intestine and its environment which may have negative health impact on infants and immuno compromise patient because of their ability to grow and produce various toxins (Noori et al., 2012). Under a stressful environmental conditions, the organism are reduce to dormant spores but remain viable without reproducing or forming vegetative cell over a long period. Most Bacillus species that can withstand extreme environmental conditions also produces enzymes that are used in food, pharmaceuticals

and beverage industries (Libonatti et al., 2014; Tawiah et al., 2012; Stenfors et al., 2008; Schoeni and Wong, 2005). Bacillus species are good sources of these genetic engineerable and biochemical diverse enzyme used in biotechnology (Xu and Cote 2003; Yarza et al., 2010).

These bacteria produce enzymes which have proteolytic effect on humans and animals (Yarza et al., 2010; Ryan and Ray, 2004).The growth of some Bacillus genra causes bio deterioration in food while some others have also been isolated and identified as insect pathogens such as Bacillus mycoides and Bacillus thuringiensis. The emetic toxin produced by Bacillus organism especially cereulide toxin is resistant to heat, pH, and some other enzymes. Cerulide toxin can cause poisonings when implicated in ingested food with symptoms characterized by vomiting, hepatic failure and death.

However some other strains of Bacillus species have also been occasionally implicated in emetic and diarrheal cases (Mc Intyre et al., 2008).

Several studies with honey have focused more on its antimicrobial potentials (Sabate, et al., 2009). There is dearth of information concerning identifying the dominants spores, roles, benefits and there public health importance (Esawy et al., 2011). Spore organisms possess adaptive features that enable them survive extreme environmental conditions such as desiccation, pressure and pH etc. The uniqueness of these organisms suggests that their products will also have some constituent that will enable them survive extreme cases. This study is focused on identifying Bacillus species in honey and there frequency of occurrence in the samples.

## MATERIALS AND METHODS

### Sample Collection

Samples collected were harvested through traditional and modern method; the former involves the use of naked flames to rid off or even destroy honey bees and the comb, while the latter involves use of smoke to suppress bees' aggressiveness to extract the honey without killing the Bees or even destroying the comb (Babarinde et al., 2011). .Honey samples used for this study were collected from different locations in Abia State (three samples from Michael Okpara University of agriculture Apiary Unit using modern harvesting method and three others from commercial dealers in Ubani market in Umuahia) Benue State (Three locally harvested samples from farmers in Jato Aka and three from commercial dealers in Kastina Ala market) Enugu State (three locally harvested samples from Opi Nsukka, other three From commercial dealers in Ogbete Main Market) and Rivers State (Three samples were harvested and collected through modern method from Songai farm in Tai LGA, three from commercial dealers in Elele and other three were branded product of Nigeria origin) in Nigeria. Twenty seven samples of honey were collected in different sterilized bottles carefully covered and stored in cupboard. These samples were analyzed at the Microbiology laboratories in Federal Industrial Research Institute Oshodi (FIRO), Lagos State Nigeria.

### Preparation of Diluents and Media

The honey samples were serially diluted aseptically by taking 1.0 ml amount of sample with a sterile adjustable pipette and transferring into 9.0 ml amount of sterile distilled water and mixed. From the diluents, 1.0 ml of the aliquot was equally diluted in ten folds serial dilution up to 10-3 dilutions. (Adebayo and Davies, 2012).The culture media (Hichrome Bacillus agar was prepared as indicated by the manufacturer (media) instruction respectively.

### Inoculation

Inoculations of samples were carried out using pour plate method, with a sterile adjustable pipette one millilitre aliquot (1.0ml) of inoculum from appropriate dilution was inoculated into sterile disposable petridishes which had been labelled accordingly and respectively in duplicates. The cooled molten agar of Hichrome Bacillus agar (HIMEDIA-M165) was poured onto the inoculums respectively, and mixed. The inoculated plates were allowed to set and subsequently incubated at 35oC for 24-48h.

### Characterization and Identification of Bacterial Isolates

Pure cultures of Bacillus bacterial isolates were identified based on their morphological and biochemical characteristics. The organisms were subsequently characterized according to the taxonomic scheme of Buchanan and Gibbons (1999). The following tests such as gram staining, Casein hydrolysis, Spore staining, Motility test, Catalase production, Oxidase test, Indole production, Citrate utilization, Nitrate reduction, Voges – Proskauer, Gelatin hydrolysis, Starch hydrolysis, Urease activity and Methly Red Test were performed on each isolate.

### Analytical Profile Index Analysis (API)

Identification of these organisms were further carried out using Analytical Profile Index kit, (API 20E and API CHB50 Biomerieux France). These kits were used in line with instructive manual provided by the manufacturer. The results were obtained from API-web program through software reading. These method of identification compares the isolates profile with the profile of organisms in data base and its result are reported with comments such as excellent identification; very good identification; good identification or acceptable profile. In low percentage cases, statement or comments like not reliable identification; doubtful identification profile are obtained (Erem et al., 2009).

### Enumeration of Bacillus species

The average number of colonies multiplied by the dilution factor was considered for the counting of the spore .Results was expressed as colony forming unit (cfu) per millilitre of honey. The analyses were carried out in duplicate. (Cappuccino and

Sherman, 2001., Barrow and Feltham 2004., Deemah, 2007., Cheesbrough, 2000., Sneath et al., 1986).

**Statistical Analysis**

All data obtained in this study were subjected to statistical analysis using one way Analysis of variance (ANOVA). Turkey's multiple range test was used to test for differences between administration groups. All analyses were done using statistical package for social sciences (SPSS) version 20.0 (IBM Statistics, UK).All the values were reported as mean ± standard error of the mean (SEM) and the results were considered significant at p-value of less than 0.05($p<0.05$) i.e. at 95 % confidence level

**RESULTS AND DISCUSSION**

**Bacillus species Isolated from Honey Sample**

A total of (27) twenty seven honey samples were screened for the presence of Bacillus species through conventional culturing method .The samples were appropriately diluted and plated on Hicrome Bacillus agar. The samples were incubated aerobically respectively. Total of (11) Eleven colonies representing different colony morphologies were observed and picked up for analysis, observation indicated that among the samples obtained from the Apiary, local farmers, Packaged honey and commercial dealers harbored different species Bacillus.

**Density of Bacillus species in Honey samples.**

The average number of colonies multiplied by the dilution factor was considered for the counting of the spore. Result was expressed as colony forming unit (cfu) per millilitre of honey.

The analyses were carried in duplicate. Results obtained showed that commercial honey sample had the highest Bacilli count. The respective range of Bacilli count for samples were as follows; Abia 3.48 -2.96cfu/ml, Benue 3.44 - 3.20 log cfu/ml, Enugu 3.36 - 3.15 log cfu/ml and Rivers state 2.91-3.40 log cfu/ml and while 2.69 log cfu/ml was also observed for the branded product. Bacillus species were significantly higher ($p<0.05$) in samples from commercial traders compared to Apiaries, locally harvested and the branded products. Additionally, Bacilli count in locally harvested samples were significantly higher ($p<0.05$) than others, meanwhile when compared to branded product to samples obtained from the Apiary, no significant difference ($p>0.05$) was observe, though they were higher in values.

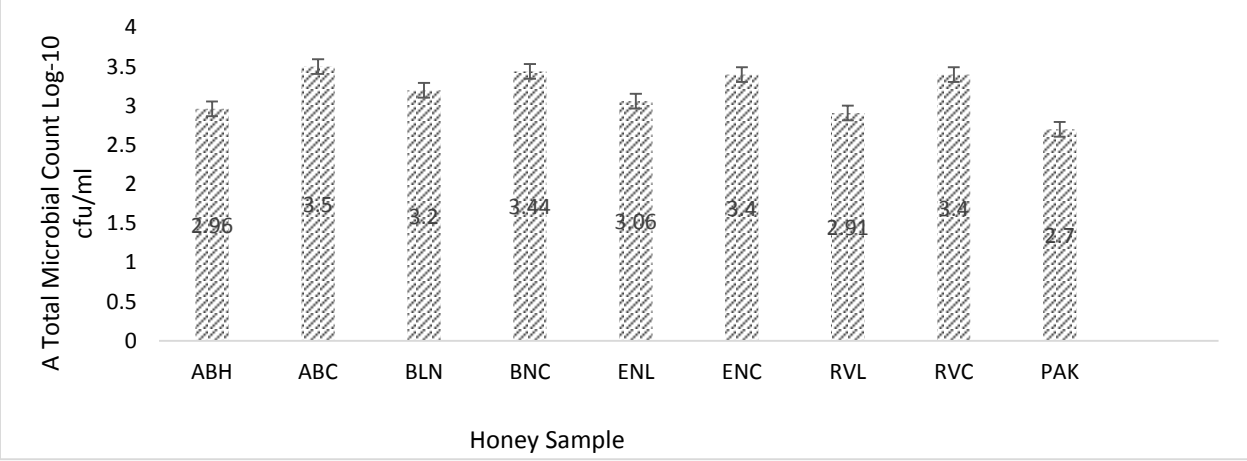


Fig.1. Total Bacilli count in honey samples collected from various state in Nigeria.

Key: ABH=Abia Hive(Umudike Apiary), ABC=Abia(Ubani Market)Commercial Product, BNL=Benue(Jato Aka)locally Harvested product, BNC=Benue(Kastina Ala)Commercial Product, ENL=Enugu(Opi Nsukka)Locally Harvested Product, ENC=Enugu(Ogbete Main Market)Commercial Product, RVL=Rivers State(Songai Farm Tai L.G.A), RVC=Rivers State (Elele Market)commercial Product and PAK=Package Honey(Supermarket).

**Characterization and Identification of Bacillus Isolates**

A total of 9 isolates were identified through conventional Biochemical methods (base on their reactions in different biochemical reagents and represented with (-) negative or (+) positive sign) and the use of API identification kits (API 20E and API CHB50 Biomerieux, France) as shown in Table 1. All of the species isolated were determined as Gram-positive and rod-shaped bacteria as both methods provided the same identification and with confirmation in percentage match from API web for isolates (A – I) as Bacillus badius (94.5 %), Bacillus cereus (99.9 %), Bacillus circulans (91.1%),Bacillus laterosporus(99.9 %), Bacillus lecheniformis (99.9 %), Bacillus megaterium (99.8 %), Bacilus mycoides (94.4 %), Bacillus polymxa (98.9 %) and Bacillus subtilis (94.5 %).

Table 1**.** Biochemical characterization of *Bacillus* species from Honey sample collected in different state in Nigeria.

| **Isolate code** | **Colour** | **Gram reaction** | **Catalase test** | **Oxidase test** | **Indole test** | **Motility test** | **MR** | **VP** | **Urease** | **Citrate** | **Starch** | **Gelatin** | **Casein** | **$No_3$ reduction** | **Spore test** | **Glucose** | **Sucrose** | **Lactose** | **Xylose** | **Mactose** | **Mannitol** | **Sorbitol** | **Salicin** | **Fructose** | **Raffinose** | **Growth in 10% nacl** | **API** 20E and API CHB50 Kits | **Probable Identity** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | Cream | +ve | + | + | - | + | - | + | - | - | - | - | + | - | + | + | - | - | - | - | - | - | - | + | - | _ | 94.5 % | *Bacillus badius* |
| **B** | Pink | +ve | + | + | - | - | - | + | - | + | + | + | + | + | + | - | + | + | | + | - | - | - | - | + | + | 99.9 % | *Bacillus cereus* |
| **C** | Cream | +ve | + | + | - | + | + | - | - | + | + | + | + | + | + | + | - | + | + | + | - | - | - | + | - | _ | 91.1% | *Bacillus circulans* |
| **D** | Cram | +ve | + | - | - | + | - | + | - | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + | _ | 99.9 % | *Bacillus laterosporus* |
| **E** | Cream | +ve | + | - | - | + | - | + | - | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + | + | 99.9 % | *Bacillus licheniformis* |
| **F** | Yellow | +ve | + | + | - | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | + | _ | 99.8 % | *Bacillus megaterium* |
| **G** | Cream | +ve | + | + | - | - | - | + | - | - | + | + | + | + | + | - | + | + | - | - | + | - | - | - | - | _ | 94.4 % | *Bacillus mycoides* |
| **H** | Blue | +ve | + | + | - | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | _ | 98.9 % | *Bacillus polymyxa* |
| **I** | Green | +ve | + | + | - | + | - | + | - | + | + | + | + | + | + | + | + | + | + | - | + | - | - | + | - | + | 94.5 % | *Bacillus subtilis* |

**Frequency of occurrence of Bacillus species in Honey obtained from Different Sources**

Percentage occurrence of the isolated Bacillus species from 27 Honey samples obtained from various sources presented in Table 2. Shows that B. megaterium (90%) was the most predominant species in all the Honey samples. Other abundant species were B. subtilis (89%), B. laterosporus (85%), Bacillus polymxa (74%), Bacillus cereus (70.4%), Bacillus badius and Bacillus mycoides had equal percentages (66.6%), Bacillus circulans (65%) whereas B. licheniformis recorded the least percentage (59.3%) in the samples.

**Table 2: Species of Bacillus isolated and their Percentage Occurrence**

| Isolates | Occurrence(n=27) | Frequency of Occurrence (%) |
|---|---|---|
| *Bacillus megaterium* | 25 | 93 |
| *Bacillus subtilis* | 24 | 89 |
| *Bacillus laterosporus* | 23 | 85 |
| *Bacillus polymxa* | 20 | 74 |
| *Bacillus cereus* | 19 | 70.4 |
| *Bacillus badius* | 18 | 66.6 |
| *Bacilus mycoides* | 18 | 66.6 |
| *Bacillus circulans* | 17 | 65 |
| *Bacillus lecheniformis* | 16 | 59.3 |

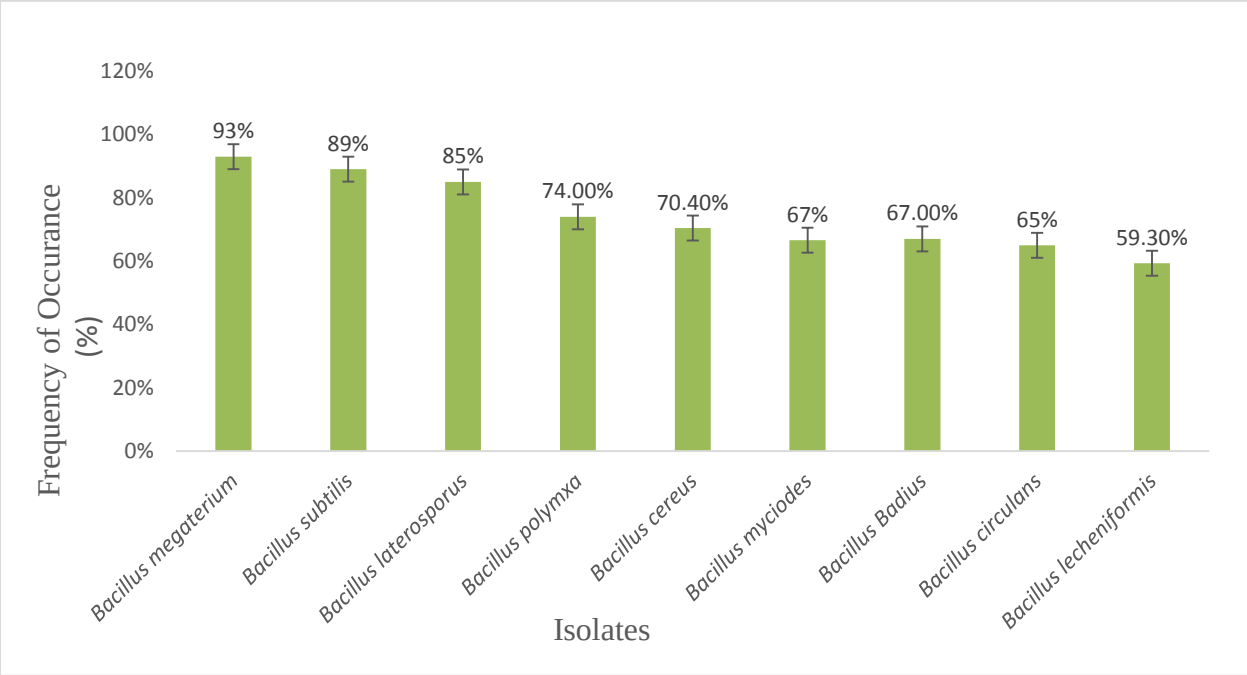


Fig: 2: Frequency of occurrence of *Bacillus* species in honey samples collected from the different Sources

**DISCUSSION**

Significant variation noted in the population of *Bacillus* species in apiary, locally harvested and samples from commercial dealers in the various markets places showed no significant difference in the population ($p$>0.05) of *Bacillus* species in the samples obtained from Apiaries and local farmers, though higher in samples from local farmers. Samples from local farmers were shown to be significantly higher ($p$<0.05) when compared with the packaged honey sample, while there was no significant difference ($P$<0.05) when compared with samples from Apiaries. However, samples from Apiaries and local farmers when compared with samples from commercial traders were significantly lower ($P$<0.05). This could be as result of further exposure to the external environment, unhygienic handling and packaging. *Bacillus* has also been isolated frequently on the external surfaces, crop and intestinal tract of the honey bees (Esawy *et al*., 2011).All the honey samples obtained from these states of Nigeria harbors *Bacillus* organism, This confirm other findings that spore forming bacteria are present in honey (Agbagwa *et al* .,2011; Buba *et al*.,2013;Kacaniova *et al.,* 2007;Hanna,2011 and Amy *et al* .,2011). These microorganisms also have been observed as normal flora in the gut of honey bee (ECSC, 2005; Kacaniova *et al.,* 2007). These findings shows that primary source of microbial contamination includes; bees, dust, pollen and flowers. Biochemical characterization for *Bacillus* genus using conventional (Bergey´s Manual of Systematic Bacteriology) method and analytical profile index (CHB 50 and API 20E) also revealed the presence of Nine species namely; *B. badius, B. cereus, B. circulans ,B. laterosporus, B. lechiniformis, B. megaterium, B. mycoides, B. polymyxa, and B. subtilis* with different percentage occurrences across the samples from different states shown in Figure 2, *B. cereus* which is of clinical significance are widely distributed in nature and are frequently isolated from soil, growing plants and also adapt and grow very well in the intestinal tract of insects and mammals (Stenfors *et al.,* 2008). *B. cereus*, has been reported to cause food poisoning similar to that caused by *Staphylococcus* species. Finding has implicated both *B. subtilis* and *B. licheniformis* as potential food poisoning agents (Wong *et al*., 1988).

However, no vegetative forms of these species were have been found in honey samples. *Bacillus* species has played a major role in bio deterioration of food and its product due to their wide range of metabolic activities and resistant to heat (Buba *et al*., 2013). According to our findings, there was high prevalence of *Bacillus* species similar with some other researches. The *Bacilli* counts observed in the present study were higher in values compared with the values obtained from honey samples from some other studies in Nigeria, though there findings were not specifically on isolation of *Bacillus* genus. The presence of *Bacillus* species in an environment with high concentrated sugar and low water content confirms its ability to compete effectively with other bacteria.

**CONCLUSION**

This study has revealed that various species of *Bacillus* are prevalent in Honey, though no pathogenic species of medical importance were found. It's therefore important to further carry out studies on the potentials and antimicrobial susceptibility of these organism should any outbreak of disease occur.

**REFERENCES**

François, E. A., Armand, P., Cokou P. A., Victorien T. D., Christine N.,Wassiyath, M., and Lamine, B.M (2018). Physicochemical Characteristics and Microbiological Quality of Honey Produced in Benin. *Journal of Food Quality*. Volume (1)13.https://doi.org/10.1155/2018/1896057

Afroz, R.,Tanvir E.M.,Zheng,w., and Little,P.J (2016).Molecular Pharmacology of Honey. *Journal of Clinical and Experinmental Pharmacology.*6:3

Libonatti, C., Soledad, V and Marina, B (2014). Antibacterial activity of honey: A review of honey around the world, *Journal of Microbiology and Antimicrobials* 4; 306-317.

Buba, F., Gidado, A and Shugaba, A (2013). Physicochemical and Microbiological Properties of Honey from North East Nigeria. *Biochem Anal Biochem* 2: 142.

Adebayo, A. A. and Davies, B. A. (2012). Microbial examination of honey marketed in south western Nigeria. *Journal of Basic and Applied Scientific Research:* 2(2)1701-1705.

Tawiah, A. A., Stephen, Y. G., Francis, A., Vivian, E. B and Kofi, A (2012). Antibiotic producing microorganisms from River Wiwi, Lake Bosomtwe and the Gulf of Guinea at Doakor Sea Beach, Ghana. BMC Microbiology.

Noori, S. A., Khelod, S.,Ahmed,A and Mohammad, J. A. (2012). Antibiotic, Pesticide, and Micobial Conaminant of Honey:Human Health Hazards. The scientific world Journal.(7):930849

Agbagwa, O.E, Otokunefor T.Vand Frank-Peterside N (2011). Microbiological quality of selected Nigerian honey. *Global Resource Journal of Microbiology*. 2: 039-042.

Amy, K. B., Brandi, M. M., Wendy, S. D., David, F. V., and Andrew, V.G (2011). Enumeration and identification of bacterial contaminants in commercial and locally produced honey.*journal of microbiology* (82) 103-113

Esawy, M.A., Ahmed, E.F., Helmy, W. A., Mansour, N.M., El-Senousy,W.M and El-Safty , M..M. (2011). Production of levansucrase from novel honey *Bacillus subtilis* isolates capable of producing antiviral levans. Carbohydrate Polymer, 36, 823-83

Babarinde,G.O.,Babarinde,S.A.,Adegbola,D.O (2011) Effects of harvesting methods on physicochemical and microbial qualities of honey. *Journal of Food Science and Technology.* Volume 48 (5): 628 - 634.

Hanna Rozanska (2011). Microbiological quality of polish honey. *Bull Vet Inst Pulawy* 55, 443-445.

Yarza, P., Ludwig, W., Euzéby, J., Amann, R., Schleifer, K. H., Glöckner, F. O and Rosselló-Móra, R. (2010). Update of the All-Species Living Tree Project based on 16S and 23S rRNA sequence analyses. *Systematic and Applied Microbiology* 33 (6): 291–299.

Erem, F. C and Barçın K (2009). Identification of Bacillus species isolated from ropey breads both with classical methods and api identification kits", Akdeniz Üniversitesi,

Sabate, D. C., Carrillo, L and Audisio, M. C. (2009). Inhibition of Paenibacillus honey samples. Research Microbiology, 160, 193–199.

Bogdanov, S., Jurendic, T., Sieber, R. and Gallman, P. (2008). Honey for Nutrition and Health: a review. *American Journal of the College of Nutrition* 27: 677 – 689.

McIntyre L, Bernard K, Beniac D, Isaac-Renton J.L, Naseby D.C (2008). Identification of *Bacillus cereus* group species associated with food poisoning outbreaks in British Columbia, Canada.*Appllied Environmental Microbiology* .74:7451–7453.

Stenfors, A. L.P., Fagerlund, Aand Granum P.E (2008). From soil to gut: *Bacillus* cereus and its food poisoning toxins. FEMS *Microbiology* Rev.;32 (4):579-606.

Deemah M. Al-Dabbagh (2007) Practical bacteriology manual Demonstrator, CLS413 Department King Saud University.

Kacaniova, M., Sudzina, M., Sudzinova, J., Fikselova, M., Cubon, J and Hascík, P (2007). Microbiological and physico–chemical quality of honey collected from different Slovak habitats. *Slovak Journal Animal Science.* 40, (1): 38 – 43.

Lawton, E.M., Ross R.P., Hill, C *and* Cotter, P.D *(*2007*)* Two-peptide lantibiotics*:* a medical perspective. *Mini Rev Med Chem* 7*:* 1236–1247.

European Commission (2005). Scientific Committee on Veterinary Measures Relating to Public Health on Honey and Microbiological Hazards. Management of Scientific Committees;Scientific Co-Operation and Networks. 1- 15.

Iurlina, M.O. (2005) Characterization of microorganisms in Argentinean honeys from different sources. *International Journal of Food Microbiology*. (12)151-160.

Schoeni J.L, Wong A.C (.2005) *Bacillus* cereus food poisoning and its toxins.*Journal of Food Prot*;68(3):636-48.

Barrow, G.I. and Feltham, R.K.A (2004) (eds.) Manual for the identification of medical bacteria (3rd edn.). Cambridge: Cambridge University Press.

Rayan K.J and Ray C.G (2004).*Sherris Medical Microbiology* (4th ed.). McGraw Hill.ISBN 0-8385-8529-9.

Xu, D., Cote, J.C. (2003). "Phylogenetic relationships between *Bacillus* species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences". *International Journal of Systematic and Evolutionary Microbiology*53 (3): 695–704.

Bryant, Vaughn M., J (2001). "Pollen Contents of Honey".*CAP Newsletter***24** (1): 10–24.

Cappuccino, J.G. and Sherman, N. (2001). Microbiology:A Laboratory Manual. 6th Edition. Benjamin Cummings. CA.

Cheesbrough, M (2000). Distirict laboratory practice in tropical countries Part 2. Cambridge University Press, UKp434.
Buchanan, L.E. and Gibbons, M.E. (1999).Bergey is manual of determinative bacteriology, Baltimore, the William and Willing Sco.

Wong, H.C., Chen,Y.L.,Fan,J.Y (1988).Incidence and Characterization of Bacillus cereus Isolates contaminating dairy product. Applied Environmental Microbiology.54, 699 -702.

Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G., (1986). Bergey's Manual of Systematic Bacteriology, Vol. 2 Baltimore: Williams and Wilkins ISBN 0-683-07893-3