ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.05 pp 200-206, 2016

Molecular Characterization of *Rhodotorula* Spp. Isolated From Poultry Meat

Randa. M. Alarousi[1]*, Sohad M. Dorgham[1], El-Kewaiey I. A.[2], Amal A. Al-Said[3]

[1]**Department of Microbiology, National Research Center, Dokki, Giza, Egypt.**
[*]**Department of Medical Laboratories, College of Applied Medical Sciences, Majmaah University, KSA**
[2]**Food Hygiene Unit,Damanhour Provincial Lab., Animal Health Research Institute, Egypt .**
[3]**Microbiology Unit, Damanhour Provincial Lab., Animal Health Research Institute, Egypt .**

Abstract: A total of 50 *Rhodotorula* spp strains were isolated from 200 fresh Chicken and quail products, represented by chicken breast and thigh (Chicken: n=50 breast, n=50 thigh and quail: n=50 breast, n=50 thigh) with an incidence 25%. PCR was applied on 5 positive samples out of 15 samples derived from chicken breasts; 5 positive samples out of 12 samples from chicken thigh meat; 5 positive samples out of 13 from quail breasts and 5 samples out of 10 positive of quail thigh meat samples, *CrtR* gene was detected at 560bp. High incidence of *Rhodotorula* spp, it may be due to un hygienic measures during rearing and slaughtering of poultry, which represent potential public health risk.
Key words: *Rhodotorula*, PCR, *CrtR* gene, quail.

Introduction

Microbial nourishment security and sustenance borne diseases are critical general wellbeing concern worldwide.There have been various sustenance borne illuness coming about because of ingestion of contaminated nourishments, for example, chicken meats. Poultry meat is one of the exceedingly expended creature started nourishment thing. With high nutritive quality, having both vital large scale and micronutrients. The vast majority of the pathogens that assume a part in sustenance borne maladies have a zoonotic starting point[1]. Most wellbeing powers have considered the importance of yeasts in nourishments, including meat items, in perspective of the general wellbeing. *Rhodotorula* is a basidiomycetous yeast in the fungal family *Sporidiobolaceae* (Phylum *Basidiomycota*)[2]. The family *Rhodotorula* consolidates 8 species, of which *R. mucilaginosa*, *R. glutinis*, and *R. minuta* are known not infection in individuals, it was beforehand considered non-pathogenic; nonetheless, it has developed as a deft etiologic operator amid the most recent two decades[3].

Among the few references to the pathogenicity of *Rhodotorula* spp. in animals, there are several reports of an outbreak of skin infections in (chickens and sea animals), lung infections and otitis in (sheep and cattle), dermatitis in a cat that had crusted lesions and mastitis[4]. Most of the cases of infection due to *Rhodotorula* in humans were fungemia associated with central venous catheter (CVC) use. Not at all like fungemia, a portion of the other limited infections caused by *Rhodotorula*, including meningeal, skin, visual, peritoneal, and prosthetic joint diseases, are not as a matter of course connected to the utilization of CVCs or immunosuppression[5].

Routine yeast distinguishing proof taking into account phenotypic qualities is frequently deceptive and uncertain, and for the most part should be underlined by sub-atomic strategies.

Differentiation of all types of the family *Rhodotorula* in view of morphological characters and physiological tests is conceivably tedious, costly and requires extensive aptitude. Subsequently, a more adequate strategy rose through the presentation of a coordinated perspective of the variety taking into genus based on molecular data and this, however not a simple errand**.**

Use of improved methods for identification such as the application of molecular techniques to species identification, has also contributed significantly. Since the study of [6] through which they described seven more new species of the genus *Rhodotorula*, according to differences in the D1and D2 region of the large-subunit rDNA, many studies were conducted. [7] demonstrated the effectiveness of using cytochrome *b* gene sequence for both species identification and the investigation of phylogenetic relationships among basidiomycetous yeasts.

Therefore, the present work aimed to: (a) Isolation and identification of *Rhodotorula* spp. field isolates from chicken and quail meat (b) Detection of cytochrome oxidase (*crtR*) gene (c) Evaluate their public health significance through the incidence of isolation.

Material and Methods

(i) Samples collection and preparation:

Two hundred fresh poultry products (Chicken and quails), represented by chicken breast and thigh (Chicken: n=50 breast, n=50 thigh and quail: n=50 breast, n=50 thigh). All samples were collected from different markets at Bahaira governorate from January to March 2015. The samples were transferred directly to the laboratory in ice boxes without any delay. Twenty-five grams of each sample were mixed with 225 ml of sterile peptone water 0.1% and thoroughly homogenized under aseptic conditions [8].

(ii) Isolation and identification of yeast:

A loop full of each sample were inoculated on Sabouraud`s dextrose agar then incubated at 25°C for 3 days [9].

Yeast colonies of different morphological appearance were re-cultivated on Sabouraud´s dextrose agar slopes and incubated at 25°C for 5 days and kept for identification.

Identification of isolated yeasts morphologically and biochemically were carried out according to methods recommended by [10]**.**

(iii) DNA extraction and PCR Amplification:

Genomic DNA of *Rhodotorula* spp. was extracted by using an extraction kit (QIAamp mini kit, Qiagen). Specific oligonucleotide primer for Cytochrome oxidase (*crtR*) gene was used as described in table (2). The amplification conditions for *crtR* gene included initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, synthesis at 72°C for 3 min and a final extension step at 72°C for 10 min. Samples were kept at 4°C until checked. The amplicons were separated by 0.8 % agarose gel electrophoresis in TAE buffer containing 0.5 μg /ml Ethidium bromides [11].

Table.1: Primer sequences and product size of *crtR* gene:

Target	Primer sequence	Product size	Reference
crtR	F.CARACTGGKACDGCHGARGATT R. WGGDCCRATCATGAYRACTGG	560 bp	Alcaíno *et al.* [12]

(iv) *CrtR* gene detection:

A total of 20 *Rhodotorula* spp. strains have been selected randomly for detection of *CrtR* gene.

(v) Statistical analysis:

Data were analyzed statistically by Chi-square test using statistical package of social science SPSS (version 18.0).

Result

1. Isolates:

All suspected orange colored colonies were examined under light microscope. The results of traditional biochemical tests indicated that all suspected isolates are *Rhodotorula* spp. The incidence of isolation was mentioned in tables (2&3).

Table (2): Number and percentage of *Rhodotorula* spp. in chicken and quail:

Groups	+ve	-ve
Chicken (n=100)	**27 (27%)**	**73 (73%)**
Quail (n=100)	**23 (23%)**	**77 (77%)**
Total (n=200)	**50 (25%)**	**150 (75%)**

***Rhodoturola* was isolated from 25% of chicken and quail samples. There were no statistical differences (P> 0.05) between number of isolated samples from chicken (27/200) and quail (23/200).**

Table: 3. Number and percentage of *Rhodotorula* spp.in chicken

Chicken samples	*Rhodotorula* +ve samples	*Rhodotorula* -ve samples
Breast (n=50)	**15 (30%)**	**35 (70%)**
Thigh (n=50)	**12 (24%)**	**38 (76%)**
Total (n=100)	**27 (27%)**	**73 (73%)**

Table: 4. Number and percentage of *Rhodotorula* spp. in quail:

Quail samples	*Rhodotorula* +ve samples	*Rhodotorula* -ve samples
Breast (n=50)	**13 (26%)**	**37 (74%)**
Thigh (n=50)	**10 (20%)**	**40 (80%)**
Total (n=100)	**23 (23%)**	**77 (77%)**

Table.5. classification of *Rhodotorula* spp. In chicken:

***Rhodotorula* species**	**Breast (n=15)**	**Thigh (n=12)**	**Total (n=27)**
R. glutinis	**8 (53%)**	**6 (50%)**	**14* (51.9%)**
R. pallida	**7 (47%)**	**Not isolated**	**7* (25.9%)**
R. minuta	**Not isolated**	**6 (50%)**	**6* (22.2%)**

*** Significant difference at P> 0.05**

It is clear from table (5) that the percentage of *R. glutinis* (51.9%) isolated from chicken samples were significantly (P> 0.05) higher than both *R. pallida* and *R. minuta* (25.9% and 22.2%, respectively).

Table.6. classification of *Rhodotorula* spp. in quail

***Rhodotorula* species**	**Breast (n=13)**	**Thigh (n=10)**	**Total (n=23)**
R. glutinis	**6 (46%)**	**6 (60%)**	**12* (52.2%)**
R. pallida	**Not isolated**	**4 (40%)**	**4* (17.4%)**
R. flava	**7 (54%)**	**Not isolated**	**7* (30.4%)**

*** Significant difference at P> 0.05**

1. It is clear from table (6) that the percentage of *R. glutinis* (52.2%) isolated from chicken samples were significantly (P> 0.05) higher than both *R. pallida* and *R. flava* (17.4% and 30.4%, respectively).
2. As show in table 5 and table 6, *R. glutinis* and *R. pallida* was isolated from both chicken and quail *Rhodotorula* +ve samples. While *R. minuta* was isolated from chicken samples, *R. flava* was isolated from quail samples.

***CrtR* gene detection:**

Twenty selected strains of *Rhodotorula* spp. carried *CrtR* gene as showed in figure (1).

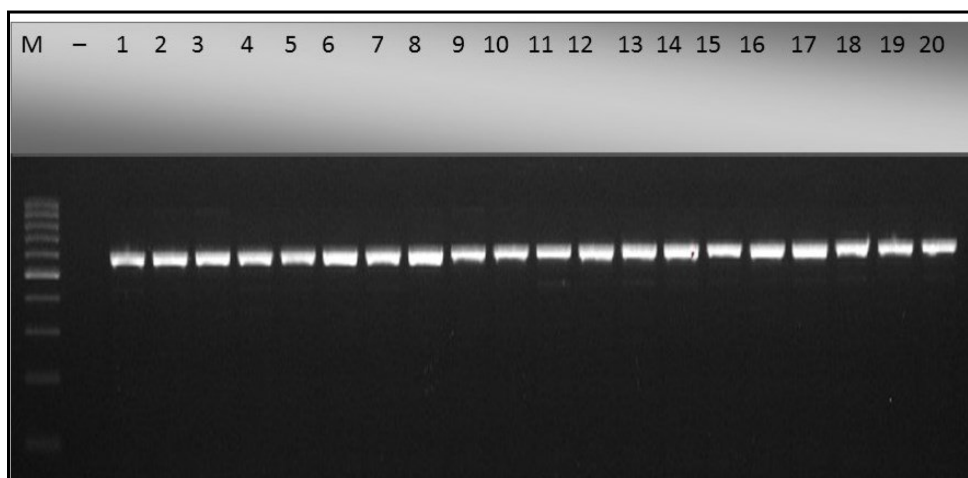

Figure (1): Ethidium bromide-stained agarose gel of PCR assay of *Rhodotorula* spp. strains. The position corresponding to *CrtR* PCR amplicons was at 560bp, an appropriate size marker in a 100 bp ladder.

Discussion

Most wellbeing powers have considered the criticalness of yeasts in nourishments, including meat items, in perspective of the general wellbeing. The determination of the defilement of nourishment fixings and of prepared sustenance with yeasts is a fundamental part of any quality affirmation or quality control program in the sustenance business [13].

According to our outcomes, morphological, cultural and biochemical recognizable proof uncovered that our suspected detaches were *Rhodotorula spp*., this result was affirmed by Fell et al.[6] who mentioned that *Rhodotorula* is a yeast which produces mucoid colonies with a characteristic carotenoid pigment.

Previously, *Rhodotorula* is considered a low virulence organism in comparison to *Candida* or *Trichosporon.* Recently, *Rhodotorula* must be considered as a potential pathogen[14].

Rhodotorula mucilaginosa has been getting expanding consideration since it can be secluded from normally matured milk [15] and other nourishment lattices[16,17], in addition to, different and extreme ecosystems, including the complex core gut microbiota of carnivore wild fish[18], marine shores, glacial core cold environments [19] and hydrocarbon-contaminated soil[20].

As indicated by our outcomes, *R. glutinis*, and *R. palida* were detached from chicken's breast with an occurrence 53%, 47%, and thigh with a frequency 50% for *R. glutinis* separately. The frequency rate of *R. glutinis* separation in quail's breast and thigh was 46% and 60% respectively. *R. palida* was confined from quail's thigh with an occurrence 40%. The past result can't help contradicting [21] who segregated *R. glutinis*, and *R. palida* from chicken filet test with low rate 5%. On the other side, our outcomes were in amicability with [22] who disengaged *Rhodotorula spp*. from chicken carcass and thigh with an incidence6 (60 %) and 4 (40 %) separately. These results show that our examples were more debased with yeast strains; this might be because of terrible hygienic measures amid the preparing steps and taking care of. At the season of butchering, the quills, bolster and assemblages of the flying creatures have been observed to be tainted with yeasts [23].

In expansion to that, yeasts have been withdrawn from the air and soil beginning from poultry repeating and raising houses, old litter and litter-containing water, wet support and winged creature droppings [24]. The high danger of Yeasts sullying is making a noteworthy commitment to the general microbial biology of poultry and might likewise add to the progressions prompting decay [25]**.** Molecular methodologies are currently being produced to give a faster and target identification of yeasts contrasted with conventional phenotypic strategies.

Carotenoids are natural pigments of yellow, orange or red color. More than 600 different chemical structures have been described to date[26]. They are terpenoids with the isopentenyl- pyrophosphate (IPP) molecule as the basic unit. Astaxanthin is a carotenoid with a high commercial interest due to its use as a food additive for trout and salmon flesh pigmentation in aquaculture[27]. Its biosynthesis is limited to a few microorganisms such as the microalgae *Haematococcus pluvialis;* the basidiomycetous yeast *Xanthophyllomyces dendrorhous* [28,29] and *Rhodotorula* spp[7]**.**

The biosynthesis of astaxanthin requires a lot of sophisticated steps, cytochrome P reductase enzyme encoded by the *cpr* gene are one of the most important requirements for this pathway [30]. Albeit a few genes for various cytochrome P450 enzymes can exist in a living being, in many species one and only *cpr* gene exists. A few special cases have been seen in plants and zygomycetes that contain a few *cpr* genes [31].

Our study, a specific primer pair based on the sequence of *cpr* gene[12] was used as conserved primers for all *Rhodotorula* spp. In this work we tried to reach a rapid diagnosis of *Rhodotorula* by purifying the DNA sample directly from grown colonies. The PCR amplification revealed 20 (100 %) positive samples out of 20 samples were chosen to apply PCR to confirm the primary conventional identification assays used on all the samples. PCR was applied on 5 positive samples out of 15 samples derived from chicken breasts; 5 positive samples out of 12 samples from chicken thigh meat; 5 positive samples out of 13 from quail breasts and 5 samples out of 10 positive of quail thigh meat samples.

Conclusion

In conclusion, this result was obtained within hours with a highly sensitive and specific result and required less efforts and time in comparison to the conventional methods used for identification of the organism; hence time factor for diagnosis of any infectious disease is very important to start as early as possible in treatment of the infection. Future prospective studies will be carried out for further studying of molecular characterization of *CrtR* gene in *Rhodotorula* species.

References

1. Busani L, Cavia GS, Luzzi I and Caprioli A. Laboratory surveillance for prevention and control of foodborne zoonosis. I[st] Super Sanita 2006; 42: 401-404.
2. Alekhova TA, Aleksandrova AA, Novozhilova TI, Lysak LV, Zagustina NA, Bezborodov AM Monitoring of microbial degraders in manned space stations. Prikl Bio khim Mikrobiol 2005; 41: 435-443.
3. Larone DH, Medically Important Fungi—A Guide to Identification, American Society for Microbiology, Washington, DC, USA, 3rd edition, 1995.
4. Kadota K, Uchida K, Nagatomoet T. Granulomatous epididymitis related to *Rhodotorula glutinis* infection in a dog Veterinary Pathology 1995; 32 (6):716–718.
5. Wilth F and Goldani LZ Epidemiology of Rhodotorula: An Emerging Pathogen. Interdisciplinary Perspectives on Infectious Diseases 2012, Article ID 465717: 7. http://dx.doi.org/10.1155/2012/465717
6. Fell J W, Boekhout T, Fonseca A, Scorzetti G and Statzell-Tallman A. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/ D2 domain sequence analysis. Int J Syst Evol Microbiol 2000; 50:1351-1371.
7. Biswas S K, Yokoyama K, Nishimura K and Miyaji M. Molecular phylogenetics of the genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial cytochrome b gene, Int J Syst and Evol Microbiol. (2001); 51: 1191–1199.
8. Russell SM, Cox NA and Bailey JS Microbiological methods for sampling poultry messing plant equipment. J Appl Poultry Res 1997; 6: 229-233.
9. Beuchat LR (ed.) Food and Beverage Mycology, 2nd ed. Van Nostrand Reinhold, New York 1987, Chapter 17: 609.
10. Kreger-Van Rij N J W The Yeasts, a Taxonomic Study. 3rd ed. Amsterdam, Elsevier, 1984.
11. Sambroock J and Russell DW Molecular cloning. A laboratory manual 3rd edition. Cold Spring Harbor NY: Cold Spring Harbor Laboratory. Press; 2001.
12. Alcaíno J, Barahona S, Carmona M, Lozano C, Marcoleta A, Niklitschek M, Sepúlveda D, Baeza M and Cifuentes V. Cloning of the cytochrome p450 reductase (crtR) gene and its involvement in the astaxanthin biosynthesis of *Xanthophyllomyces* dendrorhous. BMC Microbiology 2008; 8:169.
13. Deak T and Beuchat LR Handbook of Food Spoilage Yeasts. CRC Press, Inc., Baton, FL. 210 pp. 1996.
14. Gomez-Lopez A, Mellado E, Rodriguez-Tudela JL, Cuenca-Estrella M. Susceptibility profile of 29 clinical isolates of *Rhodotorula* spp. and literature review. J Antimicrob Chemo Ther 2005; 55:312-316.
15. Bai M, Qing M, Guo Z, Zhang Y, Chen X, Bao Q, Zhang H, Sun TS Occurrence and dominance of yeast species in naturally fermented milk from the Tibetan Plateau of China. Can J Microbiol 2010; 56:707–714. http://dx.doi.org/10.1139/w10-056.
16. Sabate J, Cano J, Esteve-Zarzoso B, Guillamón JM Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. Microbiol Res 2002; 157: 267–274. http://dx.doi.org/10.1078/0944-5013-00163.
17. Lucena-Padrós H, Caballero-Guerrero B, Maldonado-Barragán A and Ruiz-Barba JL Microbial diversity and dynamics of Spanish-style green table-olive fermentations in large manufacturing companies through culture-dependent techniques. Food Microbiol 2014; 42:154 –165. http://dx.doi.org/10.1016/j.fm.2014.03.020.
18. Raggi P, Lopez P, Diaz A, Carrasco D, Silva A, Velez A, Opazo R, Magne F and Navarrete PA Debaryomyces hansenii and *Rhodotorula mucilaginosa* comprised the yeast core gut microbiota of wild and reared carnivorous salmonids, croaker and yellowtail. Environ Microbiol 2014; 16: 2791–2803. http://dx.doi.org/10.1111/1462-2920.12397.

19. Singh P, Tsuji M, Singh SM, Roy U, Hoshino T Taxonomic characterization, adaptation strategies and biotechnological potential of cryophilic yeasts from ice cores of Midre Loven breen glacier, Svalbard, Arctic. Cryobiology 2013; 66:167–175. http://dx.doi.org/10.1016/ j.cryobiol. 2013.01.002.
20. Chandran P, Das N. Role of plasmid in diesel oil degradation by yeast species isolated from petroleum hydrocarbon-contaminated soil. Environ Technol 2012; 33:645– 652. http://dx.doi.org/10.1080/ 09593330.2011.587024.
21. Hemly A and Mahmoud YA Anti-Yeast Effects of Some Plant Extracts on Yeasts Contaminating Processed Poultry Products in Egypt. Czech. J Food Sci 2005; 23 (1): 12–19.
22. Abd-Elrahman HA, Soliman SA and Rahal EG Prevalence of yeast in chicken meat and their products. 2013: SCVMJ, XVIII (2).
23. Barnes EM Microbiological problems of poultry at refrigerator temperature, a review. J Sci Food and Agri. 1976; 27: 777–782.
24. Bryan FL Poultry and poultry meat products. In: SILLIKER JH (ed.): Microbial Ecology of Foods. Food Commodities. Academic Press, London. 1980 (2): 410–458.
25. Viljoen BC, Geornaras I, Lamprecht A, Von Holy A Yeast populations associated with processed poultry. Food Microbiology 1998; 15:113–117.
26. Takaichi S, Sandmann G, Schnurr G, Satomi Y, Suzuki A, Misawa N The carotenoid 7,8-dihydro-ψ end group can be cyclized by the licopenecyclases from the bacterium Erwini auredovora and the higher plant Capsicum annuum Eur J Biochem 1996; 241:291-296.
27. Johnson E, Conklin D, Lewis M The yeast Phaffiarhodozyma as a dietary pigment source for salmonids and crustaceans. J Fish Res Board Can 1997; 34: 2417-2421.
28. Andrewes AG, Phaff HJ, Starr MP Carotenoids of Phaffiarhodozyma. A red pigmented fermenting yeast. Phytochem 1976; 15: 1003-1007.
29. Fraser P, Miura Y, Misawa N In vitro characterization of astaxanthin biosynthetic enzymes. J Biol Chem 1997; 272: 6128-6135.
30. Van den Brink HM, van Gorcom RF, van den Hondel CA and Punt PJ Cytochrome P450 enzyme systems in fungi. Fungal Genet Biol 1998; 23:1-17.
31. Malonek S, Rojas MC, Hedden P, Gaskin P, Hopkins P, Tudzynski B The NADPH-cytochrome P450 reductase gene from Gibberellafujikuroiis essential for gibberellin biosynthesis. J Biol Chem 2004; 279:25075-25084.

*****