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# The Yeast Flora Occuring in the Trachea of Broiler Chicken

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**Summary**

The occurrence of bacteria as natural occupants of the intestinal flora of poultry is well documented. The incidence and composition of the yeast microflora, however, have received little attention. A study was undertaken with the objective of identifying the predominant yeasts associated with the trachea of chickens, isolated from broilers of a poultry-processing plant. A total of 38 representative yeast isolates were obtained and identified according to conventional methods. Species belonging to genera of *Bullera*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Rhodotorula*, *Torulaspora*, *Trichosporon*, and *Zygosaccharomyces* were isolated at various stages of the broiler program.

*Key words:* yeasts, intestinal flora, broiler chickens

## Introduction

The major ecological determinants of microbial growth, nutrient availability, water activity ($a_w$) and pH are provided near optimum levels in fresh meat as well as products produced from it (*1*). Under normal circumstances of production, meat becomes contaminated with a wide diversity of organisms. Based on this phenomenon, specific organisms are selected, depending on storage and treatment conditions, which can result in the spoilage of meat and meat products (*2*).

As spoilage organisms, yeasts are generally not considered to be of major importance, since their numbers are highly variable relative to bacterial numbers (*3,4*). However, if bacterial counts are inhibited due to environmental stresses, like a decrease in temperature, yeasts and moulds emerge as dominant spoilage organisms (*5*). The proportion of the yeast population on turkey stored at –2 °C increased drastically, whereas psychrophillic yeasts reached numbers exceeding $10^7/cm^2$ on the skin after storage for 42 days at this temperature (*6*). With the lowering of water activity, by means of dehydration or the addition of solutes for some processed meat, the spoilage microorganisms are restricted and consequently allow the development of an altered spoilage microflora. The normal gram negative spoilage bacteria are restricted at $a_w$ values below 0.98, and therefore yeasts and moulds evolve as the primary spoilage microorganisms in meat at $a_w$ levels between 0.93 and 0.85 (*7*).

Wells and Stadelman (*8*) studied the development of yeasts on antibiotic treated poultry and found that a greater percentage of the treated meat exhibited a dominant yeast flora compared to the untreated. The spoilage flora of sulphited meat also consisted mainly of sulphite tolerant yeasts due to the reduced competition of bacteria (*8*).

Based on the yeasts potential to evolve as pathogens or spoilage organisms, their origin needs to be established before any assessment can be made on their contribution to poultry meat spoilage. Therefore, in this study, yeast populations were isolated from the trachea of chickens at various stages during their live cycle and identified. The predominance of specific yeasts will be used to construct a database representative of the yeasts

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present at all stages in the broiler chicken, and their contribution to spoilage assessed.

## Materials and Methods

### *Sampling procedure*

At day 0, chickens were obtained from the hatchery after hatching and placed at a commercial density of approximately 21 birds/$m^2$ in a broiler house. Birds were killed by cervical dislocation. The birds were dipped into 70 % alcohol and aseptically opened with sterile scissors and forceps. The tracheas were removed aseptically and placed into sterile Whirlpack stomacher bags. The tracheas of 15 birds were pooled together into one bag and transported in a coolerbox on ice to the laboratory within 3 h. Microbial analyses of the tracheas were carried out over a period of six weeks (June to July) up to the time of slaughter. Surveys were conducted at weekly intervals. The birds were taken randomly from four broiler houses.

### *Processing and analysis of samples*

To compensate for variations in yeast populations between chickens, the tracheas of 15 birds were pooled and analysed in duplicate on each sampling occasion. The trachea samples were weighed and 9 mL of sterile bacto peptone (Oxoid, Basingstoke) were added to each gram of trachea. Homogenisation was performed in a stomacher for 2 min (Colworth 400, London, UK). Serial dilutions as required were prepared and spread plated onto Yeast Extract Glucose Chloramphenicol agar (Oxoid) and Plate Count agar (Oxoid).

Plates were incubated at 25 °C for 5 days under aerobic conditions after which visually distinguishable yeast colonies on the highest dilution between 30 and 300 cfu/g were counted, purified and stored on yeast extract-malt extract (YM) slants at 4 °C. The data were converted to the number of yeasts per gram of trachea. Total viable bacterial counts on the Plate Count agar were determined after incubation at 25 °C for 24 h.

### *Characterisation of yeast isolates*

The representative yeast isolates were identified by using the methods described by Van der Walt and Yarrow (*10*) and the computerized identification system of Barnett *et al.* (*11*). Each isolate was inoculated into 6 sugar fermentation media, 32 carbon source assimilation media and vitamin free medium (*10*). Additional tests performed included growth at 37 °C, in 50 % D-glucose medium, urea hydrolysis, splitting of arbutin, 0.01 and 0.1 % cycloheximide and staining of 4-week-old cultures with Diazonium Blue B salt reagent. Assimilation of nitrogen compounds, as performed by means of the auxanographic method (*12*), was also included.

Ascopore formation was examined on McClary's acetate agar, potato glucose agar, Gorodkowa agar, corn meal agar and malt extract agar (*10*). The inoculated media were incubated at 18 °C for 4 weeks and examined at 4-day intervals. Cell morphology and mode of reproduction were examined on malt extract agar (Difco) and on Dalmau plates (*10*). The formation of pseudomycelium and true mycelium was examined on corn meal agar according to the Dalmau plate technique (*10*).

## Results and Discussion

In previous papers (*13,14*) total bacterial populations, as well as yeast populations associated with fresh and spoiled refrigerated poultry, were quantified and

Table 1. Total bacterial and yeast counts associated with the tracheas of broiler chickens over a six week period, the yeast species present and their proportional percentages

| Age of chickens (weeks) | Species isolated | Fraction of total yeast population/% | Total yeast count/(cfu/g) | Total bacterial count/(cfu/g) |
|---|---|---|---|---|
| 1. | *None* | NA | – | – |
| 2. | *Debaryomyces hansenii* | 100 | $8.75 \cdot 10^3$ | $5.24 \cdot 10^8$ |
| 3. | *Debaryomyces hansenii* | 87.63 | | |
| | *Debaryomyces vanrijiae* | 3.18 | | |
| | *Rhodotorula mucilaginosa* | 4.95 | | |
| | *Torulaspora delbrueckii* | 4.24 | $7.08 \cdot 10^3$ | $8.25 \cdot 10^9$ |
| 4. | *Cryptococcus laurentii* | 0.15 | | |
| | *Candida blankii* | 96.62 | | |
| | *Debaryomyces hansenii* | 2.57 | | |
| | *Debaryomces vanrijiae* | 0.56 | | |
| | *Zygosaccharomyces rouxii* | 0.75 | $1.6 \cdot 10^5$ | $1.23 \cdot 10^9$ |
| 5. | *Cryptococcus laurentii* | 4.69 | | |
| | *Debaryomyces vanrijiae* | 2.04 | | |
| | *Debaryomyces hansenii* | 63.08 | | |
| | *Torulaspora delbrueckii* | 30.18 | $2.98 \cdot 10^5$ | $9.77 \cdot 10^7$ |
| 6. | *Bullera variabilis* | 0.85 | | |
| | *Debaryomyces hansenii* | 36.75 | | |
| | *Debaryomyces vanrijiae* | 6.41 | | |
| | *Torulaspora delbrueckii* | 6.41 | | |
| | *Trichosporon beigelii* | 49.57 | $5.85 \cdot 10^3$ | $1.47 \cdot 10^8$ |

identified. High numbers of yeasts were observed on the spoiled and fresh poultry carcasses of 5.14 and 3.13 cfu/g log counts, respectively (*13*). In order to extend the taxonomic survey, the yeasts occurring naturally in the tracheas and intestines of broiler chickens were quantified and identified. These yeasts may be responsible for contamination of the meat or may contribute to oral thrush resulting in poor growth of the chickens and diseases.

Gram-negative bacteria predominated (*3,13,14*) in poultry samples obtained from the carcasses, whereas the yeast numbers were highly variable relative to bacterial numbers. Similar results were obtained in this study from samples obtained from the tracheas of broilers (Table 1). Maximum bacterial counts were observed 3 weeks after hatching of $8.25 \cdot 10^9$ cfu/g, whereas the yeast population reached a maximum count of $2.98 \cdot 10^5$ cfu/g only after 5 weeks. Despite the predominance of bacterial loads, the increase in yeast numbers clearly indicated that the yeasts also contributed to the overall microbial ecology and therefore may also play a substantial role during spoilage. The major difference between total bacterial counts and yeast counts may be due to competitive interactions with the gut flora and therefore only representing the stable yeast microflora within the trachea of the adult chicken.

The identities and proportional percentages of incidence of the yeast isolates are shown in Table 1. No yeasts were observed in chickens directly after hatching and within the first week after hatching. The yeasts only reached detectable numbers after two weeks ($8.75 \cdot 10^3$ cfu/g) with *Debaryomyces hansenii* being the only yeast species isolated. In the third week after hatching, the yeast levels remained stable ($7.07 \cdot 10^3$ cfu/g), although a wider diversity of yeast species was isolated. Strains of *D. hansenii* remained predominant (87.6 %) but low numbers of *Debaryomyces vanriji, Rhodotorula mucilaginosa* and *Torulaspora delbrueckii* were also encountered. Five different yeast species were isolated from four-week-old chickens with *Candida blankii* being the predominant yeast species (96.6 %). Maximum yeast numbers were observed in the chickens in the fifth week after hatching, reaching counts in excess of $10^5$ cfu/g. *D. hansenii* (63.08 %) and *Torulaspora delbrueckii* (30.18 %) were the dominant yeast species. Strains of *D. hansenii* were frequently found in the previous study on the carcasses of fresh poultry and spoiled products (*14*), whereas no strains of *T. delbrueckii* were obtained. During the sixth week after hatching, prior to slaughtering, the yeast numbers, however, decreased to $5.85 \cdot 10^3$ cfu/g. Predominant yeast species obtained during this period are represented by isolates of *D. hansenii* (36.75 %) and *Trichosporon beigelii* (49.57 %).

The presence of high proportions of *Candida*, *Debaryomyces* and *Trichosporon* in the tracheas of the broilers is in correspondence with similar high numbers found on the carcasses of birds (*14*). These yeasts, therefore, may contribute to the spoilage of the carcasses after slaughtering since they already adopted to the immediate environment and being frequently found in spoiled meat products (*15*). Poultry spoilage is mainly restricted to the surface of the meat. The inner portions of the tissue are generally regarded as sterile or contain relative few organisms. Spoilage microflora, therefore, are found mainly on the surface where it is deposited from water, processing and handling (*16*). Contaminating microflora, including yeasts, have also been isolated from the air and soil of poultry brooding houses, wet feed and bird droppings (*17*). In addition, yeasts have also been isolated from the feathers, feed and bodies of the birds during the time of slaughter (*18*). All of these contaminants, contribute to the spoilage of the carcasses of chickens.

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# Flora kvasaca u traheji pilića brojlera

**Sažetak**

Dobro su poznate bakterije kao prirodni sastojci intestinalne flore u peradi. Malo se zna o rasprostranjenosti i sastavu kvaščeve mikroflore. Provedena su ispitivanja kako bi se identificirali kvasci koji prevladavaju u traheji pilića brojlera s jedne peradarske farme. Dobiveno je ukupno 38 tipičnih izolata kvasaca identificiranih uobičajenim postupcima. Tijekom životnoga ciklusa pilića izolirane su vrste koje pripadaju rodovima: *Bullera, Candida, Cryptococcus*, *Debaryomyces*, *Rhodotorula, Torulaspora, Trichosporon* i *Zygosaccharomyces*.