Yeast Growth and Tetracyclines

H. W. Walker and J. C. Ayres

Department of Dairy and Food Industry, Iowa State University, Ames, Iowa

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Characteristics of Yeasts Isolated from Processed Poultry and the Influence of Tetracyclines on Their Growth

Investigations of types of microorganisms occurring on eviscerated poultry have indicated that yeasts comprise only a small percentage of the total population. However, numbers of yeasts have been appreciably larger on carcasses treated with antibiotics such as chlortetracycline (CTC) or oxytetracycline (OTC) than on control birds (Ziegler and Stadelman, 1955; Ayres et al., 1956; Silvestrini et al., 1958; Wells and Stadelman, 1958). In addition, these workers have shown that the tetracyclines inhibit the rapid development of bacteria on processed poultry. Ayres et al. and Wells and Stadelman have proposed that if the bacterial population is maintained at a minimum, substrate is then available for the growth of yeasts. Additional factors probably are involved; for example, Whitehill (1957) has presented evidence that the bacterial flora on meat produced substances which limited the growth of yeasts. Thus, the inhibitory action of the antibiotics caused changes in the bacterial population favoring development of yeasts. No direct evidence has been presented to show that the tetracyclines per se act as growth stimulants for yeasts occurring on poultry; however, stimulation of growth of Candida albicans, a pathogenic yeast, has been attributed to the tetracyclines (Moore, 1951; Pappenfort and Schnall, 1951; Huppert et al., 1953; Carpenter, 1955a, b).

Since both OTC and CTC have been approved by the United States Food and Drug Administration for use on commercially processed poultry, yeasts that develop on the skin and lean surfaces have assumed greater importance than before because they may contribute to spoilage of the product and be of public health significance. Rhodotorula, Torulopsis, Saccharomyces, and Candida have been some of the genera frequently isolated from poultry meat (Walker and Ayres, 1956; Njoku-Obi et al., 1957; Wells and Stadelman, 1958).

The purposes of this study were to characterize yeasts isolated from eviscerated poultry in refrigerated storage and to determine if tetracycline antibiotics acted as growth stimulants for yeasts when these antibiotics were used to prolong the storage life of processed poultry.

REFERENCES


Young, H. P., Jr. and Christensen, C. W. 1942 Hydrogenation of nitriles such as those formed from fatty acids. U. S. Patent 2,287,219.
COMMERCIALLY PROCESSED CHICKENS WERE IMMERSED FOR 120 MIN IN AN AQUEOUS SOLUTION CONTAINING 10 OR 30 µG PER ML OF CTC. BEFORE PACKAGING THE CHICKEN IN POLYETHYLENE BAGS, THE SURFACES WERE SAMPLED BY SWABBING. SAMPLES WERE TAKEN ALSO AFTER 2, 4, 6, 8, 10, 12 AND 14 DAYS OF STORAGE AT 4 C. YEASTS WERE ISOLATED ON ACIDIFIED MALT AGAR (pH 4.5) AND MAINTAINED ON MALT AGAR SLANTS STORED AT 4 C. BEFORE DETERMINING PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATES, THEY WERE PLATED TWICE SUCCESSIVELY ON ACIDIFIED MALT AGAR. SINGLE COLONIES WERE SELECTED FOR CHARACTERIZATION. TO MINIMIZE DUPLICATION, 39 OF 200 ORIGINAL ISOLATES WERE SELECTED FOR FURTHER STUDY ON THE BASIS OF COLONIAL GROWTH, APPEARANCE ON MALT AGAR STREAKS, CELL MORPHOLOGY, AND REACTION IN GLUCOSE, SUCROSE, AND LACTOSE BROTHS. CULTURAL, MORPHOLOGICAL, AND BIOCHEMICAL CHARACTERISTICS OF THESE 39 CULTURES WERE DETERMINED IN ACCORDANCE WITH METHODS OF LODDER AND KREGER-VAN RIJ (1952). CARBOHYDRATE AND NITRATE ASSIMILATION WERE DETERMINED BY THE AUXONOGRAPHIC METHOD (LODDER AND KREGER-VAN RIJ, 1952) AND BY GROWTH IN LIQUID MEDIA (WICKERHAM AND BURTON, 1948).

FOUR DIFFERENT MEDIA, MALT AGAR, V-8 AGAR (WICKERHAM ET AL., 1946), CASPEK'S AGAR, AND GORODKOWA'S AGAR, WERE INOCULATED IN DUPLICATE IN ORDER TO INDUCE SPORE FORMATION. ONE OF EACH PAIR OF PLATES WAS INCUBATED AT ROOM TEMPERATURE (24 ± 2 C); THE OTHER AT 30 C.

THE EFFECT OF CTC, OTC, AND TETRACYCLINE (TC) ON GROWTH OF THESE ISOLATES WAS DETERMINED BY THE SENSITIVITY DISC TECHNIQUE (CARPENTER, 1955A) AND BY VOLUME OF CELLS PRODUCED. THE SENSITIVITY DISC TECHNIQUE CONSISTED OF MAKING A STEAK PLATE OR A POUR PLATE AND THEN PLACING ON THE PLATE ½ IN. ANTIBIOTIC ASSAY DISCS SATURATED WITH AQUEOUS SOLUTIONS CONTAINING 10, 30, 50, OR 100 µG PER ML OF THE RESPECTIVE ANTIBIOTICS. THE PLATES WERE INCUBATED AT 15 C AND OBSERVED FOR GROWTH ENHANCEMENT AROUND THE DISCS AFTER 1, 2, AND 3 WEEKS. THIS TEMPERATURE WAS SELECTED FOR INCUBATION AS THE PRODUCT FROM WHICH THE YEASTS WERE ISOLATED WAS HELD IN REFRIGERATED STORAGE AND ALSO BECAUSE THE ANTIBIOTICS WERE MORE STABLE AT THIS TEMPERATURE THAN AT HIGHER TEMPERATURES. SINCE PLATES WERE POURED TO OBTAIN A THICK LAYER OF AGAR, DEHYDRATION WAS NOT EXCESSIVE AND DID NOT LIMIT GROWTH. THREE DIFFERENT MEDIA WERE USED: NUTRIENT AGAR, MALT AGAR, AND TRYPATICEASE SOY AGAR.

CELL VOLUME WAS DETERMINED BY CENTRIFUGING THE CELLS FROM EQUAL AMOUNTS OF BROTH IN CALIBRATED SEDIMENTATION TUBES. COMPARISONS WERE MADE OF THE VOLUME OF CELLS OBTAINED FROM BROTH CULTURES CONTAINING 0, 10, 30, 50, OR 100 µG PER ML OF THE RESPECTIVE TETRACYCLINE ANTIBIOTICS. MEDIA USED WERE MALT EXTRACT BROTH, NUTRIENT BROTH, AND TRYPTICASE SOY BROTH. TO OBTAIN SUFFICIENT CELLS FOR COMPARISON, A 3-WEEK INCUBATION PERIOD AT 15 C WAS USED.

IN ADDITION TO THE TESTS DESCRIBED ABOVE, PLATE COUNTS WERE MADE OF THE TOTAL NUMBERS OF YEASTS GROWING IN THE PRESENCE AND IN THE ABSENCE OF CTC. A REPRESENTATIVE OF EACH OF THE EIGHT TYPES OF NONGROWING YEASTS WERE INCULCATED INTO TUBES CONTAINING NUTRIENT BROTH PLUS 0, 1, 2, OR 4 µG CTC PER ML. THE CULTURES WERE INCUBATED AT 15 C. TOTAL COUNTS OF YEASTS WERE MADE AT 0, 1, 3, 5, 7, 10, AND 14 DAYS ON MALT AGAR ACIDIFIED TO pH 4.5 AND INCUBATED AT 30 C FOR 4 DAYS.

TWO OF THE EIGHT YEASTS USED ABOVE, A TORULOPSIS SP., AND A CANDIDA SP., WERE EXAMINED FOR COMONITANT GROWTH WITH PSEUDOMONAS FLUORESCENS. NUTRIENT BROTH OR NUTRIENT BROTH CONTAINING CTC WERE INOCULATED AS FOLLOWS: (A) P. FLUORESCENS ONLY, (B) P. FLUORESCENS PLUS 4 µG CTC PER ML, (C) TORULOPSIS SP. OR CANDIDA SP. ONLY, (D) TORULOPSIS SP. OR CANDIDA SP. PLUS 4 µG CTC PER ML, (E) TORULOPSIS SP. OR CANDIDA SP. PLUS P. FLUORESCENS, AND (F) TORULOPSIS SP. OR CANDIDA SP. PLUS P. FLUORESCENS PLUS 4 µG CTC PER ML. EIGHTEEN- TO 24-HR CULTURES INCUBATED AT 30 C WERE USED FOR INOCULA. THE TUBES WERE INCUBATED AT 15 C. TOTAL NUMBERS OF YEASTS WERE DETERMINED ON MALT AGAR ACIDIFIED TO pH 4.5 AND INCUBATED AT 30 C FOR 4 DAYS. TOTAL NUMBERS OF BACTERIA WERE DETERMINED ON NUTRIENT AGAR INCUBATED AT 15 C FOR 4 DAYS. SODIUM TUNGSTATE WAS ADDED AT A CONCENTRATION OF 32 µG PER ML BEFORE POURING THE MEDIA IN ORDER TO NEUTRALIZE ANY CTC TRANSFERRED DURING SAMPLING (JAY ET AL., 1957).

RESULTS AND DISCUSSION

THE TYPES OF YEASTS ISOLATED FROM EVISCERATED POULTRY ARE SHOWN IN TABLE 1. THESE ORGANISMS ARE NONNACEPORE- FORMING, PIGMENTED, AND NONPIGMENTED YEASTS. THREE

<table>
<thead>
<tr>
<th>Species</th>
<th>No. Characterized</th>
<th>Differences from Species as Described by Lodder and Kreger-Van Rij (1952)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichosporon pullulans</strong></td>
<td>14</td>
<td>Starch formation in 9 of the cultures</td>
</tr>
<tr>
<td><strong>Torulopsis glabrata or T. inconspicua</strong></td>
<td>7</td>
<td>Assimilated nitrate</td>
</tr>
<tr>
<td><strong>Torulopsis famata</strong></td>
<td>4</td>
<td>Slight acid in glucose, sucrose, maltose, and galactose</td>
</tr>
<tr>
<td><strong>Candida rugosa</strong></td>
<td>1</td>
<td>Assimilated nitrate</td>
</tr>
<tr>
<td><strong>Candida scottii</strong></td>
<td>3</td>
<td>Slight acid from glucose and maltose; 2 cultures did not peptonize milk</td>
</tr>
<tr>
<td><strong>Candida krusei</strong></td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td><strong>Candida intermedia</strong></td>
<td>2</td>
<td>Assimilated nitrate</td>
</tr>
<tr>
<td><strong>Candida pelliculosa</strong></td>
<td>2</td>
<td>No ester odor; acid from glucose, galactose, sucrose, and maltose</td>
</tr>
<tr>
<td><strong>Rhodotorula aurantiaca</strong></td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td><strong>Rhodotorula mucilaginosa</strong></td>
<td>2</td>
<td>—</td>
</tr>
</tbody>
</table>
different attempts were made to induce ascospore formation on four media specifically advocated for this purpose but repeated examination failed to reveal spores in any of the cultures.

Morphological, cultural, and biochemical characteristics of the pigmented yeasts were similar to those described by Lodder and Kreger-Van Rij (1952) as representative or typical of Rhodotorula aurantiaca and R. mucilaginosa. Characteristics of the nonpigmented yeasts permitted segregating them into three groups; namely (a) those organisms resembling Trichosporon spp., (b) those resembling Torulopsis spp., and (c) those resembling Candida spp.

Nine of the cultures tentatively classified as Trichosporon spp. produced starchlike material which, according to Lodder and Kreger-Van Rij (1952), is indicative of Cryptococcus spp.; however, these organisms produced mycelial formations and otherwise closely resembled Trichosporon pullulans. Such a grouping is supported by the observation of Aschner and Cury (1951) that certain species of Trichosporon produce starchlike material. In addition, Lodder et al. (1958) have stated that T. pullulans is noted for its ability to grow at low temperatures and have identified certain yeasts isolated from refrigerated beef as being strains of T. pullulans and Candida scottii. Therefore, the growth of these species on poultry held under refrigeration could easily occur.

No distinction was made between the organisms resembling Torulopsis glabrata and T. inconspicua as these species are similar in many of their characteristics. The main difference between these two species is in their fermentative capacity (Lodder and Kreger-Van Rij, 1952).

Some of the characteristics that help to distinguish the genera Candida, Trichosporon, Torulopsis, and Cryptococcus from one another have been pointed out by Lodder and Kreger-Van Rij (1952). Torulopsis spp. produce no pseudomyelia or starchy material and seldom form a pellicle. Candida spp. produce chlamydo- spores, blastospores, true mycelia, and pseudomyelia. Cryptococcus spp. produce starchlike material, and occasionally form a pellicle with the entire contents of the flask becoming a slimy mass but form no pseudomyelia. Members of the genus Trichosporon form abundant pseudomyelia, true mycelia, blastospores, and arthropores. Torulopsis spp. generally have fermentative ability; Candida spp. have limited fermentative capacity, whereas Cryptococcus and Trichosporon spp. rarely, if ever, ferment sugars.

Although in this work only four genera (Rhodotorula, Trichosporon, Torulopsis, and Candida) were observed, other types have been reported. For example, Ziegler and Stadelman (1955) noted from stained smears that yeasts having the shape and budding characteristics of the family Saccharomycetaceae together with gram negative rods, formed the predominant flora of spoiled chicken meat pretreated with CTC. Later Wells and Stadelman (1958) identified the predominant yeasts at the time of spoilage as species of Rhodotorula and Torulopsis. Njoku-Obi et al. (1957) found both Saccharomyces cerevisiae and S. dairenensis in addition to species of Rhodotorula, Torulopsis, and Candida on poultry. The results of these studies would indicate that several genera of yeasts are common contaminants of eviscerated poultry.

Njoku-Obi et al. (1957) isolated Candida parapsilosis from eviscerated poultry and suggested that under certain conditions this organism could be of public health significance. However, Lodder et al. (1958) did not consider this organism ordinarily to be pathogenic in nature. In the present study, none of the isolates were identified or characterized as pathogenic species of yeasts; and, therefore, none were considered to be of public health significance.

With the prolonged use of broad spectrum antibiotics for therapeutic purposes, Candida albicans has frequently developed and become predominant in certain infections. This predominance, in some instances, has been attributed to growth stimulation by tetracycline antibiotics (Moore, 1951; Pappenfort and Schnall, 1951; Huppert et al., 1953; Carpenter, 1955a, b). Since increased numbers of yeasts occur on eviscerated poultry treated with tetracyclines, the question has been raised if these antibiotics serve as growth stimulants when used for preservative purposes. However, with both the sensitivity disc technique and cell volume measurements, no growth enhancement was observed in any of the 39 isolates in the presence of CTC, OTC, and TC at levels of 10, 30, 50, or 100 µg per ml.

In raw poultry tissue, the concentration of antibiotic found after several days of storage has been reported to be approximately 1 µg per g when solutions of 10 µg per ml have been used to immerse the birds (Anderson et al., Silvestrini et al., 1958; Walker and Ayres, 1958). Therefore, concentrations that were more in line with those observed in poultry tissue were examined for their effect on growth. Figure 1 shows that throughout a 14-day period there was no increase in the total numbers of yeasts growing in the presence of 1, 2, or 4 µg CTC per ml when compared to the total number growing in the absence of the antibiotic. Similar results were obtained and plotted for the other 6 isolates but are not shown. In all instances, the maximal count for all levels of antibiotic seldom exceeded 5 x 10^7 yeasts per ml when grown in nutrient broth.

Figure 2 shows that, when either a Candida sp. or a Torulopsis sp. was grown in the presence of P. fluorescens, the maximal number of yeasts was not as high as in the absence of the bacteria. However, if CTC were added to a mixture containing one of the yeasts and P.
fluorescens, the maximal number of yeasts became the same as that found in the control. The presence of yeasts had no apparent effect on the growth of the bacteria. Addition of CTC caused a pronounced lag in growth of the bacteria; however, the degree of inhibition was not as great when CTC was added in the presence of yeasts. The curves showing the growth of P. fluorescens in the presence of either a Torulopsis sp. or a Candida sp. and 4 μg per ml of CTC are not shown but these growth curves were similar to those for the yeasts growing alone or in the presence of CTC.

None of the tests showed that the addition of CTC to the growth medium resulted in increased numbers of yeasts. This agrees with the findings of Mandel et al. (1958) who could not demonstrate inhibition or stimulation of growth of two strains of C. albicans in the presence of concentrations of CTC as high as 100 μg per ml. It is conceivable that the growth of certain strains of yeast not included in this study and ordinarily not associated with processed poultry are stimulated and produce increased numbers of cells in the presence of tetracyclines. For instance, Huppert and co-workers (1953) demonstrated that concentrations of CTC of 100 μg per ml or higher caused an increase in the total growth of the strain of C. albicans which they were using.

Evidence indicates that an organism such as P. fluorescens will retard the growth of certain yeasts isolated from poultry; however, with the addition of CTC, the bacteria are inhibited while the number of yeasts increases. Whitehill (1957) observed that the bacterial flora developing on meat seemed to produce antifungals which controlled growth of yeasts. Further, it would appear that, in poultry, the increased numbers of yeasts can be explained on the basis of suppression of growth of certain bacteria by the tetracyclines and that these bacteria are then not present in large enough numbers to limit the growth of yeasts.

![Figure 1](image1.png)

**Figure 1.** The effect of chlortetracycline added to nutrient broth on the growth of two yeasts isolated from poultry.

![Figure 2](image2.png)

**Figure 2.** The effect of *Pseudomonas fluorescens* and chlortetracycline in nutrient broth on the growth of two yeasts isolated from poultry.
SUMMARY

Thirty-nine cultures of yeasts were isolated from eviscerated poultry and characterized. Pigmented yeasts had characteristics typical of Rhodotorula; non-pigmented yeasts were species of Trichosporon, Torulopsis, or Candida. Even though greater numbers of yeasts have been found on antibiotic treated birds than on control birds, none of the tetracyclines (chlortetracycline, oxytetracycline, and tetracycline) were shown to stimulate growth of these yeasts in laboratory media. However, in mixed cultures, Pseudomonas fluorescens suppressed growth of yeasts. The addition of chlortetracycline to the cultures inhibited the bacteria and permitted increased growth of yeasts. Since none of the isolates had characteristics typical of pathogenic yeasts, none were considered to be of public health significance.

REFERENCES


