Indole-Induced, Green to Brown-Black Pigment Formation by an Acinetobacter Strain from Beef

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ABSTRACT

In determining aerobic plate counts of surfaces of beef carcasses, green to brown-black colonies of an Acinetobacter sp. appeared on tryptic soy agar plates. Pigmentation was induced by indole-producing organisms in close proximity of or overlapping with the Acinetobacter sp. Addition of indole to culture media also supported pigmentation of the Acinetobacter sp. Indole appeared to be a key intermediate compound in pigment formation.

Green to brown-black colonies were observed on tryptic soy agar (TSA) plates that were used to determine aerobic plate counts (APC) of surfaces of spray-chilled beef carcasses.

MATERIALS AND METHODS

Separate cotton swabs (Hardwood Products Co., Guilford, Maine) moistened with sterile 0.1% peptone solution were used to swab each of five 10 cm² locations (inside round, strip loin, boneless rib, clod, inside neck) of each side of a carcass. The swabs were placed in 25 ml of sterile peptone and mixed thoroughly by shaking 25 times. One milliliter (divided over four plates), 0.1 ml and 0.1 ml of appropriate decimal dilutions were plated on the surface of prepoured TSA plates. Plates were incubated at 25°C. After plated incubation for 2 d some colonies were green and gradually turned brown-black after 3 to 5 days at 25°C (Fig. 1a). This phenomenon had not been observed in our laboratory previously. Sources of the pigmented colonies were 2 of 10 carcasses from a meat packing plant in Texas. When the brown-black colonies were subsequently streaked on TSA plates for purification and identification, colonies were very similar, however, some were clear and others slightly opaque. Where the two types of colonies were well-separated no pigmentation was noticed, when they were in close proximity or when overlap occurred pigmentation occurred (Fig. 1b).

The isolates were streaked onto brain-heart infusion (BHI) agar for purification and to produce isolated colonies. Isolates then were again streaked onto BHI agar and incubated for 24 h at 25°C. Following incubation, the cultures (gram-negative, oxidase-negative rods) were identified to species by inoculating the GNI (Gram Negative Identification) card of the AutoMicrobic System (AMS) of Vitek Systems, Inc. (Hazelwood, Mo.). The GNI card consists of 29 biochemical broths and one growth control broth and performs a series of conventional and nonconventional biochemical tests. The GNI card was inoculated in triplicate according to the manufacturer’s instructions. The AMS system has been evaluated and shown to be accurate for the identification of clinical (2) and food (1) isolates.

RESULTS AND DISCUSSION

Culture E (clear colonies) was identified as a strain of Escherichia coli, and A (opaque colonies) as Acinetobacter calcoaceticus (Fig. 1). When the two organisms were cross-streaked on TSA plates as shown in Fig. 2, results indicated that the E. coli strain effected pigmentation of the Acinetobacter strain. When plate count agar (PCA, Difco), Standard Methods Agar (SMA, BBL) or nutrient agar (Difco) was substituted for TSA, little or no pigmentation was observed.

Subsequent experiments showed that the green to brown-black pigmentation of the Acinetobacter strain on TSA could by effected by a series of indole-producing organisms including other E. coli strains, Citrobacter diversus, Klebsiella oxytoca, Erwinia herbicola, Proteus morganii and indole-producing Yersinia enterocolitica.

Addition of 1 to 1.5% tryptone (Difco) or 0.1 to 0.5% tryptophan to PCA caused dark green to brown-black pigmentation of the Acinetobacter species on PCA when cross-streaked with E. coli. Elimination of soytone from
Figure 1. (a) (Top) Brown-black pigmented colonies of the Acinetobacter strain (A) in the presence of Escherichia coli (E) on tryptic soy agar; (b) (bottom) pigmentation of Acinetobacter in close proximity of or when overlap occurred with E. coli.

Figure 2. Acinetobacter strain (A) cross-streaked with Escherichia coli (E) on tryptic soy agar.

TSA (15 g of tryptone, 5 g of soytone, 5 g of NaCl, 15 g of agar) did not affect pigment production; without tryptone no pigment was produced. When tryptone and soytone in TSA were replaced with an equal amount of protease-peptone (Difco) or thiotone peptone (BBL), pigment production was small to moderate, respectively. Pigment production on each of these media was evaluated at 20, 30 and 35°C. When pigment production was observed, it would occur at 20 and 30°C but not at 35°C.

Pigmentation of Acinetobacter colonies on PCA plates without the presence of E. coli or an indole-producing culture took place when indole was added to PCA. After 24 h of plate incubation at 25°C, colonies on PCA with 0.001% indole were green; those on PCA with 0.01% indole were brown-black. Based on these observations, indole appears to be a key metabolite in pigment formation by the Acinetobacter strain. It is possible that the brown-black pigment represents melanin formation since indole derivatives after undergoing several reactions can polymerize to form melanin (6).

Acinetobacter-Moraxella spp. are frequently mentioned as part of the microflora of refrigerated fresh red meats. However, according to Eribo and Jay (4), the incidence of Acinetobacter spp. in fresh ground beef is considerably lower than is generally believed. Of 1409 gram-negative isolates selected from 19 samples of fresh and spoiled ground beef, only 20 were Acinetobacter spp., all of which were recovered from fresh meat samples. Gill (5) also reported that Acinetobacter-Moraxella spp. contributed little to the spoilage of aerobically stored fresh meat of normal pH.

When the pigment-producing Acinetobacter calcoaceticus was replaced with other Acinetobacter strains previously isolated from raw beef, no pigmentation was observed. Characterization of the pigment and mechanisms involved in its production are objectives of subsequent research efforts.

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REFERENCES