of natural products such as grapefruit seed extract (Xiong et al., 1998). However, it has not been developed an efficient method that is completely devoid of chemicals and that might not cause food safety concerns.

In a previous study, we isolated and characterized Salmonella-lytic bacteriophages (Fiorentin et al., 2004). Some of these bacteriophages have been administered in vivo to SE PT4-infected broilers and there was a reduction in the colony forming units of SE PT4 per gram of cecal contents by 3.5 orders of magnitude (Log CFU/g) (Fiorentin et al., 2005). Other authors have also successfully reported reductions in Salmonella counts by using bacteriophages in chicken internal organs and feces (Toro et al., 2005), skin (Goode et al., 2003) or poultry products (Whichard et al., 2003). The positive results led to the hypothesis that the bacteriophage isolated previously in our laboratory might also be efficient in reducing SE PT4 in chicken skin. Some advantages of administering phages onto the skin of poultry carcasses are that phages would not recycle in the host and therefore selection of resistant strains would be avoided. Besides, methods of biological control pose fewer risks to the consumer compared to chemical methods.

In the present study, chicken thighs and drumsticks were contaminated with SE PT4 and later treated with a panel of salmonellae-lytic bacteriophages isolated from free-range chickens.

MATERIAL AND METHODS

Chicken thighs and drumsticks

The study evaluated three groups as described in Table 1. The groups were comprised of 25 whole legs (thighs and drumsticks) with mean weight between 300 and 350 grams. The legs were collected during slaughter of a Salmonella-negative flock previously monitored using drag swabs (Waltman et al., 1998). Chicken cuts (or parts) were experimentally contaminated by immersion in a suspension of phosphate buffered saline (PBS pH7.2) containing 10^6 CFU/mL of SE PT4 and allowed to dry for a few minutes. The samples of the three groups were transferred to sterile plastic bags, sealed and kept at 5°C. Afterwards, five samples per treatment were randomly taken at each three days for Salmonella and bacteriophage counts.

Contamination with *Salmonella*

SE PT4 isolate P125589 was kindly provided by Dr Paul Barrow (ARFC Institute for Animal Health, Houghton Laboratory, Cambridge, England). It was originally isolated by Dr B. Rowe (Central Public Health Laboratory, London, UK) from a case of human food poisoning (Barrow & Lovell, 1991).

A fresh colony of SE PT4 was inoculated into 10mL of nutrient broth (NB, 1g/L beef extract, 2g/L yeast extract, 5g/L peptone, 5g/L sodium chloride, pH 7.0) and incubated overnight at 37°C under shaking (200rpm). The culture was frozen at 80°C and one aliquot was used for enumeration of viable cells, by counting colonies grown from tenfold dilutions streaked onto nutrient agar and incubated for 24h at 37°C. After counting, the original culture was diluted with sterile buffered saline (1 L) to produce a solution containing 10^6 CFU/mL that was used to contaminate the chicken cuts.

Table 1 Treatments used to assess the effect of bacteriophages on the reduction of Salmonella Enteritidis phage type 4 contamination in broiler skin samples.

Group	Cuts	Treatment
1	25	Non-contaminated and non-treated
2	25	Contaminated by immersion on a suspension containing 10^6 CFU/mL of Salmonella Enteritidis phage type 4 at slaughter day
3	25	Contaminated as in group 2, treated one day later by immersion in a suspension containing 10^9 PFU/mL of a mixture of bacteriophages CNPSA1, CNPSA3 and CNPSA4

Treatment with bacteriophages

Bacteriophages CNPSA 1, CNPSA3 and CNPSA4 were isolated from feces of free-range chickens in Brazil and characterized as described elsewhere (Fiorentin et al., 2004). Since resistance to bacteriophages may emerge in growing populations of bacteria, we decided to use a pool of three different viruses. Frozen bacteriophage stocks were amplified on overlay cultures of SE PT4 prepared with Nutrient Broth (NB) containing 0.7% agarose. Enough bacteriophage particles were then used to inoculate one liter of SM buffer (5.8g/L NaCl, 2.0g/L MgSO4-7H2O, 5.0mL/L of a 5% solution of gelatin, 50mL/L of 1M Tris-HCl pH 7.5) to a concentration of 10^9 plaque forming units per milliliter (PFU/mL), which results in a multiplicity of infection of 1,000 (MOI: 1,000), i.e., a thousand PFU of bacteriophages per CFU of SE PT4 was used to contaminate the chicken parts. Bacteriophage titers were determined using tenfold dilutions of the virus preparation mixed to SE PT4 in log-phase growth (10µL : 250µL). The mixtures were

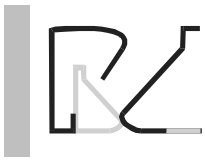

Figure 1 - Mean – standard deviation of total colony forming units (CFU x 10^8) of SE PT4 recovered from cuts contaminated with Salmonella (Group 2) or contaminated and treated with bacteriophage (Group 3). Differences between means were statistically significant on days 3, 6 and 9.

to achieve Salmonella reduction by several orders of magnitude. Goode

bacteriophages did not target any other bacteria present on the chicken skin, otherwise they would have shown higher PFU per cut with longer shelf storage time.

CONCLUSIONS

A panel of bacteriophages reduced SE PT4 countings in experimentally contaminated chicken parts stored at 5°C. We demonstrated that Salmonella CFU was reduced in the bacteriophage-treated cuts on days 3, 6 and 9 post-treatment when compared to their non-treated counterparts.

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