Research Article

Identification of bapA in Strains of Salmonella enterica subsp. enterica Isolated from Wild Animals Kept in Captivity in Sinaloa, Mexico

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bapA, previously named stm2689, encodes the BapA protein, which, along with cellulose and fimbriae, constitutes biofilms. Biofilms are communities of microorganisms that grow in a matrix of exopolysaccharides and may adhere to living tissues or inert surfaces. Biofilm formation is associated with the ability to persist in different environments, which contributes to the pathogenicity of several species. We analyzed the presence of bapA in 83 strains belonging to 17 serovars of Salmonella enterica subsp. enterica from wildlife in captivity at Culiacan’s Zoo and Mazatlan’s Aquarium. Each isolate amplified a product of 667bp, which corresponds to the expected size of the bapA initiator, with no observed variation between different serovars analyzed. bapA gene was found to be highly conserved in Salmonella and can be targeted for the genus-specific detection of this organism from different sources. Since bapA expression improves bacterial proliferation outside of the host and facilitates resistance to disinfectants and desiccation, the survival of Salmonella in natural habitats may be favored. Thus, the risk of bacterial contamination from these animals is increased.

1. Introduction

Biofilms, composed of cellulose, fimbriae, and biofilm-associated protein A (BapA, encoded by bapA), are communities of microorganisms that grow in a matrix of exopolysaccharides and can adhere to inert surfaces or living tissues [1]. Biofilm formation is associated with the ability to persist in different environments [2], which contributes to the pathogenicity of several species [3]. It has been shown that bacteria growing in biofilms are more resistant to antimicrobial agents than those growing in planktonic cultures due to their physical structure and the formation of multilayer biofilms [4]. Whereas acute bacterial infections can be eliminated after a brief antibiotic treatment, infections by biofilm-producing bacteria normally fail to be completely eliminated and lead to recurrent infections, which can only be resolved by replacing the initial antibiotic therapy [3].

Salmonella are rod-shaped bacteria commonly found in biofilms [5]. This genus includes flagellated, Gram-negative bacteria without spores that thrive in animals’ digestive tracts and environments that facilitate long periods of survival, which makes elimination difficult [6].

Fimbriae, or pili, are important for biofilm formation by Salmonella [7]. These protein structures recognize a wide range of molecular targets, allowing the bacteria to interact with various surfaces and adhere to specific tissues in the host [8]. For example, type 1 fimbriae are thin, rigid, adhesive structures that express FimH adhesins, which promote bacterial adhesion to and invasion of epithelial cells [9]. Type 1 fimbriae also mediate interactions with abiotic surfaces [9].
Thus, the objective of this study was to determine the type of antibiotic therapy necessary to treat animal health problems. Identifying the genes involved in bacterial resistance will particularly help in considering treatments in closed environments within infected animals. To prevent outbreaks of disease in both animals and humans, bacterial genuses will allow us to establish preventive measures against antibiotic resistance. This is especially important in salmonellosis, a zoonotic disease [13]. Additionally, bacteria in biofilms can have greater resistance to antibiotics due to several factors. For example, these bacteria can hide in food for long periods of time in both stool and water, making it difficult to eradicate them. Enteric pathogens can be present in soil, insect, and food. The structure of the biofilm, therefore, impedes the action of the antimicrobial agent [10].

Understanding the capacity of biofilm formation in this bacterial genus will allow us to establish preventive measures to prevent outbreaks of disease in both animals and humans, particularly those in close contact with infected animals. Identifying the genes involved in bacterial resistance will determine the type of antibiotic therapy necessary to treat animal health problems. Thus, the objective of this study was to detect the presence of bapA in *Salmonella* strains isolated from wild animals in captivity.

### 2. Material and Methods

#### 2.1. Strains
Eighty-three strains of *Salmonella* spp. belonging to 17 different serovars (Table 1) obtained from enclosures, food, and feces from zoo and aquarium animals in captivity in Culiacan and Mazatlan, Sinaloa, Mexico, were used in the study. All isolates were confirmed through biochemical and serological methods by the Enteric Bacteriology Laboratory, Institute of Epidemiological Diagnosis and Reference (InDRE), DF, Mexico, and maintained on nutrient freezing medium until being tested. *Salmonella* Typhimurium 14028S from the American Type Culture Collection (ATCC) was used as a reference control strain.

#### 2.2. Recovery and Purity Verification of Strains
*Salmonella* strains were recovered from preservation medium containing soy broth-glycerol (freezing medium), transferred to tryptophane soy broth, and incubated at 37°C for 18 h. The bacterial suspensions obtained were plated on MacConkey and XLT4 agar to confirm the negative reaction of *Salmonella* strains to lactose and to visually analyze the purity of the strains grown at 37°C for 24 h. Inclined tubes containing blood agar base (BAB) were inoculated with confirmed strains until further use.

### Table 1: List of *Salmonella* serovars used in the study.

| Identification number | Serovar | Source (# of isolates) |
|-----------------------|---------|------------------------|
| 1 Typhimurium         | Reference strain |
| 2 Albany              | *Leopardus pardalis* (f), *Panthera leo* (f), *Felis concolor* (f), *Panthera tigris sumatrae* (f), *Panthera tigris tigris* (f), *Lynx rufus* (f), *Ursus americanus* (f), *Hippopotamus amphibius* (f), *Ara macao* (f), *Carassius auratus* (w)⁴, aquatic birds (f), aquatic bird (s)⁵, *Rattus spp.* (f), *Periplaneta americana* (i)⁶, *Musca domestica* (i), raw chicken (f)⁷ |
| 3                      | *Hippopotamus amphibius* (f), *Bassariscus astutus* (f), aquatic birds (f), aquatic birds (w), *Cebus apella* (f) |
| 4                       | Aquatic birds (f), aquatic birds (s), *Python regius* (b)⁷, *Rattus spp.* (f) |
| 5 Braenderup            | *Mephitis macroura* (f), *Felis concolor* (f), *Panthera tigris* (f), *Procyon lotor* (f), *Ateles geoffroyi* (f) |
| 6 Weltevreden           | *Columbia flavirostris* (f), *Columbia fasciata* (f), *Sus scrofa domestica* (f), aquatic birds (f), aquatic birds (s) |
| 7 Derby                 | *Cebus apella* (f), *Panthera onca* (f), *Panthera tigris* (f), *Rattus spp.* (f) |
| 8 Oranienburg           | *Urocyon cinereoargenteus* (f), *Saimiri sciureus* (f) |
| 9                       | *Hippopotamus amphibius* (w), *Crocodile acutus* (w) |
| 10 Poona                | *Psittaciformes birds* (f), *Rattus spp.* (f) |
| 11 Saint Paul           | Aquatic birds (f) |
| 12 Panama               | *Crocodile acutus* (w), *Rana spp.* (f) |
| 13 Pomona               | *Ramphastos sulfuratus* (f), biological filter |
| 14 Newport              | Aquatic birds (f) |
| 15 Enteritidis          | Psittaciformes birds (f) |
| 16 Javiana              | *Rana spp.* (f) |
| 17 Give                 | *Iguana iguana* (f) |
| 18 Agona                | *Ara spp.* (f) |

⁴Feces. ⁵Water. ⁶Soil. ⁷Insect. ⁸Food. ⁹Rectal *Hyssopus*. ¹⁰BAB.
2.3. **Bacterial DNA Extraction.** DNA was extracted from isolated bacterial strains with a commercial matrix (InstaGene Matrix, Bio-Rad®).

2.4. **PCR Identification of bapA.** The oligonucleotide primers for PCR were synthesized according to the published DNA sequences of the bapA gene [10] and have, respectively, the following nucleotide sequence: forward, 5’-GCCATGGTGCTGGAAGGCCTGGCCTT-3’; reverse, 5’-GGTCGACGGGAAAGGTTAAAATGACCTTC-3’. Amplification was carried out in a thermocycler (Bio-Rad, MJ Mini Personal Thermal Cycler) with a reaction mixture of 25 μL, which contained 5 μL of template DNA, 1 μL (10 pmol L⁻¹) of each of the forward and reverse primers, 12.5 μL PCR SuperMix (22 mM Tris-HCl, 55 mM KCl, 1.65 mM MgCl₂, 220 μM dGTP, 220 μM dATP, 220 μM dTTP, 220 μM dCTP, and 22 U/mL recombinant Taq DNA Polymerase), and 1.5 μL MgCl₂ (50 mM). The final volume was prepared with nuclease-free water. The PCR program included an initial denaturation step at 94 ˚C for 5 min followed by 30 cycles of denaturation (94˚C for 1 min), annealing (50˚C for 45 s), and extension (72˚C for 1 min). Final extension was carried out at 72˚C for 5 min. Amplification products were separated by submarine gel electrophoresis on 1.5% agarose gel with prestained GelRed (solution at 1:10,000) in 0.5x Tris-EDTA buffer. A 100 bp DNA ladder (Bio-Rad) was used as a molecular weight marker. The gels were visualized in Gel Documentation System™ EZ GelDoc and photographed for analysis.

2.5. **Statistical Analysis.** The frequency of the presence of bapA was determined according to the previously reported formula [14]. To determine whether there were significant statistical differences among the different serovars examined, chi-square tests were performed using the epidemiological data analysis program, Epidat 3.1.

3. **Results and Discussion**

PCR reactions of the 83 isolates belonging to 17 different serovars with oligonucleotides to bapA amplified a product of 667 bp, which corresponds to the expected size of the bapA initiator (Figure 1). Importantly, there were no differences detected in this initiator element between different serovars tested. Of the strains analyzed, 65 were isolated from animal feces (mammals, birds, and reptiles), 6 were isolated from American cockroach and Musca domestica, 2 were isolated from food, 1 was isolated from a biological filter, and 4 and 5 were isolated from enclosures of water and soil, respectively.

All serovars amplified bapA, consistent with previous results [10], which suggests that bapA is a very conserved gene both between and within different serovars with a high degree of identity (99%) [15]. This conservation offers diagnostic advantages because the presence of bapA can be used to identify the Salmonella genus in different environments [16]. BapA belongs to a family of large surface proteins involved in bacterial adhesion to various surfaces and maturation of biofilms [17]. The protein, which was previously named Stm2689, plays an important role in the mouse model of intestinal colonization as well as bacterial spread to other organs [18]. In this study, strains were isolated from the stool of wild animals lacking gastroenteric disorders, which suggests that intestinal colonization in these animals is associated, in part, with the presence of the bapA gene. Supporting this notion, previous research compared the propensities of Salmonella strains with or without bapA to colonize the intestine and demonstrated that mutated strains exhibited lower colonization rates than those with wild-type bapA [19].

The mechanisms that allow these pathogens to persist in animals’ digestive tracts are poorly understood. However, the intestinal persistence of Salmonella spp. observed in clinically healthy animals increases the risk of bacterial contamination because bapA expression ensures that more bacteria survive outside of the host and retain their infective ability. This allows the bacteria to resist desiccation and the action of the disinfectants; thus, the survival of Salmonella in natural habitats may be favored [20, 21]. Further understanding of the mechanisms involved in bacterial intestinal persistence will facilitate the development of innovative strategies that safeguard the public population against salmonellosis, a natural zoonotic disease.

4. **Conclusions**

bapA was identified in all 83 strains belonging to 17 different serovars isolated from wildlife in captivity which suggests that it is a highly conserved gene in Salmonella and can be targeted for the genus-specific detection of this organism from different sources and diagnostic potentials, which need to be explored. Additionally, most animals that tested positive were asymptomatic carriers. This poses a challenge for...
professionals in the health care area to overcome, because the capacity of *Salmonella* to survive in many environments suggests that its dissemination will likely continue to increase in the future.

**Competing Interests**

The authors declare that there are no competing interests regarding the publication of this paper.

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