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MOTILITY INDUCTION OF *Salmonella pullorum* UNDER MODIFIED CONDITIONS IN SUDAN

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المستخلص

حفزت حركة السالمونيلا بلورم تحت ظروف معدلة بالسودان و ذلك بتحوير الوسط المنبتي باستعمال وسط شبه جامد بدلا من المنبت الهلامي جامد الحركة الذي كان يستعمل في العادة. الوسط المعدل يحتوي على 53 جرام جلاتين, 25 جرام شوربة قلوب و 2 جرام هلام و اضيف اليها 0.5% ديكستروز, كما زيدت درجة حرارة التحضين الى 42.5 درجة مئوية من 37 درجة مئوية و مدت فترة الحضانة من 24ساعة الى 48 ساعة. لتحديد هذه الحركة تم استيراد جرثومة السالمونيلا بلورم من الخارج (Health Protection Agency Culture Collection, UK. Taxonomy Tax Link: S9096) للاختبار كما استعملت جرثومة السالمونيلا ملهبة الامعاء كمحكم ايجابي وهي معزولة محليا من معمل ربك للبحوث البيطرية, بعد 24 ساعة لوحظ وجود نمو تمثل في خط منتشر في كلا الانبوبتين المزروعتين. هذا الانتشار اصبح اكثر وضوحا عند درجة حضانة 42.5 درجة مئوية و فترة حضانة 48 ساعة. اثبت هذا النمو الذي ظهر بشكل خط منتشر, إن السالمونيلا بلورم لها مقدرة للحركة و هذا دليل لامتلاكها الاسواط.

Abstract

Motility of *Salmonella pullorum* under modified condition in Sudan was performed by modifying the growth conditions. Semisolid medium instead of the usually used solid motility medium consist of 53g gelatin, 25g heart infusion broth and 2 g agar supplemented with 0.5% dextrose was used. Incubation temperature was raised increased from37C^{o} to 42,5C^{o} and the duration of incubation was increased from 24hr to 48hrs. For testing the motility, imported *Salmonella pullorum* (Health Protection Agency Culture Collection, UK Taxonomy Tax Link: S9096))

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was purchased and a local strain of *Salmonella eneritidis* isolated from Rabak Veterinary Research Laboratory was used as positive control. Diffusion line which indicated movement in the positive control *Salmonella enteritidis* inoculated tube as well as in the other tube inoculated with *Salmonella pullorum* was observed. Those lines were seen more clearly at 42,5C^{o} and incubation temperature for 48 hrs. These diffusion lines indicated that *Salmonella pullorum* can exhibit the ability of possessing flagellae and, hence motility.

Keywords: Salmonella, flagella motility, modified condition.

Introduction

Pullorum disease caused by *Salmonella pullorum* is a septicemic disease affecting primarily chickens, turkeys and Pheasants, other birds such as Quail, Ducks, Peacock and guinea fowl are susceptible. (Shivaprasad, 2000). Recently *Salmonella pullorum* and *Salmonella gallinerum* have been placed in a single species designated as *Salmonella. enterica subsp. enterica serovars gallinarum -pullorum* but debate continues as to whether they are single or different serovars. Pullorum disease produces serious economic losses in poultry industry if not adequately controlled and causes outbreaks with high morbidity and mortality rates. The disease appears mainly during the first 2 weeks of life. Most of Salmonella species possess flagellae and exhibit motility. (Ewing, 1972) and (Le Minor, 1984). *Salmonella pullorum* and *Salmonella gallinarum* are two notable exceptions, having been shown early on to lack flagellae and motility (St. John –Brooks, (1934). *Salmonella pullorum* is non-motile under normal conditions but inducement of flagella proteins has been shown in some strains of *salmonella pullorum* when grown in special media (OIE Terresterial Manual, 2008). Evidence indicated that *Salmonella pullorum* may possess flagellae. Ibrahim, *et .al.,* (1986) reported that antisera generated to Salmonella flagellae reacted with *Salmonella pullorum*, and Berchieri *et al.* (1995) stated that, serological screening of poultry flocks by a flagellum-based enzyme-linked immunosorbent assay indicated reactions with sera from *Salmonella pullorum* infected birds Kilger and Grimont (1993) found the genes for flagella in *Salmonella pullorum.* Holt and chaubal, (1997) found that antiflagellar antibody reagents reacting to strains of *Salmonella pullorum*.

Flagellin is apparently the dominant activator of protective pathway during salmonella infection. It is evident that epithelial cells exposed to microbial challenge can ultimately respond with cellular proinflammatory activation or in programmed cell death. Epithelial cells under stress by bacterial pathogens activate an apoptotic pathway in parallel with proinflammatory pathways; in an effort to increase expression of reactive flagella. Neish, (2006). This study was conducted using a special medium (semi solid media supplemented by dextrose instead of the solid media originally described by Holt and Chaubal, (1997). The objective of this study to approve the hypothesis that observable motility would indicate an expression of flagellin protein.

Materials and Methods

1. Bacterial strains.

Two strains were used:-
One is standard *Salmonella pullorum* imported from Health Protection Agency Culture Collection, UK. Taxonomy Tax Link: S9096 sent in lyophilize form. The strain was used for the detection of motility.
Two is *Salmonella enteritidis* a locally isolated strain in Rabak Veterinary Research Laboratory (Se10).

2. Media

Bacteriological peptone broth (Oxoid) consists of:-

Peptone	10 g
Sodium chloride	5 g
Disodium phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g

Final PH (at 25 C^o) 7.0 ±0.2.

It was prepared by dissolved 50 grams of powder in 1 liter distilled water (DW), mixed well and distributed into test tubes and sterilized by autoclaving at 121 C^o for 15 minutes, then stored in the refrigerator at 4 C^o until used.

Xylose lysine Deoxycholate Agar (XLD) (Himedia):

Yeast extract	3.00 g
L -lysine	5.00 g
Lactose	7.00 g
sucrose	7.00 g
xylose	3.50 g
Sodium chloride	5.00 g
Sodium deoxycholate	2.50 g
Sodium thiosulphate	6.80 g
Ferric ammonium citrate	0.80 g
Phenol red	0.08 g
agar	15.00 g
Distell water	1 L

Final PH (at 25C^{o}) 7.4±0.2

Fifty six point sixty eight grams was suspended in 1000 ml distilled water. Heat with frequent agitation until the medium boils, and then transferred immediately to a water bath at 50C^{o}. After cooling, it was there been pour into sterile Petri plates.

Semisolid motility (Himedia) consists of:-

Gelatin	53.4 g
Heart infusion broth	25.0 g
Agar	2.0 g
dextrose monohydrate	0.50%

All components were added to some cold distill water which was completed to 1.0 L. and mixed thoroughly. It was gently heated until brought to boiling. The media was distributed into tubes. Autoclaved at 121C^{o} for 15 minute.

3. Initial Growth of Salmonella :-

Salmonella pullorum and *Salmonella enteritidis* each was diluted in bacteriological peptone incubated at 37C^{o}/24hrs then subcultured onto XLD agar and incubated at 42.5C^{o}for 24hrs. Resulting colonies of each inoculums were then reinoculated in the tubes of semisolid media containing dextrose and then labeled as A and B. Tube B was inoculated with *Salmonella enteritidis* colonies and A was inoculated with *Salmonella pullorum* colonies and incubated at 42.5C^{o} per24hrs using a needle impregnated by the culture. Both tubes were observed for 24hrs as well as for 48hrs.

Results

Diffusion was observed after 24hrs of incubation on both tubes A&B that were incubated at 37C^{o} and also at 42, 5 C^{o}. This diffusion was more obvious in the tube containing *Salmonella enteritidis* rather than *Salmonella pullorum*.
At 42,5C^{o} the diffusion lines were more clear and obvious than at 37C^{o}. (FIG.1)

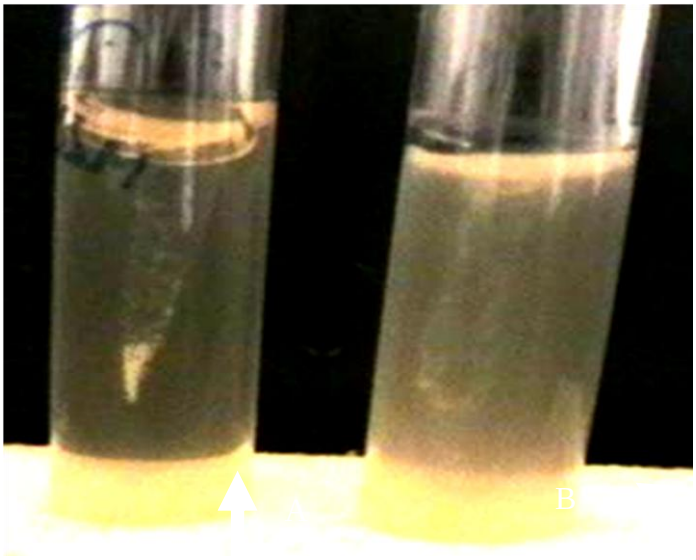

Fig. [1] Motility Test: Motile – showing a diffused growth in the medium, diffuse in tube B (*Salmonella enteritidis*), slightly diffuse in tube A (*Salmonella pullorum*)

Discussion

In this contribution, the motility of *Salmonella pullorum* was detected by using semisolid medium, raised temperature and longer duration of incubation time. It is well known that *S. entritidis* is one of the flagellated salmonellae and motile. A clear diffusion line was observed in both tubes. This diffusion expressed the motility of both cultures as far as that *Salmonella enteritidis* originally classified within the motile salmonellae, therefore, the diffusion on the tube inoculated with S. *pullorum* confirms that it is motile or possessing flagella induced gene. This finding was in agreement with Holt and chaubal (1997). Rising of the temperature from 37C^{o} to 42.5C^{o} and increase of incubation duration, both had an effect on improving the motility. This was expressed by clear diffusion line, Holt and chaubal (1997) observed similar results when using solid medium. They also observed 57% motility of *S. enteritidis* when incubated at 37 C^{O} while increase to 89% when incubation took place at 42.5C^{O}. Also 20% motility *S. pullorum* was found at 37 C^{o} and 50 % at 42.5C^{o}. From the work done by Holt and Chaubal (1997) and the result of this study no correlation between the migration capacity and the medium of isolation was

found, because (Holt and Chaubal, 1997) detected motility in solid medium and in this study motility was detected in a semisolid medium. It seems that the presence of some physiochemical factors such as the use carbohydrate substance like dextrose, temperature and increase of the duration of incubation level is necessary for expression of diffusion behavior of *S. pullorum* according to protocol of Holt and Chaubal, (1997). Since (1997) it seems that no substantive physiochemical research regarding *Salmonella pullorum* motility had been conducted (OIE, 2008) and this is attributed to two main reasons. First, *S. pullorum* is nonmotile on regular culture media (le minor, 1984) and (Snoeyenbos, G. H. , 1991.), which reduces the inclination to search intensively for flagella. Special conditions are necessary before motility can be detected. A second reason may be the status of the disease itself. *S.pullorum* seldom infects humans and thus is of little interest to those conducting human pathogenesis research, and with the declining incidence of clinical disease caused in poultry by this organism, the need for reseach on pullorum disease diminished significantly (Bullis, 1977) and (Snoeyenbos, G. H. , 1991.). however, molecular work had been conducted which aproved this hypothesis (Kliger and Grimon, 1993) and (AbdelRahman, 2011) result positive who found preciptating line to FLiC gene.

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