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CHARACTERIZATION OF THE BLOWFLY MAGGOT (*Lucilia robineau*) EXCRETION/SALIVA EXTRACT

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ABSTRACT

Maggots of the blowfly have been used for the treatment of antibiotics resistant wounds. The total protein concentration, isolation and characterization of biologically active proteins and peptides from Maggot saliva were determined by Bradford and SDS-PAGE gel electrophoresis. The total protein concentration was 430.51 mg/ml. SDS-PAGE gel electrophoresis of Maggot Excretion/Secretion revealed the presence of 5 proteins and peptides ranging from 17KDa to 121KDa. These results are obtained side by side with a proteins and peptides standard reference. The bands of proteins isolated were Beta galactosidase 121 KDa, Glycosylated bovin serum albumin 93 KDa, Ovalbumin 40KDa, Cardonic anhydrase 36 KDa, Beta lactoglobin 23 KDa and Lysozyme 17KDa.

Key words: Debridement, Maggot saliva, Gel electrophoresis, Proteins and peptides

INTRODUCTION

Blow flies (Diptera: Calliphoridae) maggots are widely distributed throughout the world and characterized as a facultative ectoparasites responsible for primary or secondary myiasis in humans and livestock [1], [2]. The adult species are considered as synanthropic, which is in close relation with human settlements. They also feed on carrion and human feces, and breeds prolifically in carrion, making them medically, veterinary, sanitationarily, and forensically important flies [1], [2].

Studies have shown that maggots of *Lucilia sericata* have been extensively used as a cleaning agent for chronic and infected wounds that are antibiotic resistant. The secretion/excretion has some beneficial effects such as debridement [3] and the removal of pathogenic bacteria [4]. In terms of debridement, maggots extretion/saliva have shown to be more effective than conventional therapies [5], while in terms of wound healing and remodelling, the outcomes of clinical trials are controversial [6]. Maggots of the green bottle blowfly have been used for the treatment of many types of wounds including venous ulcers [7], traumatic and post-surgical wounds [8], osteomyelitis [9] and burns [10].

MATERIALS AND METHODS

Estimation of total protein

Total protein concentration was estimated using the method of Bradford [11] and the standard protocols of Bradford reagent kit using BSA as a standard protein and the PHS as a blank. Concentration was measured in mg/l.

Separation of protein fractions using Gel electrophoresis

One dimensional (1D) Electrophoresis was carried out as described by Laemmli [12] using 15% gel to detect proteins and peptides with molecular weight ranging between 10-120 kDa. Maggot ES (about 15µg) and a mixture of peptides marker of 1.02–26 kDa (Sigma) were applied using Mini Protein Tetra cell instrument (Bio Rad) and power supplier. Gels (1mm thick) of 6×10 cm were used for all tests. Coomassie brilliant blue staining [13].

RESULTS

The protein concentration was 430.51mg/ml (Figure 1).

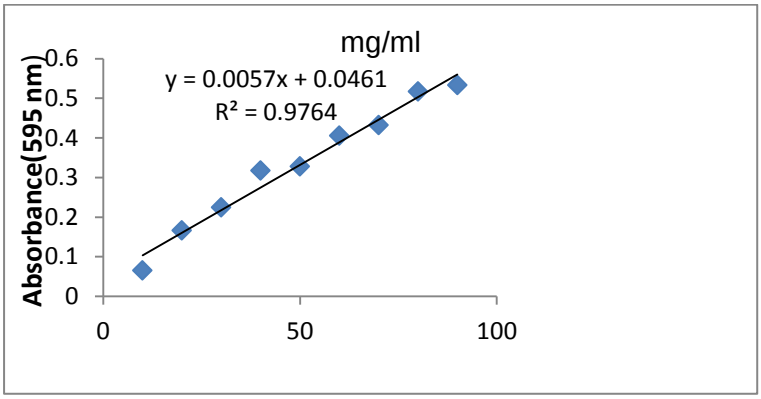
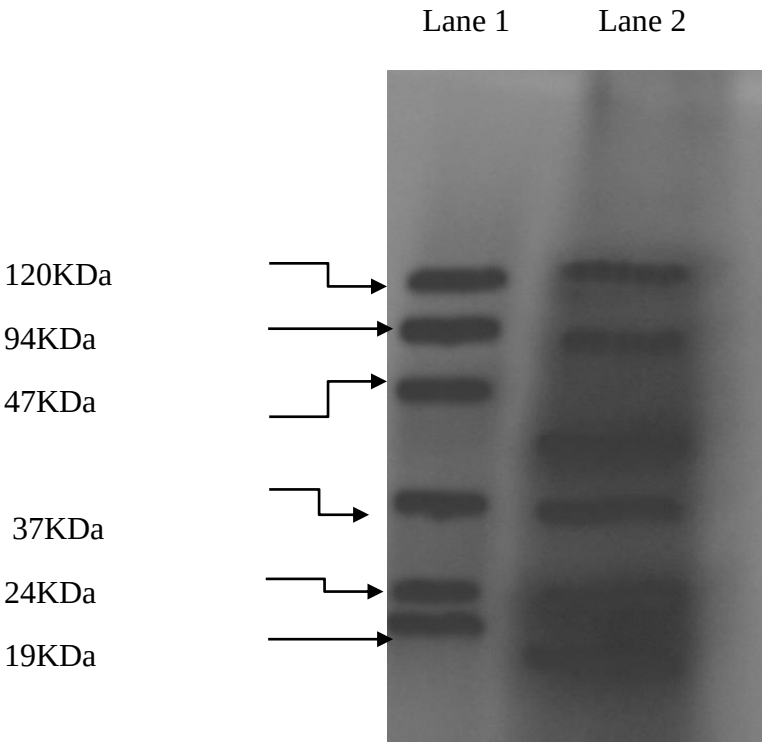


Figure 1. Total protein of maggot Excretion/Saliva standard curve.

SDS-PAGE gel electrophoresis of Maggot Excretion/Secretion with the standard makers on lane 1 and the different bands of proteins isolated from the maggot ES on lane 2 revealed the presence of 5 proteins and peptides ranging from 17KDa to 121KDa (Plate 1). These results are obtained side by side with a protein and peptide standard references. The bands of proteins isolated agrees with the bands of Beta galactosidase 121 KDa, Glycosylated bovin serum albumin 93 KDa, Ovalbumin 40KDa, Cardonic anhydrase 36 KDa, Beta lactoglobin 23 KDa and Lysozyme 17KDa (Table 1).



Line1: Pre-stained protein marker

Line2: maggot ES

Plate 1: Separation of molecular weight proteins components of the Maggot Excretion and Secretion (ES) by Tricine-SDS_PAGE

Table 1: SDS-PAGE of isolated maggot (ES) proteins and their molecular weight

Proteins isolated	Molecular weight (KDa)
Beta galactosidase	121
Glycosilated bovin serum albumin	93
Cardonic anhydrase	36
Beta lactoglobin	23
Lysozyme	17

DISCUSSION

Determination of the total protein concentration is an important tool among the procedures used in enzyme and protein characterization and purification. Physiological studies of protein expression and clinical diagnoses of altered protein levels in body fluids, is indicative of a variety of diseases. Maggots secretion/excretion protein is high compared to other animal products like leech saliva [14, 15], as studies have shown that the maggot of *Lucilia robineau* has free radical scavenging activity [16].

Proteins isolated using the SDS-PAGE of maggot ES are as follows, Beta galactosidase, glycosilated bovin serum albumin, Ovalbumin, Cardonic anhydrase, Beta lactoglobin and lysozymes in the proportions of 17 KDa and 121 KDa. β-galactosidase is a exoglycosidase which hydrolyzes the β-glycosidic bond formed between a galactose and its organic moiety. It may also cleave fucosides and arabinosides but with much lower efficiency. The interconversion of carbon dioxide and water to bicarbonate and protons (or vice versa) is caused by the enzyme Carbonic anhydrases. ß-lactoglobulin has a role in transport while Lysozyme functions primarily as an antimicrobial by breaking down the carbohydrates in bacterial cell walls, killing the bacteria.

Alaama *et al*. [17] worked on Leech saliva extract. They produced a series of bands (n= >25) with varying molecular weight ranging from 3 - 99 kDa. They detected proteins and two anticoagulants isolated by RP-HPLC with molecular weights 6.289kDa and 14.255kDa for protein1 and protein 2 respectively using trycine SDS-PAGE. Those isolated proteins inhibited the amidolytic activity of thrombin in the chromogenic substrate by 30.61% and 41.22 % respectively. The leech saliva has low molecular weight compared to the maggot saliva.

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