EVALUATION OF THE DIFFEENT BROTH MEDIA USED FOR PRODUCTION OF PASTEURELLA

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ABSTRACT

Trials to increase the yield of P.multocida was made in the present study by growing the organism in different nutrient media that vary in its composition. Dry weight of bacterial yield and absorbance were used as indices for the degree of growth. The least bacterial yield was produced by casamino acid media without enrichment or buffer: tryptic soy broth as the dry weight gains were 0.1 gm/100 ml for both media and the absorbance were 0.2. The best P.multocida yield was attained by using casamino acid media plus enrichment and buffer, in which the dry weight bacterial yield was 0.4 gm per 100 ml while the absorbance value was 0.6. By using this type of media with aeration, the dry weight was increased to 0.9 gm per 100 ml and the absorbance value was greater than 1.0. Potancy testing of the vaccine prepared from aerated cultures by mice vaccination challenge inoculation system.revealed that all four concentrations of bacterin were effective in reducing mortality caused by P.multocida challenge in the mice vaccination challenge inoculation system. Mice given 2X, X; X/2 and X/4 concentration gave acceptable protection that reached 4.36, 4.20, 3.72 and 2.250 log protective value

INTRODUCTION

Pasteurella multocida infection is one of the most serious diseases of livestock. Various techniques have been attempted to control and eliminate pasteurellosis. One of the more promising approaches is the development of immunization procedures which over long periods will protect animals exposed to P. multocida **Lu and Pakes (1981)**. **(Calnek et al,. 1997)** reported that vaccination is an ideal prevention of fowl cholera in the various avian species. Two main methods are currently used for fowl cholera vaccine production. The first method widely used in USA, the bacterin is prepared from a culture grown on agar medium, adjusting and standardizing the bacterial concentration, then the bacterial suspension after getting rid of agar shreds is transferred to a separate container for inactivation. The second method used in the European community

and some African and Asian countries (**British Veterinary Codex (1970) and Geneidy et al., 1967**) depends on the use of P.multocida organisms grown in broth cultures and inactivated with formalin. Different types of broth culture media are used by different laboratories. Bacterial yields differs greatly according to the type of media used. **Batu (1968)** found that the large scale culture of P.multocida on casein hydrolysate media yeild a culture density of $2x10^9$ organisms per ml. **Arawwewela et al., (1981)** formulated a simple and economical medium containing casein hydrolysate can sugar, yeast extract phosphate buffer, and peptic digest of blood to obtain dense cultures of P.multocida. **Rebers et al., (1989)** reported that the growth of P.multocida obtained with trypticase soy broth was equal to or better than those obtained with blood afar.

It was the aim of the present study to compare between different broth media used for cultivation of P.multocida depending on bacterial yield on dry weight basis and spectrophotometery (absorbance), to examine the antigenic composition of P. multocida grown on different broth culture media , to study the effect of aeration on bacterial yield originated from broth cultures. Finally to study the immunogenicity of a vaccine prepared under the most optimum growth conditions.

MATERIAL AND METHODS

1- Bacterial strains :

The P. multocida vaccinal strain used for prduction of fowl cholera vaccine ,Veterinary Serum and Vaccine Research Institute, Abbassia.

2- Bacterial growth studies :

Seven different types of media were used. It include nutrient broth (Oxoid, England), tryptose phosphate broth (Difco Laboratories Detroit Michigan), trypticase soy broth (Difco Laboratories, Detroit, Michigan), brain heart infusion broth (Difco), yeast extract protiose cysteine broth (YPC) (**Namoka and Murata 1961**), enriched casine hydrolysate media (Oxoid) without buffer and enriched casine hydrolysate media (Oxoid) with buffer. All these media were prepared as 1% of the volume of fresh medium, and allinoculated cultures 100 ml were incubated at 370C for 24 hours under stationary conditions.

3- Sparger Aeration:

Aeration was applied by positive pressure from an air compressor via an air filter to the media gave the best results. The reaction vessel was a 10-liter Pyrex bottle. Air bubbles was distributed finely through earthenware filter candles. Air pressure required was 8 lb per square inch using Berkefeld N filter candle. A thick stream of fine bubbles was produced. Ten liters of enriched

casamino acid broth (as gave the best results) were sterilized in the bottle which has been fitted with gas distributer (filter candles). It was incubated at 37°C, aerated for twenty four, checked for density and purity and formalin was added to a concentration of 0.25 percent.

4- Assessment of bacteria yield :

a- Dry weight estimation :

To assess yields of bacteria growth, cultures were grown for 24 hours. The cells were then sedimented by centrifugation at 6000 RPM for 20 minutes. They were then dried in three changes of cold acetone followed by 12 hours in the vacuum desiccator. Yields were expressed as grams of dry bacteria per 100 ml of medium (gm/100 ml).

b- Determination of absorbance:

2 ml sample of 24 hours culture of each medium was transferred to a cuvette to determine absorbance at 525 mu wave length (Baush and Lomb spectronic 710 digital readout). An appropriate uninoculated broth was used as a blank.

5- Sodium Dedecyl Sulphate Polyacrylamide Gel Electrophoresis : Protein profile concentration of P.multocida was determined using sodium dedecyl sulphate polyacrylamide gel electrophoresis according to the technique of **Rapp et al. (1986)** was adopted.

6- Vaccine preparation:

The technique described by **Geneidy et al. (1967)** was followed.

7- Vaccine evaluation:

The prepared vaccine was evaluated in mice using the method of **Ose and Muenster (1968).**

RESULTS AND DISCUSSION

Growth for large scale vaccine production for P.multocida, dense suspensions of bacteria are necessary. There are 2 methods of producing dense suspensions. The first is to culture on solid media in Roux flasks and harvested in formalinised normal saline. This is laborious as each flask has to be harvested separately and tested for purity. The second, and recommended, method is the use of cultures in a medium that specifically supports P.multocida **OIE . 1989).** On the other hand the yield of P.multocida in stationary liquid media is approximately constant i.e 109 organisms per ml. Trials to increase the yield of P.multocida was made in the present study by growing the organism in different nutrient media that vary in its composition. Dry weight of bacterial yield and absorbance were used as indices for the degree of growth, as shown in Table (1).

The least bacterial yield was produced by casamino acid media without enrichment or buffer: tryptic soy broth as the dry weight gains were 0.1 gm/100 ml for both media and the absorbance were 0.2. Tryptose phosphate broth, nutrient broth and brain heart infusion broth gave moderte bacterial yield where the dry weight of each media was 0.2 gm/100 ml and the absorbance was 0.3. The best P.multocida yield was attained by using casamino acid media plus enrichment with yeast extract, casitone, suchrose, magnesium sulphate,disodium hydrogen phosphate and potassium dihydrogen phosphate as buffer, in which the dry weight bacterial yield was 0.4 gm per 100 ml while the absorbance value was 0.6.

Further augmentation of bacterial growth on enriched casamino acid media was tried in the present study through aeration (as gave the best results). The purpose of aeration was to supply oxygen to the P.multocida organism for increasing its metabolism **(Bain, 1963)**. Oxygen is not very soluble in aqueous media, only 7 parts per million can go into solution at 37°C. This can be increased by raising the total pressure in culture media, or by increasing the partial pressure of oxygen in the gas mixture. Plain air in the present trial was satisfactory provided that it is effectively dispersed throughout the medium in abundant small bubbles and it is pushed through the culture vessel by positive pressure. As shown in Table (2) the dry weight of bacterial yield, by using this type of media with aeration, was increased to 0.9 gm per 100 ml and the absorbance value was greater than 1.0. Two-fold dilution of this culture media resulted in 0.6 gm per 100 ml dry weight and 0.76 absorbance.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was used to examine the protein profiles of P.multocida organism grown in different media. Results indicated that there were qualitative and quantitative differences in protein profiles. As shown in Fig. (1) and Table (4) P.multocida organisms grown in tryptose phosphate broth, enriched casamino acid media, brain heart infusion broth and YPC media expressed protein bands at the region of 80 KDa but in different quantities. Tryptose phosphate broth grown P.multocida expressed 80.8 KDa (1.5%) and 80.13 KDa (2.3%). Brain heart infusion broth expressed (2.9 %) protein band, enriched casamino acid expressed 83.52 Kda (1.5%) and 82.15 KDa (2.7%) while YPC media expressed 81.13 KDa (2.3%). Meanwhile this protein band was not expressed by P.multocida grown in non-enriched casamino acid media, tryptic soy broth and nutrient broth.P.multocida organisms grown in tryptose phosphate broth lacked protein bands 69 up to 79 KDa protein regions, meanwhile brain heart grown P.multocida lacked protein from 70-60 KDa region. These results agreed with those of **Davis et al., (1992)** who stated that the protein bands of pasteurellae varied from one media to another, also our results agreed with that obtained by **MacInnes and Rosendal (1987)** who observed minor changes in profiles of proteins from Haemophilus pleuropneumonia by altering the conditions of growth. These variations in protein profiles could be attributed to

the amount of iron in media as it was recently reported that P.multocida grown in non-depleted medium secreted a siderophase termed multicidin **(Hu et al., 1986)**.

All four concentrations of bacterin were effective in reducing mortality caused by P.multocida challenge in the mice vaccination challenge inoculation system. Mice given 2X; X; X/2 and X/4 concentration gave acceptable protection that reached 4.36, 4.20, 3.72 and 2.250 log protective value.

Table 1 : Yields of P. multocida in still cultures of different media expressed in gm/100 ml and absorbance.

Media	Dry weight (gm/100 ml)	Toxin
Tryptose phosphate broth	0.2	0.3
Casamino acid	0.1	0.2
Enriched casamino acid media with buffer	0.4	0.6
Tryptic soy broth	0.1	0.2
Nutrient broth	0.2	0.3
Brain heart infusion broth	0.2	0.3
YPC media	0.2	0.3

Table 2 : Yields of P. multocida in aerated enriched casamino acid media expressed in gm/100 ml and absorbance in undiluted and 1/2 dilution.

Media	Dry weight (gm/100 ml)		Absorbance	
	Undiluted	Dil. 1/2	Undiluted	Dil. 1/2
Enriched casamino acid media with buffer	0.9	0.6	>1.0	0.76

Table 3 : Log protection in mice group vaccinated with different concentration of bacterin prepared from aerated culture.

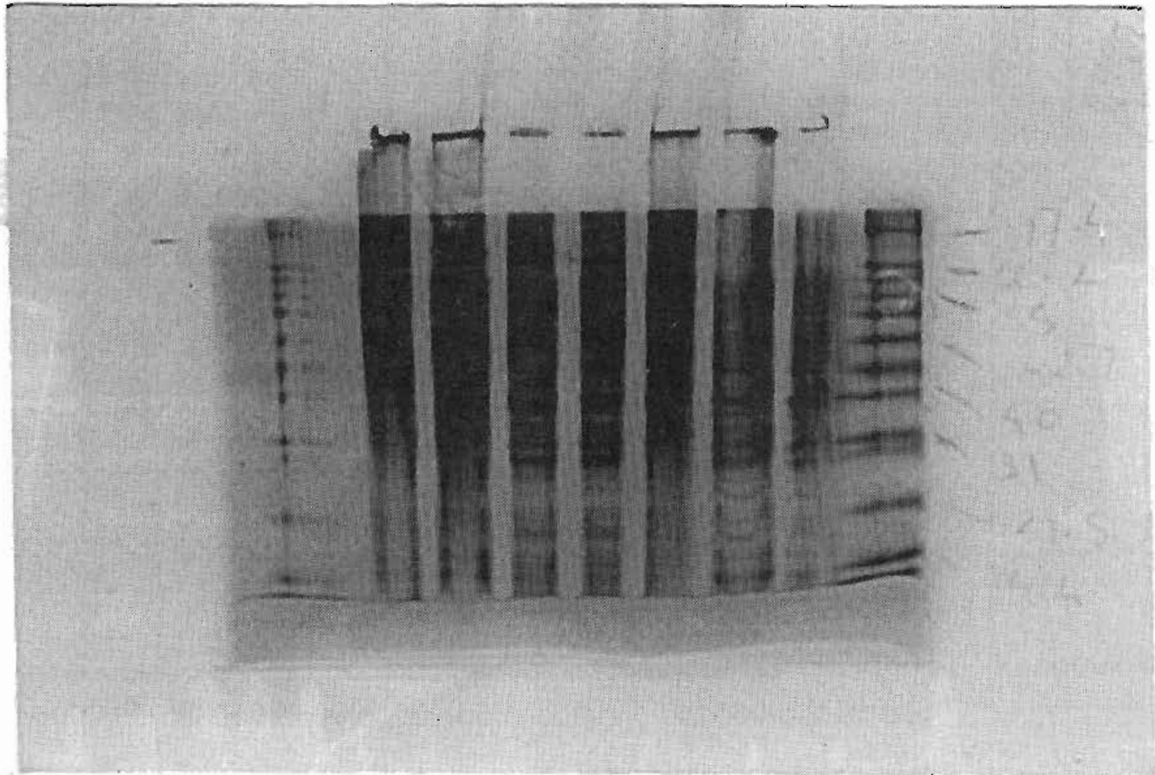
Concentration of aerated culture vaccine	LD_{50}	Log protective value
2 X	$10^{-7.01}$	4.36
X	$10^{-6.85}$	4.20
X / 2	$10^{-6.37}$	3.72
X / 4	$10^{-5.20}$	2.50
Unvaccinated control	$10^{-2.65}$	-

Table 4 : Protein profile of P. multocida in different media.

Lames	Lane 1		Lane 2		Lane 3		Lane 4		Lane 5		Lane 6		Lane 7		Lane 8	
Rows	Band%	mol.w.	Band%	mol.w.	Band%	mol.w.	Band%	mol.w.	Band%	mol.w.	Band%	mol.w.	Band%	mol.w.	Band%	mol.w.
R1															7.46	97.4
R2	2.91	80 8			1.45	83.529					2.74	82.154	2.39	81.137		
R3			1 86	77.193	2.3	80.132	1.89	79 8								
R4					1.41	75.294	2.68	76.873	1.88	74.362	0.169	75.607	0.135	75.922		
R5			0.89	69.007	0.793	69.294	1.43	89.582	2.66	70.162			0.0445	71.337	5.28	66.2
R6	4.51	66.034	4 5	62.182	0.437	64.079	0.766	64.24	1.43	66.034			0.0842	65.213		
R7	6.4	61.524	1.96	59.144	1.52	60.19	1.32	60.644	3.84	61.254	4.57	59.144	1.49	61.562		
R8	12	56.678	1.92	57.106	4.09	57.97	6.72	57.681	7.46	57.681			4.4	58.701		
R9			3.46	54.754	2.65	53.782	3.03	53.782	3.31	53.782	4.85	53.782	4.26	54.144	3.48	55
R10	5.73	52.708	3.49	52.945	0.598	51.541	2.97	53.064					1.53	51.888		
R11	2.06	49.949	4 39	51.425	1 17	51.31					1.1	51.425	2.52	49.949		
R12					1.81	49.393	1.73	49.393	1.6	49.393	2.7	49.625				
R13			5.77	48.191			1.41	48.516			0.921	47.975	1.74	48.191		
R14					0.921	47.229	1.27	47.123	2.39	47.229						
R15	1.58	46 286			0.585	45.771	0.596	45 566					2.32	45.976		
R16					0.187	44.958			1.26	45.261						
R17	0.9	43.765	6 59	44.757	1.5	43.278	1.41	43.57	1.09	43.278	0.995	43.667	2.44	43.375		
R18			1.47	42.66	1.24	42.463	1.56	42.444	1.25	42.444			2.15	42.444	7.83	42.7
R19	2.78	42.365	0.94	42.385	1.4	42.247	0.644	42.228			0.449	42.424				
R20	4.89	41.897	5.59	42.072	2.16	41.684	1.98	41.703	1.83	42.013	1.6	41.974	3.06	42.033		
R21			4.97	41.529	1 37	41.491			0.57	41.472	3.75	41.453	4.59	41.626		
R22	0.311	41 242			2.07	41.089	1.87	41.089	0.913	41.108	3.8	41.089	2.52	41.089		
R23			1 16	40.899	0	40.673										
R24					0.316	40.503	0.109	40.597	0.148	40.485	0.0841	40.56				
R25	0.268	40.372	0.601	40.354	0.496	40.204	0.513	40.13	0.263	40.056	1.28	40.167	2.54	40.056		
R26	0.378	38.447			4.11	37.322	5.34	37.322	0.959	37.322	7.26	37.322	8.44	37.322	6.23	40
R27			1.75	37.138	2.57	36.141	0.972	35.873	0.141	36.141						
R28	0.0746	34.996	2.45	34.481	1.02	34.566	0.618	34.738	0.36	34.481						
R29	3.25	32.252							0.553	32.172	2.63	34.31	0.884	34.31		
R30			0.847	32.014	3.01	31.777	2.11	31.934	0.89	31.308	1.8	31.689	0.846	31.62		
R31					5.95	29.206	0.446	30.809	1.77	29.751	3.48	29.206			8.75	31
R32	1.09	28.848	0.775	28.789			5.24	29.026			1.47	28.494	6.09	29.146		
R33	0.157	26.9	0.352	26.9	6.44	26.9	5.5	26.9	1.63	27.629	4.53	26.9	3.94	26.57		
R34					0.696	25.976	0.91	25.976	0.0571	26.137	0.0202	25.553	0.0477	25.658		
R35	0.0189	25 5	0.0409	24.223	1.34	24.123	0.797	24.123	0.519	24.777	0.00243	24.024	0.456	24.223		
R36	0.125	23.681	0.0303	22.402	0.298	22.449	0.794	22.358	0.1	22.634	0.901	22.219	1.28	22.311		
R37	0.181	21.992					0.57	20.15							7.32	21.5
R38	1 08	19.053	1.55	19.109	2.34	18.885	1.06	19.109	1.03	19.393			3.07	19.222		
R39							1.06	17.809			2.25	18.229	4.91	17.084		
R40	2 05	16.934	0.0553	16.686	2 91	16.984	3.24	16.835	1.34	16.736	1.96	16.298	1.11	15.824		
R41	0.294	14.787	4.82	14.064	0.313	14.744	0.934	14.485	0.609	14.315	4.19	14.064	3.74	14.657	11.4	14.4
R42	2.41	13.616			3.91	13.417	2.33	13.22	1.2	13.536	0.972	13.104				
R43	4.28	12.318			6.19	12.281	3.47	12.281	2.83	12.464	7.56	12.209	8.24	12.574		
R44			9.19	11.995	0.478	11.375	1.77	11.75	0.615	11.012	4.02	11.925	7.33	12.137		
R45	5.54	10.581	1.67	10.851	2.6	10.98	3.81	10.948	1.64	10.948						
Sum	65.2		73 1		74.7		75		49 1		72.8		88.6		57.8	
in Lane	100		100		100		100		100		100		100		100	

Lane 1 = Tryptose phosphate broth   Lane 2 = Casamino acid   Lane 3 = Enriched casamino acid media with buffer
Lnae 4 = Typtic soy broth   Lane 5 = Nutrient broth   Lane 6 = brain heart Infusion broth   Lane 7 YPC media

Fig. 1 : Protein profile of P. multocida grown in different media.



Lane (1) (from left) Tryptose phosphate broth.
Lane (2) (from left) casamino acid.
Lane (3) (from left) Enriched casamino acid media with buffer.
Lane (4) (from left) tryptic soy broth.
Lane (5) (from left) Nutrient broth.
Lane (6) (from left) brain heart infusion broth.
Lane (7) (from left) YPC media.
Lane (8) (from left) Molecular weight marker.

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الملخص العربى

تقييم لنمو الباستريللا ملتوسيدا على بعض الميديات السائلة لاستخدامها فى إنتاج لقاحات الباستريللا ملتوسيدا

المشتركون فى البحث

سوزان فخرى چورچى سيد محمد أبوالسعود ساهر مكين جرجس

نظراً لحاجتنا كمنتجين للقاحات الباستريللا ملتوسيدا بكميات كبيرة، فإننا نريد التعرف على أفضل الأوساط الغذائية التى تعطى أعلى درجة إنماء لميكروب الباستريللا ملتوسيدا، فقد تم فى هذا البحث دراسة نمو ميكروب الباستريللا ملتوسيدا فى الأوساط الغذائية السائلة التى تختلف فى تركيبها، وقد تم إستعمال الوزن الجاف والإدمصاص الضوئى لقياس درجة النمو، وتبين من هذه الدراسة أن أعلى درجة نمو (٤ر٠جم/ ١٠٠مللى، ٦ر٠ إدمصاص) تم الحصول عليها بإنماء ميكروب الباستريللا ملتوسيدا فى وسط حامض الكازامينو، ونتج عن تمرير الهواء فى هذا الوسط زيادة فى درجة النمو وصلت إلى ٩ر٠ جم/ ١٠٠ مللى، وأكثر من ١ فى الإدمصاص.

وعند تقييم اللقاح المحضر باستخدام تمرير الهواء باختبار تحديد المناعة فى الفئران كان لوغارتم الحماية ٢ر٤ ولم ينتج عن زيادة التركيز مرة أو مرتين زيادة معنوية فى لوغارتم الحماية (٦ر٤). وعند تخفيف التركز مرتين أو أربعة مرات للقاح المحضر باستخدام تمرير الهواء ظلت تحدث حماية مقبولة فى الفئران المحصنة.