Arch. Inst. Razi, 1968, 20, 147 - 151

STUDIES ON BACTERIOPHAGE AND METABOLIC IDENTIFICATION OF BRUCELLA STRAINS *

By :

M. Keyhani and F. Entessar

Department of Microbiology, State Razi Institute, Hessarak Iran

Brucella organisms have been divided into three species by conventional biochemical and serological methods (Huddleson, 1929; Wilson & Miles, 1932). Attempts to correlate the biochemical method of identification with serological typing have shown contradictory results (Veazie & Meyer, 1936). There are strains of **B. abortus** that are serologically indistinguishable from **B. melitensis,** and there exists strains of **B. melitensis** which can not be differentiated from **B. abortus** by conventional biochemical methods (Kabler & Maclanahan, 1936; Cruickshank, 1954; Pickett & Nelson, 1955). Thus, neither biochemical tests nor serological typing can properly classify all Brucella isolates. Using oxidative metabolism methods, Meyer & Cameron (1961) have shown that it is possible to differentiate the organisms in the genus **Brucella** by patterns on substrates of amino acids and carbohydrates. It was found (Meyer, 1961; Meyer & Morgan, 1962) that all strains that were metabolically classified as **B. abortus** were susceptible to Brucella bacteriophage, type abortus, strain 3. Cultures which were not susceptible showed a metabolic pattern characteristic of another species.

To obtain further information about the bacteriophage and metabolic identification of Brucella strains, a total of 148 strains was studied. These had been isolated from cattle, sheep, goat, man and dairy products. There were 43 strains of **B. abortus** and 105 strains of **B. melitensis** as determined by conventional biochemical and serological methods. Brucella strains were examined for their susceptibility to lysis by Brucella bacteriophage, type abortus, strain 3. Several strains whose identity was uncertain were selected and examined for their oxidative metabolic activity on amino acids and carbohydrates. The oxidative metabolism tests were generously performed by Dr. M. E. Meyer, University of California, Davis.

Materials and Methods

Brucella bacteriophage was obtained from Dr. Meyer and propagated on **B. abortus** S. 19. A dilution of 10^6 phage particles per ml was added to a culture containing approximately 10^4 cells per ml in semi-solid trypticase soy broth (containing 0.7% agar) at 44 C. The medium was gently mixed and poured over agar plates. After incubation at 37 C. for 36 hours, the phage was harvested in peptone

water and left for 48 hours at 4 C. The bacterial lysate was centrifuged at 3000 RPM for 15 minutes to deposit the agar and cells. The supernatant was filtered through a Millipore filter (type HA, 0.45 pore size). The cell free filtrate was stored at 5 C. or lyophilized. Experiments in this laboratory had shown that a high titer was obtained by this method and there was no loss in phage titer by filtration. The lytic activity of Brucella bacteriophage was increased from titer of 10^{-8} to 10^{-14} by consecutive passage in trypticase soy broth and solid culture of sensitive **B. abortus** S. 19. The assessment of susceptibility to lysis by Brucella bacteriophage was performed as described by Morgan et al (1960). The phage titer was determined by the agar layer method (Adams, 1959 and McDuff et al, 1961). A double agar layer method was also used to determine susceptibility of Brucella strains to lysis by bacteriophage.

Results and Discussion

The 43 smooth **B. abortus** strains tested were lysed by bacteriophage at routine test dilution (RTD) and 10,000 x RTD. The 71 **B. melitensis** strains tested were not lysed by bacteriophage at either dilution. This confirms the results obtained by Meyer (1961) and Meyer & Morgan (1962). However, by using a double agar layer method, several strains of **B. melitensis** were lysed by Brucella bacteriophage at routine test dilution (RTD) and 10,000 x RTD. Small dark plaques were observed. This demonstrates that the double agar layer method is more sensitive in detecting lysis by bacteriophage. Table I shows that these strains of Brucella were examined with oxidative metabolism tests and displayed the metabolic patterns of **B. melitensis.**

Table I. Oxidative rate (Q02N) on amino acids and carbohydrates of 6 **B. melitensis** strains that have shown susceptibility to bacteriophage by using double agar layer method.

Strain No.	Substrates											
	Carbohydrates								Amino acids			
	D-Alanine	L-Alanine	L-Asparagine	L-Glutamic acid	DL-Ornithine	DL-Citrulline	L-Lysine	L-Arginine	L-Arabinose	D-Galactose	D-Ribose	I-Erythritol
1—731	190	123	157	115	50	12	20	33	33	36	23	143
2—796	364	199	285	267	26	16	16	29	60	110	41	300
3—779	244	153	175	194	19	0	0	0	60	59	0	223
4—729	288	278	287	230	62	33	20	33	43	13	40	205
5—780	201	222	194	158	63	25	10	16	51	61	18	195
7—801	209	303	124	249	14	0	13	37	47	80	112	183

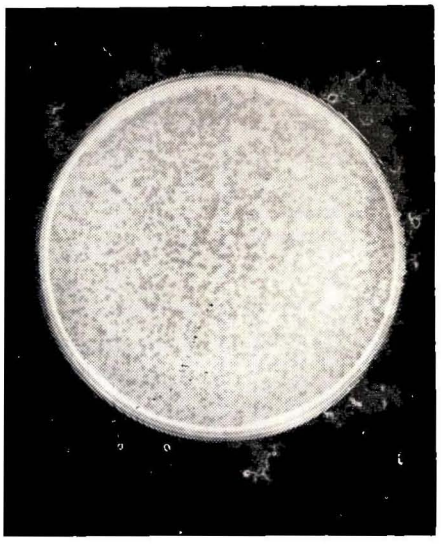

Fig. 1. The effect of Brucella bacteriophage at RTD on the Br. abortus.

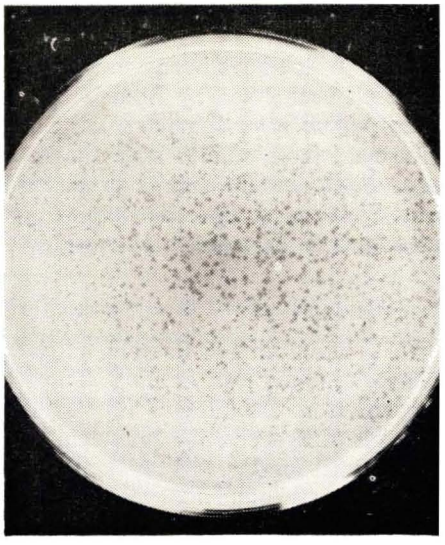

Fig. 2. The effect of Brucella bacteriophage at RTD on Br. melitensis.

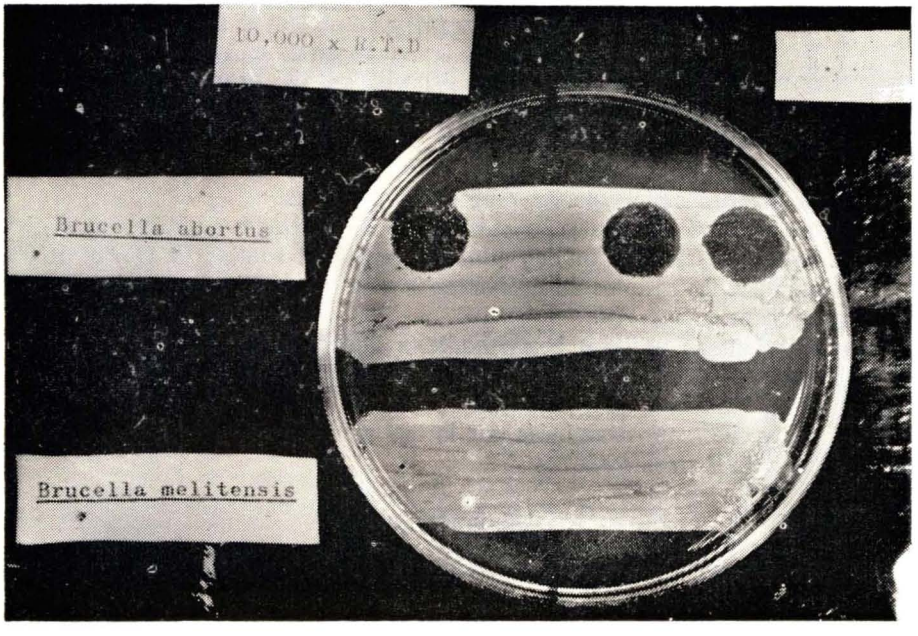

Fig. 3. The effect of Brucella bacteriophage at RTD and 10,000 X RTD on the Br. abortus and Br. melitensis.

From these and other studies it can be concluded that the use of Brucella bacteriophage is an important laboratory procedure for identifying Brucella species. Isolates which have been shown to be **B. abortus** by oxidative metabolic procedures have all been lysed by Brucella bacteriophage, type abortus, strain 3. Conversely, those which have shown patterns of **B. melitensis** have not been lysed by phage. However, these studies have shown that with a more sensitive method of double agar layer, certain strains of **B. melitensis** are lysed. This can be important in identification of Brucella species and may suggest antigenic or other differences in strains of B. melitensis showing varying susceptibility to phage.

Summary

A total of 148 Brucella cultures was examined for lysis by Brucella bacteriophage, type abortus, strain 3. The lytic activity of Brucella bacteriophage was increased from the titer of 10^{-8} to 10^{-14} by consecutive passage in fluid and solid cultures of **Brucella abortus** strain 19. This bacteriophage was used in phage typing of Brucella strains.

It was found that all smooth cultures of **B. abortus** examined were lysed by the Brucella bacteriophage used at routine test dilution (RTD) and 10,000 x RTD. Cultures of B. melitensis were not lysed at either dilution. However, by using a double agar layer method, several strains of **B. melitensis** were lysed by Brucella bacteriophage which appeared as small dark plaques. These strains were examined with oxidative metabolic tests and displayed the metabolic pattern of **B. melitensis.**

RESUME

Les nombres de 148 cultures de Brucella ont été examinés pour lyser avec phage de Brucella, type abortus, souche 3. L'activité lytique de bactériophage a été augmenté selon le titre 10^{-8} a 10^{-14} par passage consécutif sur les milieux liquides et solides de culture de B. abortus souche 19. Ce bactériophage est employé pour déterminer le type des espèces de Brucellas.

On a observé que toutes les cultures de **B. abortus** examinées ont été lysées par bactériophage en utilisant les dilutions pour test routine (RTD) et 10,000 x RTD, tandis que les cultures de **B. melitensis** n'ont pas été lysées par les deux dilutions mentionées.

Cependant en utilisant la méthode de double gélose, plusieurs souches de **B. melitensis** ont été lysées par Brucella bactériophage en presentant des petites plaques noires.

Ces souches sont examinées par le test métabolisme oxydatif qui montrent les caracteres de **B. melitensis.**

Acknowledgment

The authors wish to express their appreciation to Dr. Mever of University of California for performing the oxidative metabolism tests and Dr. Nicoletti of F.A.O. of the United Nations for reading the manuscripts. They also wish to thank Dr. Ardalan, Dr. Amjadi and Dr. Ghazarian of Razi Institute for their technical support.

REFERENCES

(1) Adams, M. H. : Bacteriophages. Interscience Publishers, Inc., New York. (1959).

(2) Cruickshank, J. C. : Observations on Brucella species based on the examination of 800 strains, J. Hyg. 52, (1954) : 105.

(3) Huddleson, I. F. : The differentiation of species in the genus **Brucella**, Mich. State Univ. Agr. Expt. Sta. Tech. Bull. (1929) : 100.

(4) Kabler, P., and MacLanahan, M. : A differential study of forty Brucella strains isolated in Minnesota. J. Infectious Diseases, 58, (1936) : 293-298.

(5) McDuff, C. R. **et al.** : Characteristics of Brucellaphage, J. Bact., 83, (1962) : 324-329.

(6) Meyer, M. E. : Metabolic characterization of the genus **Brucella.** III. Oxidative metabolism of strains that show anamalous characteristics by conventional determinative methods, J. Bact., 82, (1961) : 401-410.

(7) Meyer, M. E. and Cameron, H. S. : Metabolic characterization of the genus **Brucella**, J. Bact., 82, (1961) : 387-395.

(8) Morgan, W. J. B. **et al.** : Brucella bacteriophage. Nature 188, (1960) : 74-75.

(9) Meyer, E. M. and Morgan, W. J. B. : Metabolic characterization of Brucella strains that show conflicting identity by biochemical and serological methods. Bull. Wld. Hlth. Org., 26, (1962) : 823-824.

(10) Pickett, M. J., and Nelson, E. L. : Speciation in the genus **Brucella.** J. Bact., 69, (1955) : 333-336.

(11) Veazie, L. and Meyer, K. F. : The serologic classification of the Brucella group, J. Infec. Dis. 58, (1936) : 280.

(12) Wilson, G. S. and Miles, A. A. : The serological differentiation of sooth strains of the Brucella group, Brit. J. Exper. Path. 13, (1932) : 1.