

قسم المراقبة الصحية على الأغذية
كلية الطب البيطرى - جامعة أسيوط
رئيس القسم : د. ا. د / على يوسف لطفى

تقييم عدد من المنابت البكتريولوجية لعد وتصنيف البكتريا المحبة للبرودة من اللبن

توليف البسيونى ، فوزى أبو الخير ، نجاح محمد

تم جمع عدد ٤ عينة من اللبن بمدينة أسيوط لعد وتصنيف البكتريا المحبة للبرودة وكذلك تقييم عد ثلاث منابت بكتريولوجية لعد ذلك النوع من الميكروبات . وقد أثبتت النتائج أن نسبت Heart infuaion agar أحسن من المنبتين Standard methods agar and Trypticase soya agar; الآخرين

فى عد الميكروبات المحبة للبرودة كما أن إضافة صيغة Crystal violet على المنابت المستخدمة تقلل من الوقت وتعتبر طريقة سريعة لعد تلك الميكروبات وكان متوسط عدد البكتريا المحبة للبرودة فى اللبن على المنابت الثلاث هى ١٣٨ × ٦١٠ ، ٣٠٣ × ٦١٠ ، ٤٦٦ × ٦١٠ على التوالى .

وأمكن عزل عترات مختلفة من الميكروبات المحبة للبرودة .

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Dept. of Food Hygiene,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. A.Y. Lotfi.

**EVALUATION OF VARIOUS PLATING MEDIA FOR ENUMERATION
OF PSYCHROTROPHIC BACTERIAL IN MILK***
(With 3 Tables)

By
T.A. EL-BASSIONY; F. ABOUL-KHIER and N.M. SAAD
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SUMMARY

40 random samples of market milk sold in Assiut City markets were collected and examined bacteriologically for enumeration and isolation of psychrotrophic bacteria using heart infusion agar, standard methods agar and trypticase soya agar.

Our study revealed that heart infusion agar is more reliable for direct enumeration of psychrotrophic bacteria if compared with standard methods agar and trypticase soya agar. Moreover it is apparent that media which contain crystal violet (2 mg/Litre) is evaluated as more rapidly in order to obtain psychrotrophic count.

The mean psychrotrophic count on heart infusion agar, standard method agar, and trypticase soya agar with crystal violet were 7.02×10^6 , 2.2×10^6 and 4.5×10^6 respectively, while without crystal violet were 13.8×10^6 , 3.5×10^6 and 4.66×10^6 respectively. *Pseudomonas* species, *Alcaligenes faecalis*, *Acinetobacter antiratus*, *Flavobacter*, coliforms, proteous species and *Serratia* species were isolated in different percentage.

INTRODUCTION

Spoilage or deterioration is one of the most important microbiological problems of milk and milk products. Psychrotrophic bacteria have been and still considered among the major problems of dairy industry. This group of microorganisms can flourish and grow well during extended periods of storage in cold temperatures (RICHTER, 1981). Moreover, they can produce a variety of off-flavorous as well as physical defects (HUMMER & BABEL, 1957 and THOMAS, 1958, 1959). Furthermore, individual members of psychrotrophic bacteria have been implicated from time to time as a causal agents of food poisoning (HOBBS, 1975).

Many selective plating media are developed and evaluated for enumeration and isolation of psychrotrophs from milk and milk products. Of these standard methods agar, heart infusion agar and trypticase soya agar (A.P.H.A., 1972 and COELHO and COELHO, 1978).

Since the psychrotrophic bacteria have their optimal growth temperature in the mesophilic range, many attempts were made to decrease the incubation time by raising the incubation temperature and using some inhibitors (BAUMANN & REINBOLD, 1963 and SMITH & WITTER, 1979). Crystal

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violet has been documented as an inhibitor for gram positive mesophilic bacteria (SMITH & WITTER, 1979).

This work was performed to evaluate three different specific media for enumeration and isolation of psychrotrophic bacteria from milk.

MATERIAL and METHODS

Collection and preparation of samples:

40 random samples of market milk sold in Assiut City, were collected from different sources.

Handling and preparation of collected samples were done according to A.P.H.A. (1972).

Enumeration of psychrotrophic bacteria:

The technique adopted is that recommended by A.P.H.A. (1972). Standard methods agar, heart infusion agar and trypticase soya agar with and without 2 mg/L. of crystal violet (SMITH & WITTER, 1979) were used. The plates containing crystal violet were incubated at 20°C for 4 days (THOMAS, 1969), while the other plates without crystal violet were incubated at 7°C for 10 days (A.P.H.A. 1972 and OLSON, 1961 & 1963).

Isolation and identification of psychrotrophs:

A significant number of colonies were inoculated onto agar slant and pure cultures were prepared for further identification according to COWAN & STEEL (1974) and BAILEY & SCOTT (1978).

RESULTS

All results obtained from the examined samples of milk are presented in Tables (1-3).

DISCUSSION

Table (1) shows the maximum, minimum and average counts of psychrotrophic bacteria recovered from the examined samples on the three media used.

The obtained findings agree to a certain extent with those reported by RANDOLPH *et al.* (1973), while lower figures were recorded by MOUSTAFA (1978). This variation may be attributed to the different media used for enumeration of psychrotrophic bacteria or the hygienic measures adopted during production of milk.

From the results obtained it is evident that psychrotrophic count recovered more frequently on heart infusion agar with and without crystal violet than did standard methods agar and trypticase soya agar. These results suggest that heart infusion agar is more reliable than standard methods agar and trypticase soya agar for direct enumeration of psychrotrophic bacteria from milk. These findings substantiate what has been reported by COELHO & COELHO (1978). Moreover, it is apparent that media which contain crystal violet (2 mg/Litre) are evaluated as more rapid in order to obtain psychrotrophic colony count.

Tables (2 & 3) show the incidence percentage of isolated psychrotrophic bacteria in milk. Such organisms played a role in deteriorating the manufactured products of milk through production of proteolytic or lipolytic enzymes during growth.

PSYCHROTROPHIC BACTERIA IN MILK

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Table (1): Statistical analytical results of psychrotrophic count/ml. in examined milk samples on different media

Media	Count				
	Maximum	Minimum	Mean	+ S.E.	
Heart infusion agar	without crystal violet	24 x10 ⁷	1.7x10 ³	13.8 x10 ⁶	7.1x10 ⁶
	with crystal violet	64 x10 ⁶	1.2x10 ⁴	7.02x10 ⁶	2.2x10 ⁶
Standard methods	without crystal violet	2.2x10 ⁷	1.2x10 ³	3.5 x10 ⁶	1.2x10 ⁶
	with crystal violet	10.6 x10 ⁶	8.6x10 ³	2.2 x10 ⁶	1.8x10 ⁶
Trypticase soya agar	without crystal violet	2.8x10 ⁷	2.2x10 ³	4.66x10 ⁶	1.3x10 ⁶
	with crystal violet	2.8x10 ⁷	1.3x10 ⁵	4.5 x10 ⁶	1.1x10 ⁶

Table (2)
Frequency distribution of isolated psychrotrophs
in examined milk samples

Isolates	No. of isolates	Percentage
Pseudomonas	29	25.21
Alcaligenes faecalis	22	19.13
Acinetobacter anitratus	5	4.34
Flavobacter	6	5.21
Coliforms	27	23.47
Proteus species	17	14.79
Serratia species	9	7.82
Total	115	

Table (3)
Frequency distribution of isolated Coliforms
in examined milk samples

Isolates	No. of isolates	Percentage
E. coli	6	22.2
Enterobacter species	12	44.4
Citrobacter species	6	22.2
Klebsiella species	3	11.1
Total	27	