*Assiut Vet. Med. J. Vol. 36 No. 72, January 1997*

Dept. of Food Hygiene,
Fac. Vet. Med., El-Mansoura Univ.
Head of Dept. Prof. Dr. A.M. El-Ashmawy.

# PREVALENCE OF PSEUDOMONADS IN FARM BULK-MILK

(With 4 Tables)

By

***A.M. AL-ASHMAWY; A.M. EL-GAMAL* and A. EL-KHALEK****

* Animal Health Resarch Institut, El-Mansour, Egypt.

(Received at 16/6/1996)

## انتشار الزوائف فى لبن المزارع المجمع

***عبده العشماوى ، أحمد الجمل ، عادل عبدالخالق***

تم الفحص البكتريولوجى لعدد ١٥٠ عينه من لبن المزارع المجمع جمعت أسبوعياً من مزارع الألبان الخاصه والحكوميه بالمنصوره – مصر وذلك لدراسة مدى انتشار ميكروب السيدوموناس باستخدام المستنبتات المختلفه واختبار كفاءة المعزولات لنشاطها الانزيمى للبروتين والدهون. وقد أسفرت النتائج عن تواجد السيدوموناس فى العينات المختبره بنسبة ٣ر٨٩٪ ومتوسط العدد الكلى ٤٠٩ x ٤١٠ ميكروب /مل باستخدام مستنبت PSM وكانت ٧ر٩٨٪ ومتوسط العدد الكلى ٢٠٢ x ٥١٠ ميكروب /مل باستخدام مستنبت PAM وكان معامل الارتباط بين المستنبتين (ر) ٦٢ر٠ كذلك أمكن عزل وتصنيف ثمانية أنواع من ميكروب السيدوموناس. وقد أظهرت الدراسه أن مع مستنبت PAM كان عدد مستعمرات مشابهات السيدوموناس أكثر من تلك عند استعمال مستنبت PSM أيضاً أوضحت النتائج عن ايجابية ١٧٧ر١٦٣ من ٢١٥ عترة من معزولات السيدوموناس لنشاط تحلل البروتين والدهون بالترتيب.

## SUMMARY

One hundred and fifty random raw milk samples were collected weekly from farm bulk-milk tank at different private and governomental dariy farms at El-Mansoura city, Egypt. The samples were bacteriologically examined for enumeration and isolation of pseudomonas organisms as well as detection of proteolytic and lipolytic activity of the isolates. Pseudomonads were detected in 89.3% milk samples with a mean count of $4.9x10^4$ cfu/ml on Pseudomonas selective medium (PSM), while the incidence was 98.7% with

a mean count of $2.2x10^5$ cfu/ml on Pseudomonas Aeromonas medium (PAM). Accepted correlation was computed between the two media (r=0.62). Eight species of pseudomonas could be isolated. PAM gave a higher frequency of false positive competitors than PSM. Out of 215 pseudomonas isolates, 177 and 163 were produced proteolytic and lipolytic activity respectively.

***Key words: Milk - Farm - Pseudomonads***

## INTRODUCTION

Pseudomonas species are ubiquitous in nature. They are the most important psychrotrophic microflora found in cold stored raw milk not only because they predominate (Muir et al., 1979), but also because many strains elaborate extracellular enzymes such as proteases and lipases which are extremely thermostable (Cogan, 1977). These enzymes are able to withstand pasteurization causing a range of flavour defects in milk and diversity of dairy products as rancid, fruity, bitter, putrid, cheesy, unclean, soapy, musty and fishy (Bassette et al., 1986). In addition slime and pigment production were reported (Walker, 1988).

*Ps.aeruginosa* has emerged as a major opportunistic pathogen accounting for approximately 11% of all nosocomial infections mainly of the lower respiratory tract, urinary tract and in surgical wounds (Botzenhart and Ruden, 1987). Moreover, the organism is responsible for several cases of mastitis and remains in the undder for a number of years (Hawell, 1972).

In Egypt serveral cases of food poisoning due to *Ps aeruginosa* have been reported which have been traced to the consumption of contaminated dairy products (Ahmed et al., 1989).

In recognition of the economic and public health significance of pseudomonas spp., this study was planned to investigate the incidence of pseudomonas spp. in farm bulk-milk using two types of media and also to study the proteolytic and lipolylic activities of the isolated pseudomonas spp.

## MATERIALS and METHODS

One hundred and fifty random raw milk samples each of approx, 250 ml were collected weekly after agitation from farm bulk-milk tanks at different private and governomental dairy farms around El-Mansoura City, El-Dakahlia province, Egypt. All the samples were immediately submitted to

the laboratory in insulated ice box and held in refrigerator until examined bacteriologically as follows:

**Enumeration and isolation of pseudomonads:**

Each sample was thoroughly mixed and serial decimal dilutions were made in sterile 0.1% peptone water. 0.1 ml aliquots were spread onto the surface of pre-dried plates of the following media (a) Pseudomonas selective agar base (PSM) supplemented by CN supp (Anon, 1990) with incubation at 30°C ± 1°C for 48h. (B) Pseudomonas Aeromonas selective agar base (PAM) (Kielwein, 1971), with incubation at 25°C ± 1°C for 3 days. The plates showed countable colonies were selected and counted.

Suspected colonies were selected from each plate, purified and maintained on tryptone soya agar slope at refrigerator for identification according to the methods of Stanier et al., (1966) and Krieg & Holt, (1984).

**Detection of proteolytic and Lipolytic activity:**

Two hundred and fifteen identified strains of the pseudomonas spp. were evaluated for their proteolyic activity using skim agar, incubated at 25°C for 3 days and for lipolytic activity using victoria blue butter fat agar, incubated at 25°C for up to 7 days (Harrigan and MacCance, 1976).

## RESULTS

The results are recorded in Tables 1,2,3 and 4.

## DISCUSSION

The results presented in Table 1 show that out of 150 samples tested, 134 (89.3%) were found to contain pseudomonas spp. when plated on PSM plates, compared to 148 (98.7%) when PAM was used. Significantly more pseudomonas spp. ($P < 0.001$) were isolated on PAM than on PSM. Many workers found that pseudomonads became increasing more dominant in the psychrotrophic flora during storage. It can be assumed that organisms isolated from refrigerated raw milk and identified as pseudomonads will become numerically the most important types of organisms in raw milk during storage at low temperature (Neill, 1974 and Muir et al., 1979).

The numbers of pseudomonads detected in the milk samples using PSM ranged from $10^2$ - $4.5x10^6$ c.f.u/ml with a mean value of $4.9x10^4$ c.f.u./ml. The highest frequency distribution (71.65%) lies within the range $10^4$ - $10^6$ c.f.u./ml. While in case of PAM the numbers of pseudomonads ranged from $10^2$ - $7.7x10^6$ with a mean value of $2.2x10^5$ c.f.u./ml. The

highest frequency distribution (70.95%) lies within the range $10^5$ - $10^7$ c.f.u./ml Tables 1&2. These findings simulate those reported by Gerlach (1989), who found that counts of pseudomonas spp. in tested raw milk samples were greater that $10^4$ cells/ml.

The correlation coefficient between pseudomonas counts PSM and PAM media was r=0.62 (P>0.001) which indicates an acceptable correlation between the two media.

The incidence of individual pseudomonas spp. isolated from farm bulk-milk samples on PSM and PAM is recorded in Table 3. It is evident from the Table that more population of *Ps.aeruginosa; Ps.fluorscens; Ps.fragi; Ps.putida and Ps cepacia* were isolated on PAM than PSM. *Pseudomonas fluorescens* was the species most frequently recovered on both PSM (67.3%) and PAM (42.2%) which substantiate what have been reported by Kroll et al., (1984) and kwan and Skura (1985).

The percentage of agreement of incidence of individual pseudomonas spp. detected by the two media was greatest for *Ps.pseudoalcaligens* (80.7%) and in decreasing order *Ps fragi* (78%); *Ps.cepcia* (71.3%); *Ps.alcaligenes* (68.&%); *Ps.maltophilia* (65.3%); *Ps.putida* (63.3%); *Ps.aeruginosa* (60%) and *Ps.fluroescens* (54%).

In general this level of agreement suggests that the selectivity of the two media for particular pseudomonas spp. may differ. PAM medium gave higher frequency of false positive competitors than PSM.

Table 4 reveals that out of 215 pseudomonas isolates, 177 (82.3%) and 163 (75.8%) possessed proteolylic and lipolytic activity respectively. All strains of *Ps.fluorescens and Ps.fragi* showed proteolytic and lipolytic activity, while *Ps cepacia* failed to produce both.

These findings are in agreement with those reported by Kwan and Shura (1985) and Reinheimer et al., (1990). However Rashed and Buday (1981) found that approximately 70% of pseudomonad isolates demonstrated both proteolytic and lipolytic activity.

In conclusion the results achieved in this study indicate that Pseudomonas selective medium PSM performs better than Pseudomonas Aeromonas medium PAM for the isolation of pseudomonas spp. from raw milk. The presence of pseudomonas species in raw milk samples with various levels of contamination is indicative of bad farm hygiene. Therefore, low temperature storage (below 4°C) together with good hygienic practices offer the most practical means of controlling pseudomonads in raw milk.

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**Tabel (1): Incidence and statistical analytical results of pseudomonads count (c.f.u. / ml ) from farm bulk-milk sample:**

| No. of samples | Pseudomonas selective medium PSM | | | | | | Peudomonas Aeromonas medium PAM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | % | Min. | Max. | Mean | +S EM | Positive samples | % | Min. | Max. | Mean | +S EM |
| 150 | 134 | 89.3 | $1 \times 10^2$ | $4.5 \times 10^6$ | $4.9 \times 10^4$ | 0.0847 | 148 | 98.7* | $1 \times 10^2$ | $7.7 \times 10^6$ | $2.2 \times 10^5$ | 0.0847 |

* Significant diference in percentage of positive samples:

Z = 4.81, P < 0.001

** Regression coefficient (r) between PSM and PAM Counts = 0.62, P<0.001.

**Table (2) : Frequency distribution of farm bulk-milk samples based on their pseudomonas count / ml.**

| Range | Frequency | | | |
|---|---|---|---|---|
| | PSM No. of samples | % | PAM No. of samples | % |
| $10^2 - 10^3$ | 7 | 5.22 | 4 | 2.70 |
| $10^3 - 10^4$ | 20 | 14.93 | 14 | 9.46 |
| $10^4 - 10^5$ | 52 | 38.81 | 25 | 16.89 |
| $10^5 - 10^6$ | 44 | 32.84 | 67 | 45.27 |
| $10^6 - 10^7$ | 11 | 8.20 | 38 | 25.68 |
| Tatal | 134 | 100 | 148 | 100.00 |

**Table (3) : Incidence of individual pseudomonas species isolated from farm bulk- milk samples.**

| Pseudomonas spp. | % of positive samples PSM | PAM | % of agreement |
|---|---|---|---|
| Ps. aeruginosa | 61.33 | 34.67 | 60 |
| Ps. fluorescens | 67.33 | 42.67 | 54 |
| Ps. fragi | 53.33 | 42.00 | 78 |
| Ps. putida | 36.67 | 21.33 | 63.3 |
| Ps. cepacia | 26.67 | 13.33 | 71.3 |
| Ps. maltophilia | 15.33 | 27.33 | 65.3 |
| Ps. alcaligenes | 24.00 | 19.33 | 68.7 |
| Ps. pseudoalcaligenes | 12.00 | 10.00 | 80.7 |

**Table (4) : Number of Pseudomonas spp. isolated from bulk-milk showing proteoyltic and lipolytic activity.**

| Pseudomonas spp. | No. of isolates tested | Number of proteolytic isolates | percentage | Number of lipolytic isolates | percentage |
|---|---|---|---|---|---|
| Ps. aeruginosa | 35 | 35 | 100.00 | 27 | 77.14 |
| Ps. fluorescens. | 35 | 35 | 100.00 | 35 | 100.00 |
| Ps. fragi | 35 | 35 | 100.00 | 35 | 100.00 |
| Ps. putida | 25 | 25 | 100.00 | 13 | 52.00 |
| Ps. cepacia | 25 | 00 | 00 | 00 | 00.00 |
| Ps. maltophilia | 20 | 16 | 80.00 | 19 | 95.00 |
| Ps. alcaligenes | 20 | 17 | 85.00 | 18 | 90.00 |
| Ps. pseudoalcaligenes | 20 | 14 | 70.00 | 16 | 80.00 |
| Total | 215 | 177 | 82.3 | 163 | 75.80 |