ENUMERATION OF HEAT- AND COLD-STRESSED *PSEUDOMONAS AERUGINOSA* UTILIZING SELECTIVE PROCEDURES

by

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STATEMENT AUTHOR

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

F. Eugene Nelson

F. EUGENE NELSON
Professor of Nutrition and
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16 December, 1976

Date

To my late husband, Gale L. Fuller, Ph.D.,
whose love and example helped me to become
a better person and scientist.

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ABSTRACT

Sublethally stressed cells of Pseudomonas aeruginosa were enumerated using several selective procedures. Exponential phase (5 h) cultures were stressed at 1 or 5 C in phosphate-buffered distilled water. Cold-stressed organisms plated on Acetamide Agar (ACE), King's Medium B with cetrimide (KMB), King's Medium B with nitrofurantoin (KMN), Trypticase Soy Agar with nitrofurantoin (TSN) and Naladixic Acid Cetrimide Agar (NAC) gave lower counts than those obtained on Plate Count Agar (PCA). The MPN Method gave some higher counts and some lower than those on PCA. KMB and NAC gave counts statistically different for cold-stressed cells at the 95% confidence level.

Stationary phase (24 h) cultures were stressed at 55 C in reconstituted milk-solids-not-fat. Counts of heat-stressed organisms on ACE, KMB, KMN, TSN, NAC and Pseudosel (PSE) and when enumerated by the MPN Method were significantly lower than counts on PCA. Only PSE affected counts of unstressed organisms, the counts being significantly lower than those on PCA.

Incubation of plates at 41 C reduced the count of heat-stressed cells from counts obtained at 35 or 37 C. No significant difference in count could be deomonstrated for cold-stressed cells.

P. aeruginosa was isolated from field lettuce. Several bacteria other than P. aeruginosa and yeasts and molds were also isolated from lettuce when using procedures selective for P. aeruginosa.

INTRODUCTION

Pseudomonas aeruginosa is currently considered one of the most frequent causative agents of hospital associated infections. *Pseudomonas* infections rarely occur in the healthy human host, but some strains can cause disease in debilitated patients or those with open wounds.

Earlier studies concentrated on hospital environments as the source of this organism. These sources included patients, hospital personnel and inanimate objects in the environment. More recent studies have also found *P. aeruginosa* in cooked and uncooked food served in the hospital, suggesting that establishment of intestinal flora could result from ingestion of contaminated food. The question is raised as to whether this bacterium occurs naturally in some foods or if contamination occurs during preparation as a result of human handling or from equipment.

Clinical laboratories utilize selective media and elevated temperature of incubation for isolation of *P. aeruginosa* from human infections. Human epidermal tissue, urinary tract, respiratory tract and blood provide good media and environments for the growth of these bacteria. Generally, no problems arise when *P. aeruginosa* is isolated from the body and subcultured using selective procedures. However, *P. aeruginosa* present in food may sustain sublethal injury during processing and distribution of the food. Stressed organisms are more demanding in their requirements for initiation of growth than are those that have not

been stressed. The use of selective procedures may further impede the growth of stressed cells.

Since food is considered a possible source of P. aeruginosa in hospital infections, the method of detection employed must be one which can enumerate cells which are injured but still viable, as well as normal (uninjured) cells.

The purpose of this study was to determine the effects of selective media and incubation temperature on the enumeration of P. aeruginosa which had been subjected to temperature stress. Subsequently, preferred methodology was applied in testing for the presence of P. aeruginosa in head lettuce from the field, since lettuce is a major horticultural crop in Arizona and usually is consumed without being subjected to adequate microbicidal treatment.

REVIEW OF LITERATURE

General Characteristics

Pseudomonas aeruginosa cells are gram-negative rods that are motile with polar monotrichous flagellation. The most important pigments produced are the blue chloroform-soluble pigment, pyocyanin, and the yellowish-green water-soluble pigment, fluorescein, (Buchanan and Gibbons, 1974). Loss of ability to produce the characteristic pyocyanin is a mutation that can cause difficulty in identification of this organism (Myrvik, Pearsall and Weiser, 1974).

P. aeruginosa is aerobic, utilizing only oxidative metabolism. The organism is not generally nutritionally fastidious and can survive or even grow in unlikely media such as disinfectant solutions. Favero, Carson, Bond and Peterson (1971) found that *P. aeruginosa* will grow at a rapid rate (increase from 10^0 to 10^7 cells/ml in 48 h) in distilled water.

P. aeruginosa is most commonly isolated from water, soil, dust, food and animal or human feces. Human feces are considered a natural habitat for this organism. Ringen and Drake (1952) found 11% of the humans examined were carriers, but Lowbury and Fox (1954) found only 3% of the population carried *P. aeruginosa* in their feces. Healthy volunteers had to ingest 10^6 cells before this organism showed up in the feces; then it disappeared six days after the dosage (Buck and Cooke, 1969). No gastrointestinal upset occurred.

Medical Importance

Nosocomial infections attributable to organisms formerly regarded as innocuous have become a far more serious problem than in the past (Lindberg, 1974). The principal bacterial pathogens now include organisms which were once considered nonpathogenic or saprophytic and which often affect individuals who have a significant underlying disease. In one study about seven out of every 1000 hospitalized patients developed an infection with P. aeruginosa, this organism accounting for about one-tenth of all nosocomial infections (Bennett, 1974). P. aeruginosa is not the cause of the largest number of serious infections, but mortality due to it appears to be the highest for any of the gram-negative organisms which cause life-threatening disease (Rogers, 1959; Fried and Vosti, 1968; Young and Armstrong, 1972). Once established, the infection is difficult to treat because of the limited number of antibacterial agents which are clinically effective.

Occurrence in the Hospital Environment

Several studies have cited hospital environments as the source of P. aeruginosa. Trust and Bartlett (1976) isolated P. aeruginosa from 53% of the ornamental aquarium plants examined and felt that aquariums in hospitals may be a source of this organism. Young and Armstrong (1972) suggested that cross-contamination, mediated by vectors such as medical equipment or hands of medical personnel, can occur between patients. Similar observations were made by Kominos, Copeland and Grosiak (1972), where cultures from the hands of nurses caring for the patients yielded the same types of P. aeruginosa that were isolated from infections. These

investigators suggested direct handling of patients by personnel was the principle mode of transmission. Kominos, Copeland, Grosiak and Postic (1972) isolated P. aeruginosa from uncooked vegetables in a hospital kitchen. Tomatoes had the highest count and frequency of occurrence. This organism also was found on the hands of workers, knives and cutting boards. Pyocine typing of clinical isolates revealed that many of the types were the same as those recovered from vegetables and kitchen sources. Other studies (Shooter, Cooke, Gaya, Kumer, Patel, Parker, Thom and France, 1969; Shooter, Cooke, Faiers, Breaden and O'Farrell, 1971) found P. aeruginosa in medicine and cooked and uncooked food and suggested that this organism became established as intestinal flora of patients when ingested. Wright, Kominos and Yee (1976) recovered P. aeruginosa from 44% of hospital salad samples. However, counts were low (1 to 10^3 colony forming units/g). As stated earlier, Buck and Cooke (1969) found that healthy persons had to ingest 10^6 organisms/ml before P. aeruginosa showed up in the feces. Although no studies on colonization in debilitated patients were cited, Wright et al. (1976) suggested vegetable salads as the reservoirs of P. aeruginosa for the colonization and infection of susceptible patients.

Few studies have been conducted on vegetables directly from the growing field. P. aeruginosa was detected in 24% of the soil samples but was found in only 0.13% of the vegetable samples from the various agricultural areas of California (Green, Schroth, Cho, Kominos and Vitanza-Jack, 1974). This indicates a low colonization rate of field vegetables and does not explain the high percentages found in vegetables at the hospital by Kominos, Copeland, Grosiak and Postic (1972).

Green et al. (1974) suggested that contamination may occur during harvest, handling, processing and transit.

Selective Procedures

The production of the pigments pyocanin and fluorescin is used in the isolation and identification of P. aeruginosa. Burton, Eagles and Campbell (1947) stated glycerol, salt mixture and acid-hydrolyzed casein containing alanine and leucine were required for the production of pyocyanin. Blackwood and Neish (1957) found glycerol and dihydroxyacetone were the only substrates tested that stimulated the formation of pyocyanin. Essential minerals for the production of this pigment were reported as K^{+}, Mg^{++}, $SO_4^{=}$, $PO_4^{\equiv}$, and Fe^{+++} (Burton, Campbell and Eagles, 1948; Frank and DeMoss, 1959). King, Ward and Raney (1954) suggested the use of King's Medium A, which utilizes Bacto peptone (Difco), glycerol and mineral salts.

Mineral requirements for the production of fluorescin have also been established as $PO_4^{\equiv}$, Mg^{++}, $SO_4^{=}$, and $Fe^{\equiv}$ (King, Campbell and Eagles, 1948). Georgia and Poe (1932) reported that the production of this pigment by P. pyocyanea (aeruginosa) is influenced by the type of peptone used. Of the commercial peptones more recently available, Proteose peptone No. 3 (Difco) is recommended and used in King's Medium B for the enhancement of fluorescin production (King et al., 1954). If only one medium can be employed, King's Medium B is the best choice (King et al., 1954).

Hood (1948) was one of the first to use cetavlon (Imperial Chemical, Ltd.) in a selective medium for the isolation of P. aeruginosa.

Lowbury and Collins (1955) decided on the use of 0.03% cetrimide, which was an improved version of cetavlon. They also adjusted the pH to 7.8 with phosphate buffer to improve growth, fluorescence and pigment production. Brown and Lowbury (1965) suggested addition of 0.03% cetrimide to King's Medium B for the selective growth of P. aeruginosa. This medium does not completely restrict the growth of Klebsiella species and Providencia. Goto and Enomoto (1970) modified this formula by reducing the cetrimide to 0.02% and adding 0.015% naladixic acid. They reported excellent growth-supporting activity and inhibition of all other bacteria tested. Lilly and Lowbury (1972) agreed that the medium containing cetrimide and naladixic acid was superior to that with cetrimide alone.

Pseudosel (BBL) is a commercial product that is a modification of Brown and Lowbury's (1965) formula. It contains 0.03% cetrimide. Pseudosel was termed as not a highly selective medium for P. aeruginosa by Lambe and Stewart (1972) but was recommended for good pigment production. Smith and Dayton (1972) found that acetamide broth used in conjunction with ultraviolet light scanning was superior to Pseudosel for the growth of P. aeruginosa from rectal swabs.

P. aeruginosa gives rapid hydrolysis and utilization of acetamide. Bühlmann, Vischer and Bruhin (1961) added 1% acetamide to Christensen's medium (1946), instead of 0.1% glucose and 2% urea, for the identification of P. aeruginosa. Hedberg (1969) utilized acetamide as the sole carbon source in an agar and broth used for the selective growth of P. aeruginosa. She reported that this medium without peptone prevents the growth of swarming Proteus. Mima polymorpha was the only reported contaminant.

Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1971) contains a tentative Most-Probable-Number (MPN) method for quantitative determination of P. aeruginosa in water samples. The presumptive medium is an asparagine enrichment broth and the confirmatory medium is acetamide broth. Formulas were described by Drake (1966). Evaluation of two MPN methods (Highsmith and Abshire, 1975) found the formula modification by Favero et al. (1971) to be superior to the Standard Method. Drake (1966) recommended incubation at 38-39 C. Favero et al. (1971) and Highsmith and Abshire (1975) used 37 C, but Standard Methods specifies 35 ± 0.5 C as the temperature of incubation.

King's Medium B containing 0.005% nitrofurantoin (1-[nitrofurfurylideneamino] - hydantoin) was reported to be at least as sensitive as cetrimide agar (Thom, Stephens, Gillespie and Alder, 1971). At a nitrofurantoin concentration of 0.02%, the colony size of some strains decreased. Burzynska and Maciezska (1974) found that P. aeruginosa plated on King's Medium B containing nitrofurantoin formed characteristics colonies and growth of Enterobacteriaceae was inhibited. Kominos, Copeland, Grosiak and Postic (1972) used 0.2% nitrofurantoin in trypticase soy broth, but Thom et al. (1971) recommended 0.01% in nutrient borth as a liquid medium.

P. aeruginosa will grow at 41 C but not at 4 C; optimum temperature for growth is 37 C (Buchanan and Gibbons, 1974). Seleen and Stark (1943) showed that good growth of P. aeruginosa occurs at 42 C and that this characteristic can help differentiate this organism from other fluorescent bacteria. Haynes (1951) confirmed this observation but used

41 ± 1 C in his study. He also stated that the ability of P. aeruginosa to grow well at 37 C and the inability of other fluorescent bacteria to grow at this temperature has served as a differentiating characteristic for many years. However, some other pseudomonads will grow well at 37 C. Drake (1966) recommended incubation at 38-39 C to inhibit the growth of saprophytic pseudomonads. Bühlmann et al. (1961) used growth at 0 and 42 C as one parameter in the identification of apyocyanogenic strains of P. aeruginosa.

Kominos, Copeland, Grosiak and Postic (1972) used selective incubation at 42 C in addition to selective media such as Mueller-Hinton agar (Difco) containing 0.03% cetrimide and Trypticase soy broth (BBL) containing 0.2% nitrofurantoin. Green et al. (1974) used acetamide broth and King's Medium B with 0.03% cetrimide in conjunction with incubation at 42 C. Wright et al. (1976) used Mueller-Hinton agar with 0.03% cetrimide and acetamide broth incubation at 42 C.

Highsmith and Abshire (1975) reported that 28% of their P. aeruginosa isolates would not grow on cetrimide agar at 42 C. Several methods for the isolation and identification of P. aeruginosa have been published (Haynes, 1951; Stanier, Palleroni and Doudoroff, 1966; Gilardi, 1971; Kantor, Kominos and Yee, 1975).

Heat Stress

Processing may subject food to conditions which impose sublethal heat stress upon the microorganisms. Lesions produced by heat may be repairable but require additional time or more nutrients than are required by unheated bacteria (Nelson, 1943). Ordal (1970) studied heat

injury in Staphylococcus aureus and Salmonella. He characterized some of the repairable lesions as damage to the cytoplasmic membrane, impairment of metabolic capabilities and degradation of ribosomal RNA. Bacteria which have been subjected to sublethal heat are more demanding in their requirements for growth than are unstressed organisms. Presence of heat-injured coliform cells in a sample may give a count on a selective medium that is only 10% of that obtained on standard plate count agar (Maxcy, 1970). The effects of selective components on heat-stressed cells have been reported by others (Nelson, 1971; Smolka, Nelson and Kelley, 1974).

Cold Stress

Cold injury has been studied, using P. fluorescens and several other bacteria (Straka and Stokes, 1959). These stressed cells showed an increase in nutritional requirements. Injured coliforms which were given a period of time to grow on trypticase soy agar before being overlaid with violet red bile agar gave better counts than when plated directly with the selective medium (Speck, Ray and Read, 1975). A correlation has been shown between loss of viability and leakage of endogenous solutes from chilled bacteria (Strange and Ness, 1963). Cold shock also is believed to entail damage to a permeability regulating system, thus allowing some substances which normally would not enter to penetrate into the cell (Strange and Postgate, 1964). Similar mechanisms may be involved in the injury and subsequent repair of bacteria stressed by heat and cold.

Chemical Stress

Chemicals which are inhibitory or selective may provide further injury to temperature-stressed bacterial cells and further delay growth. Escherichia coli injured by exposure to a quarternary ammonium compound showed inhibition of growth when selective agents were present in the medium (Scheusner, Busta and Speck, 1971). Favero et al. (1971) showed that P. aeruginosa grown in distilled water reacted differently to chemical stress than did cells grown in conventional laboratory culture media.

METHODS AND MATERIALS

Cultures Employed

Three strains of P. aeruginosa were employed, ATCC 10145, NRRL 3198 and a laboratory strain obtained from the Department of Microbiology and Medical Technology, University of Arizona. Cultures were held -20 C in litmus milk. Each strain was grown in nutrient broth (Difco Laboratories, Detroit, Michigan) at 37 C for 5 h to obtain exponential phase cells or 24 h to obtain stationary phase cells. These times were based on growth curves determined for each strain and illustrated in Figures 1, 2 and 3. Cultures were mixed by a vortex mixer for 1 min prior to dispensing for stress treatment, in order to diminish cell clumping.

Temperature Stress

Cold stress was accomplished by adding 1 ml of exponential phase culture to 99 ml prechilled (1 or 5 C) phosphate-buffered distilled water (Hausler, 1972) and holding at that temperature for 5 min, 30 min and 24 h before plating. All samples were immediately diluted in room temperature (23 to 25 C) phosphate-buffered distilled water and plated.

Heat stress was accomplished by adding 1 ml of stationary-phase broth culture to 50 ml rehydrated milk-solids-not-fat (110 g/l). A 5 ml aliquot was heated in a screw-capped test tube at 55 C in a thermostatically controlled bath for 5, 6 or 7 min after reaching 55 C, using a predetermined come-up time, and then cooled in ice water.

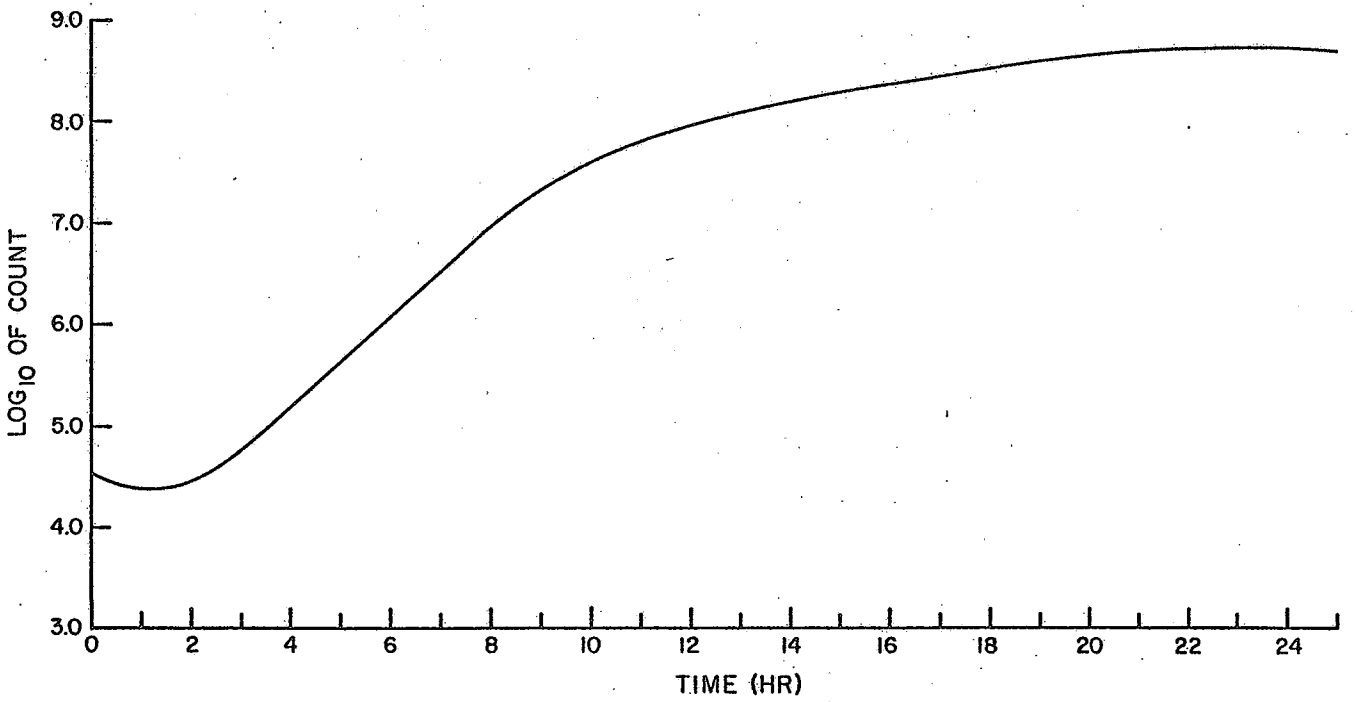

Figure 1. Growth curve of P. aeruginosa strain ATCC 10145, grown in nutrient broth and enumerated on PCA at 37 C.

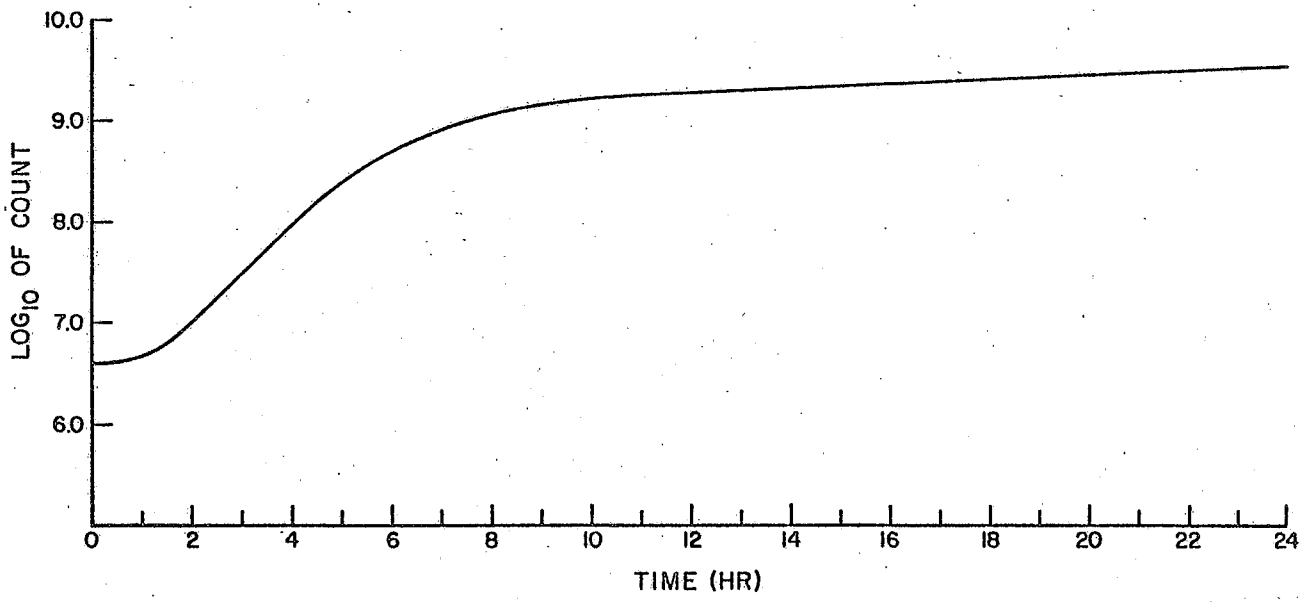

Figure 2. Growth curve of P. aeruginosa strain NRRL 3198, grown in nutrient broth and enumerated in PCA at 37 C.

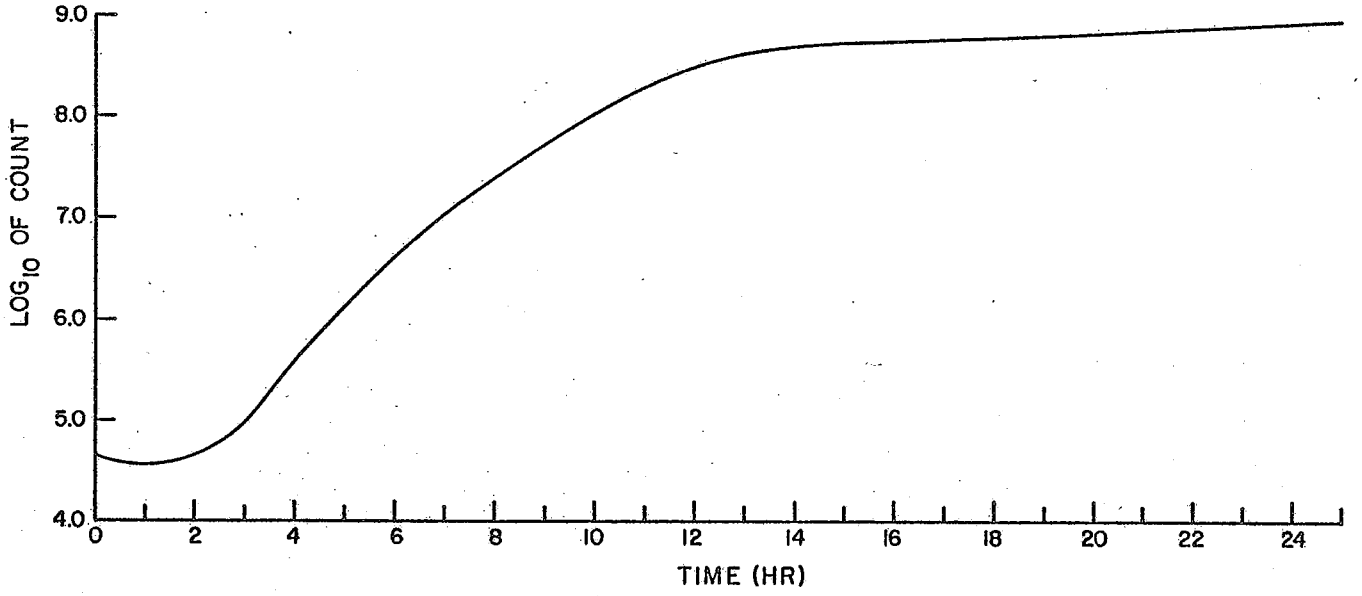

Figure 3. Growth curve of P. aeruginosa lab strain, grown in nutrient broth and enumerated in PCA at 37 C.

Unstressed cells were diluted in phosphate-buffered distilled water and plated out as the control. Platings were made in duplicate and each experiment was repeated three times. Plates were incubated at 37 C, except where otherwise indicated.

Media Employed

Standard Methods Plate Count Agar (Difco Laboratories, Detroit, Michigan) (PCA) (Hausler, 1972) and several media selective for P. aeruginosa were used. Acetamide Agar (ACE) was used by Green et al. (1974). The formula is that of Hedberg (1969), utilizing acetamide (Eastman Kodak Co., Rochester, New York) as the sole carbon source. King's Medium B (King et al., 1954) (KMB) with 0.03% cetrimide (hexadecyltrimethyl-ammonium bromide, Eastman Kodak Co., Rochester, New York) was suggested by Brown and Lowbury (1965). Naladixic acid-cetrimide Agar (NAC) of the formula of Goto and Enomoto (1970) utilizes 0.02% cetrimide and 0.005% naladixic acid (Sigma Chemical Co., St. Louis, Missouri). Trypticase soy agar plus 0.02% nitrofurantoin (N-[5-Nitro-2-furfurylidene]-1-Aminohydantoin) (Sigma Chemical Co., St. Louis, Missouri) (TSN) was used. Kominos, Copeland, Grosiak and Postic (1972) used a level of 0.2% nitrofurantoin in trypticase soy broth, but Thom et al. (1971) used a level of less than 0.02% in solid medium. King's Medium B was used with 0.02% nitrofurantoin (KMN) (Thom et al., 1971) or with 0.2% nitrofurantoin (modification of Green et al., 1974).

Pseudosel (BBL, Cockeysville, Maryland) (PSE) utilizes 0.03% cetrimide as the selective agent. The MPN Standard Method (American Public Health Association, 1971) uses an asparagine enrichment broth

(ASP) as a presumptive test and acetamide broth (ACE-B) for confirmation. Five tubes of asparagine broth were inoculated with each appropriate dilution and incubated at 35 ± 0.5 C for 48 h. All positive presumptive tubes were confirmed by placing one loopful of growth into acetamide broth and incubating for 48 h at 35 C.

Isolation of P. aeruginosa from Field Head Lettuce

In the fall of 1976, several varieties of Iceberg lettuce (Lactuca sativa L.) were collected randomly from fields in the areas of Willcox and Marana, Arizona. Samples (3-4/field/day) were placed in a plastic bag and held at room temperature. The lettuce was not vacuum cooled to avoid possible chill damage to the microorganisms. Samples were cultured within 24 h after cutting unless otherwise stated. Some heads were aseptically cut in half and one-half cultured immediately. The other half was placed in a plastic bag and chilled at 1 C for 48 h and then examined for microorganisms.

Outer leaves and 6.4 mm of the exposed end of the butt were aseptically removed. Trimmed heads were weighed (range:500-800 g), then sterile phosphate-buffered distilled water was added to attain 1:5 dilution following blending. Samples were blended for 1 min on low speed and 1 min on high speed in a Waring commercial blender. Appropriate dilutions were placed into asparagine broth tubes, and plates were poured with PCA, NAC, KMB and KMN (0.02% and 0.2% nitrofurantoin). Tubes were incubated at 35 and 42 C and confirmed in acetamide broth. Plates were incubated at 38 and 42 C. Cultures were confirmed by picking 1 to 3 colonies from a plate and identifying by fluorescence, gram-negative

reaction, growth at 42 C in Trypticase soy broth, oxidase reaction and glucose utilization. OXI/FERM Tubes (Roche Diagnostics, Nutley, New Jersey) were also used in the identification of selected cultures.

Statistical Analysis

Statistical analysis of variance was performed on the temperature-stress experiments using computer program A 3.1 of Sokal and Rohlf (1969). This program was modified to conform to the CDC 6400 System of the University of Arizona Computer System.

The "log ratio" of the count was calculated by converting the counts of duplicate plates to $\log_{10}$, averaging these logarithms from the individual plates and then calculating the difference between the $\log_{10}$ values of the control and selective procedure. As an example,

($\log_{10}$ average of count on KMB, 37 C, 6 min, 55 C) -

($\log_{10}$ average of count on PCA, 37 C, 6 min, 55 C) = "log ratio"

If the count on KMB was lower than that on PCA, the log ratio value would be a negative value; if the count was higher the value would be positive.

Statistical analysis of variance was then performed on these values for the three trials of each experiment. The values shown on the graphs are the average log ratio for the three trials. When analyzing the count on PCA at 35 or 37 C, which was the control value, the log ratio is equal to zero. However, when the effect of selective incubation is analyzed, the value for PCA does not always equal zero. As an

example,

($\log_{10}$ average of count on PCA, 41 C, 30 min, 5 C) -

($\log_{10}$ average of count on PCA, 35 C, 30 min, 5 C) = -.3312

Estimated counts and confidence levels for MPN were determined from Standard Methods (American Public Health Association, 1971) tables. Confidence levels of 95% are illustrated by dotted lines in the graphs.

RESULTS AND DISCUSSION

Temperature Stress Studies

Selective Media

Table 1 gives the average number of colonies per ml that grew in a representative trial where cold stress at 1 C was used. Selective media had no apparent effect on enumeration of unstressed cells. Cells stressed at 1 C gave lower counts on ACE, KMB, TSN and NAC than when plated on nonselective PCA, although the differences were relatively small in some comparisons.

Statistical analysis of data from three trials showed that unstressed P. aeruginosa gave the same count on all media, so it is not illustrated. KMB and NAC significantly reduced the count of bacteria chilled for 5 min, 30 min and 24 h (Figure 4). Counts on TSN and ACE were not significantly different from those on PCA. The media containing cetrimide significantly lowered the number of exponential phase cells enumerated after stress.

Data from one representative trial of cells stressed at 5 C are shown in Table 2. This treatment corresponds to possible stress encountered in foods under less rigorous conditions of refrigeration. Unchilled organisms showed little difference in counts between the several media. ACE, KMB, TSN and NAC reduced the counts of stressed organisms in varying degrees. Holding for 24 h at 5 C markedly reduced counts obtained with all media, including nonselective PCA.

TABLE 1. INFLUENCE OF PLATING MEDIUM ON COUNTS OF P. AERUGINOSA (ATCC 10145) STRESSED AT 1 C FOR 0 MIN, 5 MIN, 30 MIN AND 24 H

	Colony Forming Units/ml			
Medium	0 min	5 min	30 min	24 h
PCA	820,000	650,000	360,000	3,900
ACE	760,000	510,000	290,000	990
KMB	760,000	1,300	540	90
TSN	880,000	390,000	380,000	2,200
NAC	590,000	24,000	3,400	120

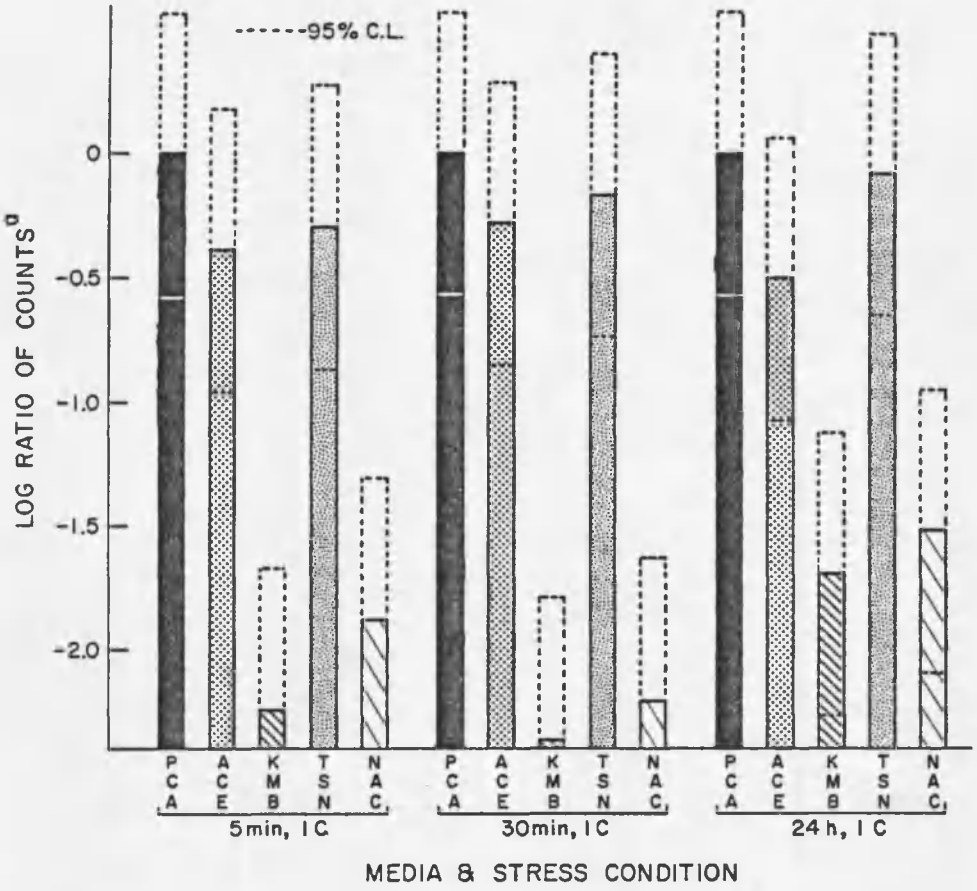

Figure 4. Comparison of enumeration of cold-stressed (1 C) exponential phase cells of P. aeruginosa (ATCC 10145).

[a]Log of count on selective medium - log of count on PCA.

TABLE 2. INFLUENCE OF PLATING MEDIUM ON COUNTS OF P. AERUGINOSA (LAB STRAIN) STRESSED AT 5 C FOR 0 MIN, 5 MIN, 30 MIN AND 24 H

	Colony Forming Units/ml			
	0 min	5 min	30 min	24 h
PCA	1,500,000	1,100,000	1,100,000	7,500
ACE	1,400,000	560,000	500,000	5,800
KMB	1,400,000	130,000	37,000	1,500
TSN	1,500,000	680,000	550,000	7,600
NAC	1,100,000	380,000	50,000	2,200

Statistical analysis of data from three trials (Figure 5) shows that KMB and NAC gave significantly lower counts than PCA when exponential phase cells are held for 5 min and, particularly, 30 min at 5 C. Counts on TSN and ACE were not significantly different from those on PCA. After 24 h of stress the total cells enumerated decreased markedly, but no significant difference between counts on PCA and selective media was found. Table 3 gives the average count of cells from a representative trial of cold stress at 5 C, utilizing pour plate and MPN Standard Method. The MPN Method gave some population estimates that were higher and some that were lower than counts obtained on PCA.

Statistical analysis of data from three trials (Figure 6) shows KMB as the only selective medium which gave a significantly lower count than that obtained on PCA. The confidence limits for the MPN Method are so large that no significant difference from counts on PCA can be demonstrated.

The present data indicate that cold shock at 1 and 5 C occurs in P. aeruginosa in the exponential phase. This effect reduces counts on selective media after short periods of time and reduces counts on all media tested after holding the cells for 24 h at 1 or 5 C. The sensitivity of exponential phase cells of P. aeruginosa agrees with work done on cold shocked cells of Clostridium perfringens (Traci and Duncan, 1974) and Aerobacter aerogenes (Strange and Ness, 1963). Work done by Gorrill and McNeil (1960) also showed that actively growing cultures of P. pyocyanea (aeruginosa) were injured when diluted into liquids at temperatures less than 18 C. Exponential cells of P. aeruginosa may also be

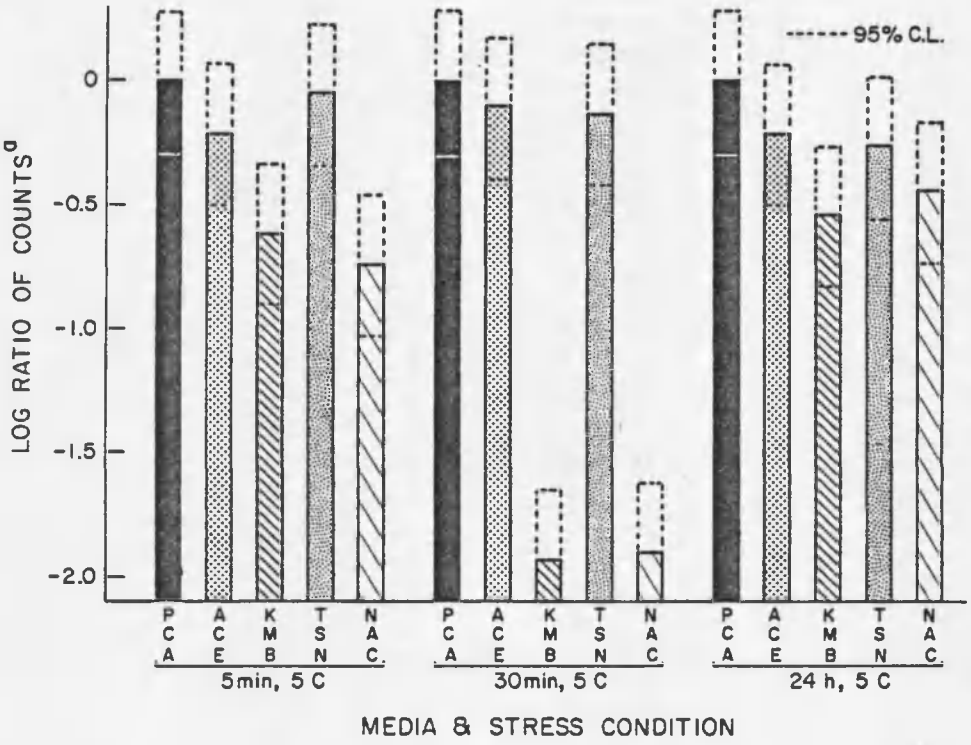

Figure 5. Comparison of enumeration of cold-stressed (5 C) exponential phase cells of P. aeruginosa (ATCC 10145).

[a]Log of count on selective medium - log of count on PCA.

TABLE 3. INFLUENCE OF INCUBATION TEMPERATURE AND SELECTIVE MEDIUM ON COUNTS OF P. AERUGINOSA (ATCC 10145) STRESSED AT 5 C FOR 0 MIN, 30 MIN AND 24 H

Medium	Colony Forming Units/ml		
	0 min	30 min	24 h
		35 C	
PCA	740,000	100,000	8,200
KMB	620,000	3,300	3,800
KMN	680,000	67,000	7,100
TSN	770,000	120,000	9,600
MPN	130,000	110,000	3,300
		41 C	
PCA	710,000	47,000	6,300
KMB	400,000	4,500	3,600
KMN	660,000	88,000	6,100
TSN	640,000	110,000	5,700

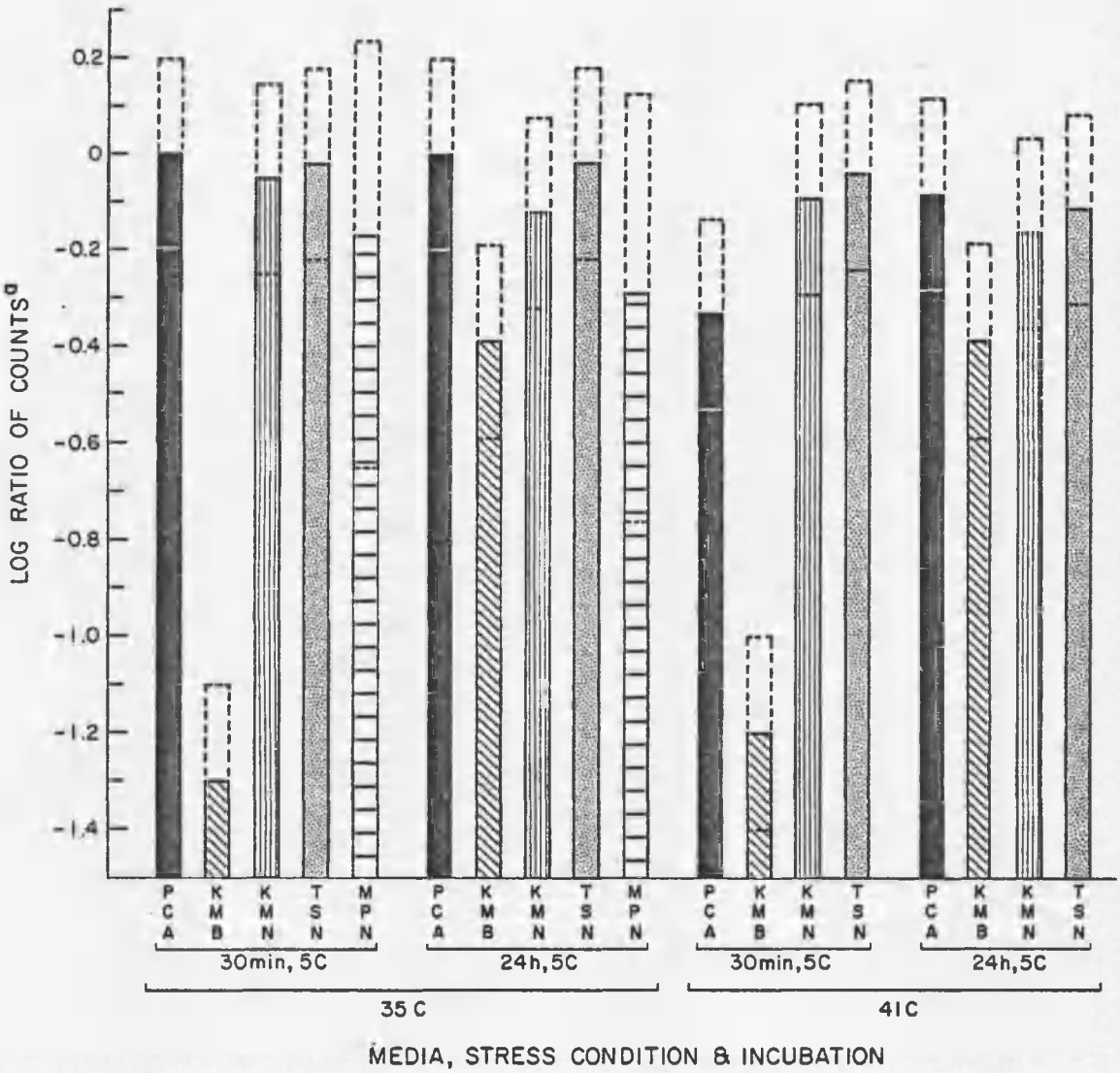

Figure 6. Comparison of enumeration of cold-stressed (5 C) exponential phase cells of P. aeruginosa (ATCC 10145) incubated at 35 and 41 C.

[a]Log of count on selective medium - log of count on PCA.

more susceptible to damage by stresses other than temperature. Skaliy and Eagon (1972) found that P. aeruginosa cells harvested during the exponential phase of growth were more susceptible to the effect of drying than were those of older cultures.

The conditions under which the bacterial cells are grown prior to stressing may also play an important role in how they react. This has been demonstrated in P. aeruginosa, when a mesophilic strain grown at 10 C showed no measurable decrease in viability after rapid chilling to -2 C, but the same strain grown at 30 C prior to chilling was susceptible to cold shock and decreased in viability (Farrell and Rose, 1968).

Selective agents have been reported to affect young cells and cold-injured cells of other bacteria. When a 4 h E. coli culture was enumerated on Violet Red Bile agar which had been boiled rather than autoclaved, the productivity was significantly higher than when autoclaved medium was used, but no difference could be shown when a 16 h culture was used (Jensen and Hausler, 1975). Jackson (1974) showed that Staphylococcus aureus that had been stored at 5 C became sensitive to mannitol-salt agar which contains selective agents.

Cold-stress experiments to determine the effect of selective media were carried out on exponential phase cells of three different strains of P. aeruginosa. Each strain reacted in a similar manner, suggesting that cold injury is not strain specific.

Preliminary experiments were carried out to examine the effect of cold shock on stationary phase cells of P. aeruginosa. No significant effect of selective media was found and data are not reported here.

During thermal processing of food, microorganisms can undergo heat-stress damage which can cause the cells to take longer to initiate growth or be more sensitive to their growing conditions. Stationary phase cells were grown to study the effect of selective media on heat-stressed cells of P. aeruginosa. When stationary phase cells were heated at 55 C, the colony count on PCA was reduced by about 98% in 5 min and by about 99% in 7 min.

Data from a representative trial (Table 4) using a laboratory strain of heat-stressed cells indicate use of PSE and KMB media results in counts considerably below those on PCA. PSE gave particularly low counts, even with unstressed control cells. Several plates employing selective media had to be incubated for 72 h to obtain countable colonies. This phenomenon may be explained by the results of Speck et al. (1975) and Ordal (1970). These investigators suggested that stressed cells require an opportunity to repair themselves before growing on selective media.

Statistical analysis of data from three trials (Figure 7) showed that PSE gave a significantly lower count of both heat-stressed and unstressed P. aeruginosa cells. PSE and KMB are very similar in composition, but the counts on KMB, while lower, were not significantly different from the counts on PCA. Counts on TSN and ACE were not significantly different from PCA.

Table 5 shows data from a representative trial involving enumeration of heat-stressed P. aeruginosa (NRRL 3198) utilizing selective media, including the MPN Standard Method. The estimated MPN for

TABLE 4. INFLUENCE OF PLATING MEDIUM ON *P. AERUGINOSA* (LAB STRAIN) STRESSED AT 55 C FOR 0 MIN, 5 MIN AND 7 MIN

	Colony Forming Units/ml		
Medium	0 min	5 min	7 min
PCA	16,000,000	270,000[a]	40,000[b]
ACE	14,000,000	370,000[c]	21,000
KMB	6,700,000	140,000	7,500
TSN	19,000,000	330,000	45,000
PSE	80,000[c]	12,000[c]	2,100[c]

[a]98% kill.

[b]99.75% kill.

[c]Counted after 72 h, due to smallness of colonies.

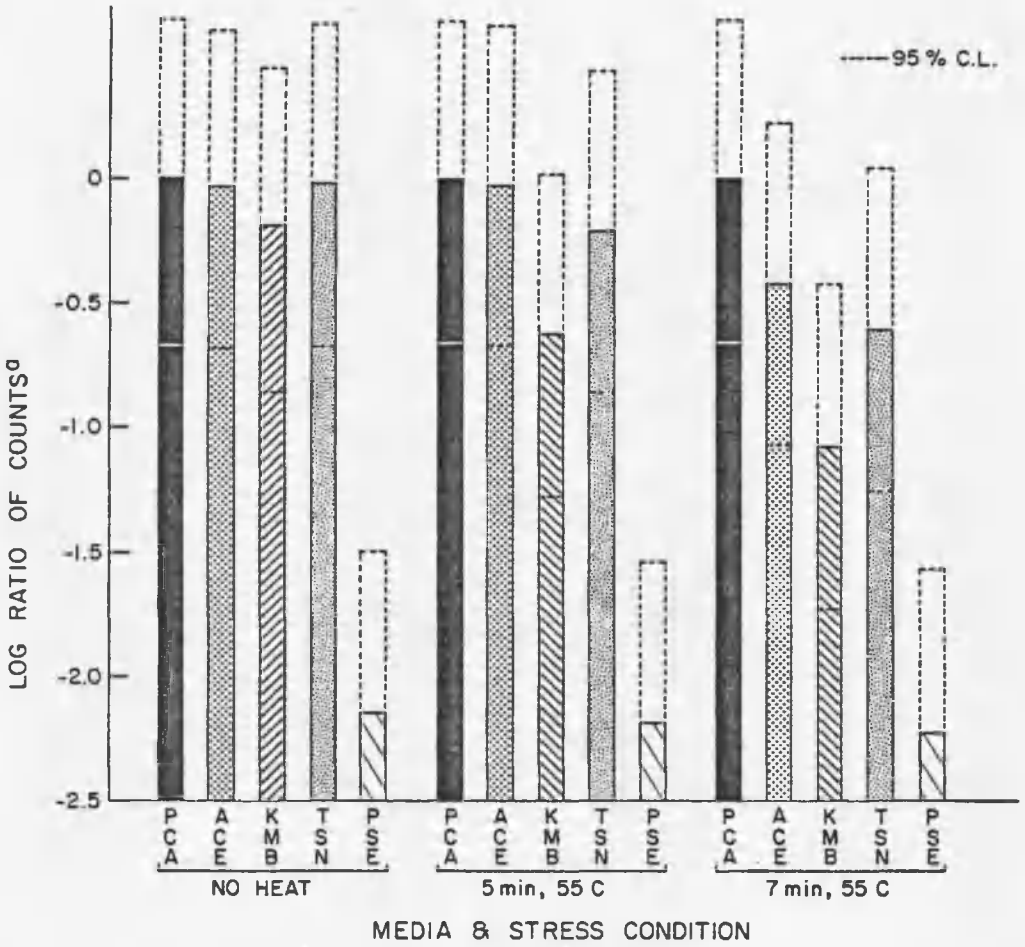

Figure 7. Comparison of enumeration of heat-stressed (55 C) stationary phase cells of P. aeruginosa (lab strain).

[a]Log of count on selective medium - log of count on PCA.

TABLE 5. INFLUENCE OF SELECTIVE MEDIUM ON COUNTS OF P. AERUGINOSA (NRRL 3198) STRESSED AT 55 C FOR 0 MIN AND 6 MIN

	Colony Forming Units/ ml	
Medium	0 min	6 min
PCA	10,000,000	550,000
KMB	10,000,000	41,000
KMN	8,000,000	39,000
TSN	8,000,000	61,000
MPN	33,000,000	23,000

unstressed cells was higher than the count obtained on PCA, but the MPN estimate for heat-stressed cells was lower than the count on PCA.

Statistical analysis of three trials (Figure 8) showed no significant difference in the counts of unstressed cells. The count of heat-stressed cells was significantly decreased by the use of KMB, KMN, TSN and MPN Standard Method when compared with counts obtained on PCA.

Counts by several selective media were essentially the same for unstressed cells. For all three strains of P. aeruginosa tested, the selective media, including MPN Method, lowered the number of colonies that grew from heat-stressed cultures. This sensitivity has been demonstrated for other bacteria. Nelson (1943) found that several heat-stressed bacteria were more difficult to enumerate on selective media than were unstressed bacteria. Maxcy (1970) showed counts to be comparable for uninjured cells of E. coli on PCA and VRB, but counts of heat-injured cells were much lower on the selective medium (VRB). These examples agree with the findings of this study.

Selective Temperature

In order to determine the effect of incubation temperature on the growth of cold-stressed (5 C) cells of P. aeruginosa, agar plates were incubated at 35 and 41 C. Table 3 shows that incubation at 41 C lowered the count of cells stressed at 5 C for 24 h, although the differences are relatively small. Statistical analysis of data (Figure 6) showed no significant difference in counts between plates incubated at 35 and those incubated at 41 C.

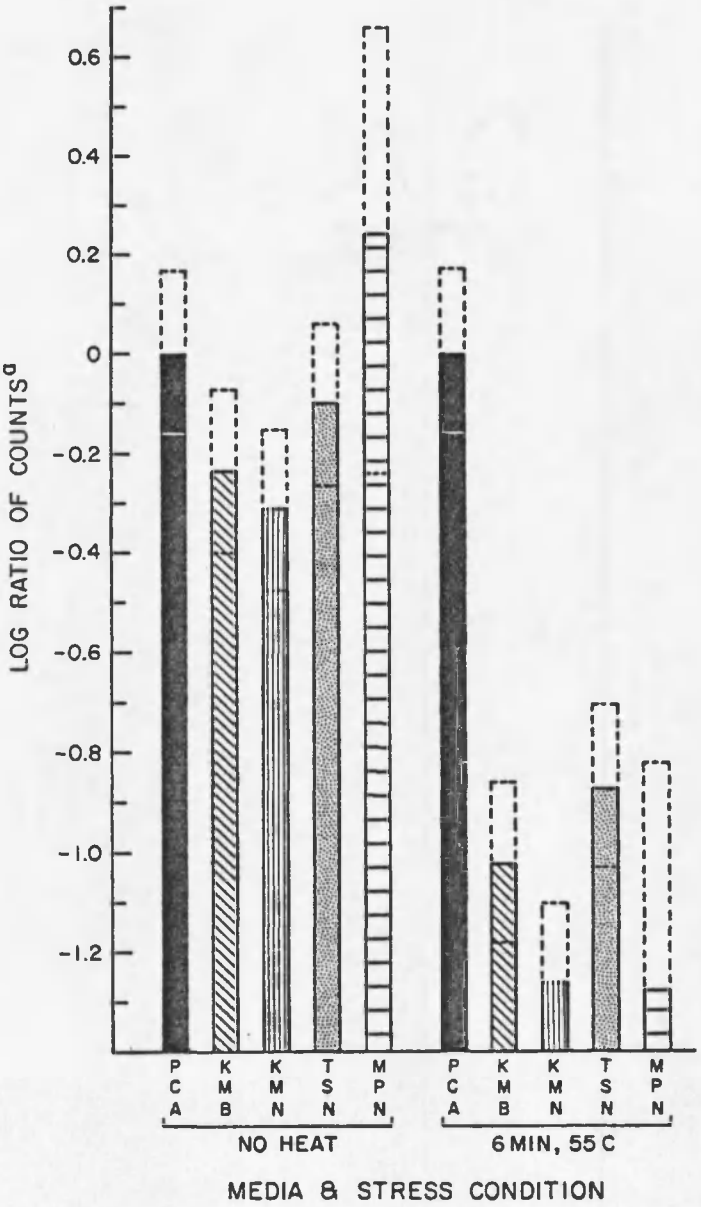

Figure 8. Comparison of enumeration of heat-stressed (55 C) stationary phase cells of P. aeruginosa (NRRL 3198).

[a]Log of count on selective medium - log of count on PCA.

Although no significant decrease in count was shown for cold-stressed (5 C) cells incubated at 41 C, it is important to note that not all strains of P. aeruginosa will grow on the selective cetrimide agar at the incubation temperature of 42 C (Highsmith and Abshire, 1975).

The effect of incubation at 41 C on the growth of cells stressed at 1 C was not examined.

Table 6 presents data from a representative trial of a heat-stress experiment when incubation at both 37 and 41 C was used. Incubation at 41 C reduced the count of heat-stressed cells on all media tested.

Statistical analysis of data from three trials showed that selective media and selective temperature had no effect on counts of unstressed cells and are not illustrated. Counts on KMB and TSN for cells stressed for 5 min and incubated at 37 C showed no significant difference from PCA, but the count was significantly lower for ACE (Figure 9). Cells stressed for 7 min at 55 C were counted in significantly lower number when plated on ACE, KMB and TSN and incubated at 37 C. When stressed cells were plated with the three selective media and incubated at 41 C, counts were significantly lower than those on PCA.

These results indicate that cold-stressed and heat-stressed cells of P. aeruginosa react differently to the elevated temperature of incubation. No significant difference could be shown for cold-stressed cells, but incubation at 41 C definitely decreased the count of heat-stressed cells.

A similar experiment was conducted using P. aeruginosa ATCC 10145 to be more certain that these reactions are characteristic of the

TABLE 6. INFLUENCE OF INCUBATION TEMPERATURE AND PLATING MEDIUM ON COUNTS OF P. AERUGINOSA (LAB STRAIN) STRESSED AT 55 C FOR 0 MIN, 5 MIN AND 7 MIN

Medium	Colony Forming Units/ml		
	0 min	5 min	7 min
		37 C Incubation	
PCA	10,000,000	1,700,000[a]	23,000[b]
ACE	13,000,000	100,000[c]	2.200[c]
KMB	6,000,000	190,000	2,200
TSN	10,000,000	240,000	2,200
		41 C Incubation	
PCA	9,500,000	270,000	1,200
ACE	14,000,000	15,000[c]	620[c]
KMB	7,000,000	25,000	1,300
TSN	13,000,000	7,000	1,100

[a]83% kill.

[b]99.8% kill.

[c]Counted after 72 h, due to smallness of colonies.

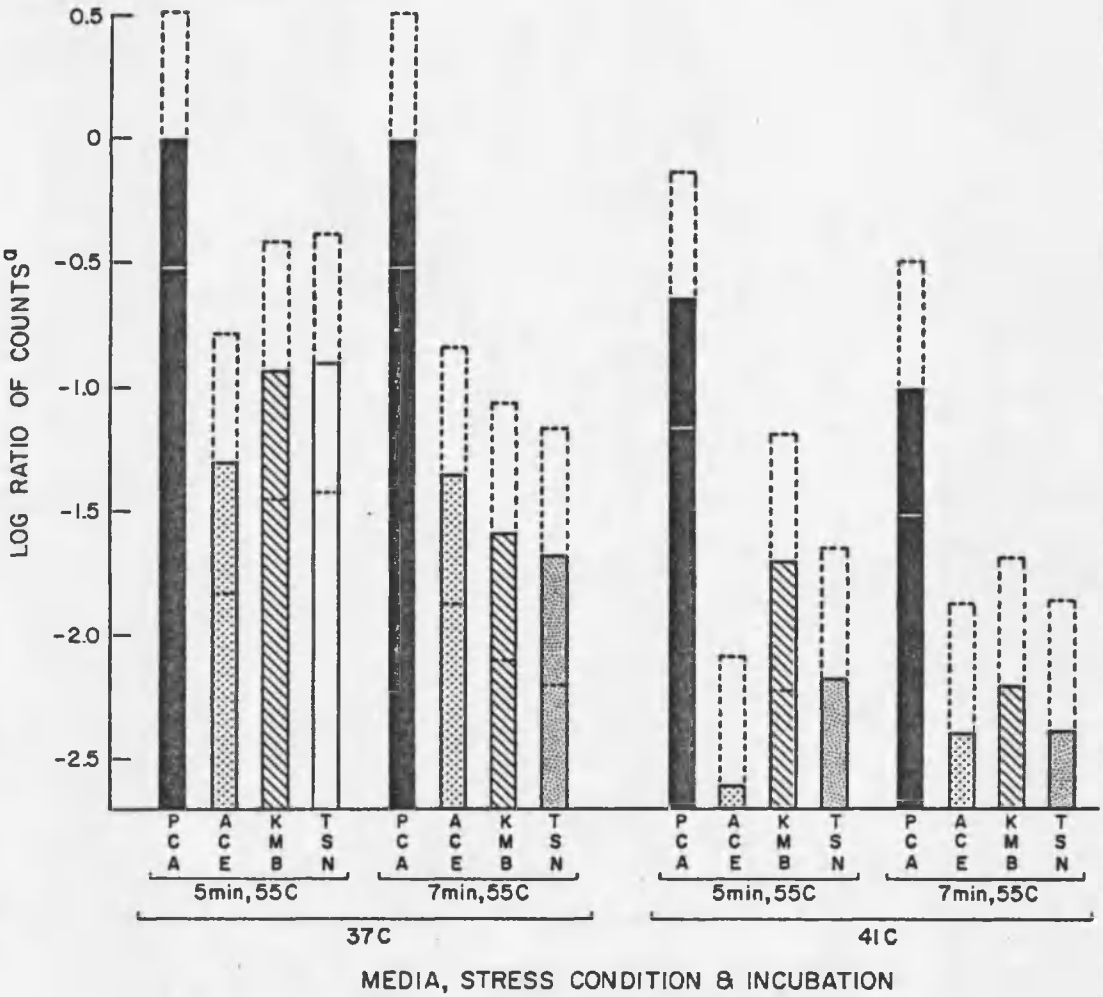

Figure 9. Comparison of enumeration of heat-stressed (55 C) stationary phase cells of P. aeruginosa (lab strain) incubated at 37 C and 41 C.

[a]Log of count on selective medium - log of count on PCA.

species. Data from a representative trial (Table 7) suggest that incubation at 22 or 41 C lowers the count of heat-stressed cells when compared with 35 C. NAC gave counts lower than those on PCA for both unstressed and stressed cells.

Statistical analysis of data from three trials showed that incubation temperatures used had no effect on enumeration of unstressed cells and these results are not shown. Counts on NAC at incubation temperatures of 35 and 41 C were not significantly different from those on PCA, but there was a significant difference with incubation at 22 C (Figure 10). The opposite was found with TSN. There was no significant difference between counts on PCA and TSN when incubated at 22 C; but when incubated at 35 or 41 C, TSN gave counts significantly lower than those on PCA.

Acetamide agar yielded counts of stressed P. aeruginosa which were comparable in most cases with results using PCA. Colonies were very small and sometimes needed an additional 24 h of incubation to develop to a size that was readily differentiated and countable. Surface colonies showed good fluorescence and development of yellow-green pigment. The medium would be difficult to use in a routine way, particularly when laboratory personnel might not be well trained and experienced.

The three media containing cetrimide (PSE, KMB and NAC) lowered the number of colonies formed by temperature-stressed cells. PSE also gave poor results for unstressed cells of P. aeruginosa. Good fluorescence is produced by growth on these three media. NAC seems to be more selective than KMB for the growth of P. aeruginosa and is probably the better one to use for isolation of P. aeruginosa from food products.

TABLE 7. INFLUENCE OF INCUBATION TEMPERATURE AND PLATING MEDIUM ON COUNTS OF P. AERUGINOSA (ATCC 10145) STRESSED AT 55 C FOR 0 AND 6 MIN

Medium	Colony Forming Units/ml	
	0 min	6 min
	22 C Incubation	
PCA	53,000,000	2,200,000
NAC	31,000,000	620,000
TSN	52,000,000	1,800,000
	35 C Incubation	
PCA	53,000,000	9,800,000
NAC	30,000,000	2,400,000
TSN	50,000,000	3,200,000
	41 C Incubation	
PCA	55,000,000	3,000,000
NAC	45,000,000	1,000,000
TSN	50,000,000	330,000

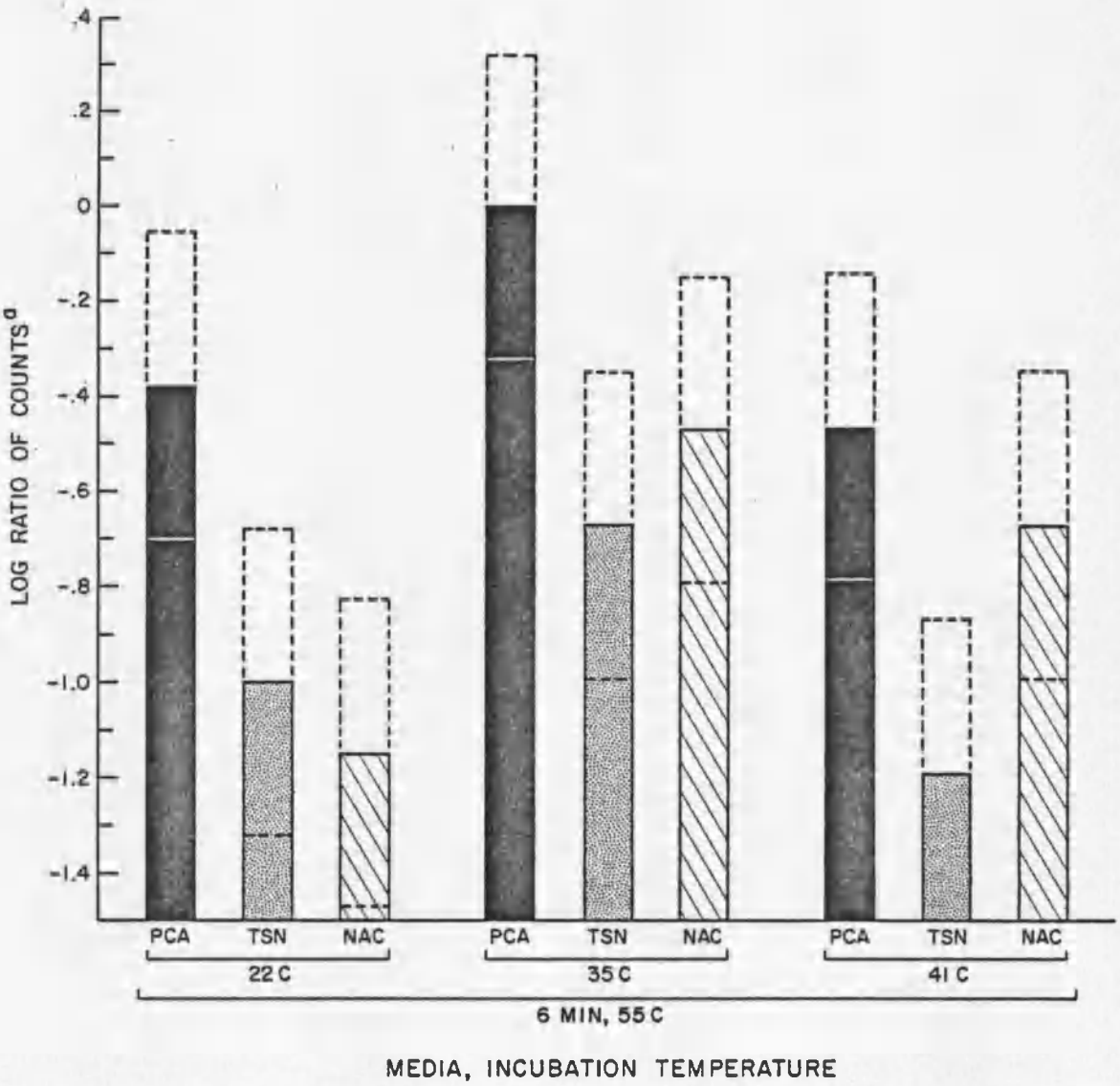

Figure 10. Comparison of enumeration of heat-stressed (55 C) stationary phase cells of P. aeruginosa (ATCC 10145) incubated at 22, 35 and 41 C.

[a]Log of count on selective medium - log of count on PCA.

Of the selective media studied, TSN gave numbers of countable colonies closest to those obtained on PCA. This might be attributed to the trypticase soy agar base used in TSN, which is high in nutritional components that may be required by injured cells for their repair and reproduction. Colonies are larger and more easily differentiated on the TSN medium than on the other selective media. The selective properties of TSN have not been sufficiently studied to be able to recommend it for use in the isolation of P. aeruginosa from food products.

KMN (0.02%) produces colonies of P. aeruginosa which have a greater degree of fluorescence than those grown on TSN. KMN gave comparable counts for cells stressed by cold, but gave significantly lower counts for heat-stressed cells.

The MPN Method of enumeration reduced the estimate number of heat-stressed cells of P. aeruginosa but not those that were cold-stressed.

Field Lettuce Studies

Head lettuce samples F1, F2, F3 and F4 were held at room temperature for 24 h prior to plating. Dilutions of 1:5, 1:50 and 1:500 were plated with KMB, KMN and also placed in one tube of ASP, then incubated at 35 C. Colonies were too numerous to count on dilution 2×10^{-3} for plates poured with KMB and KMN at 35 C. Several colonies were isolated but none was identified as P. aeruginosa. The 2×10^{-3} dilution was positive in ASP/ACE-B for all four samples but the broth was not streaked onto a plate for isolation of colonies and confirmation of type.

Lettuce samples F5 and F6 were held for 6 days at room temperature prior to sampling. No colonies grew on KMB and KMN plates poured with dilutions 2×10^{-5} to 2×10^{-7} that were incubated at 38 or 42 C. However, ASP/ACE-B tubes incubated at 35 C gave a positive reaction with the 2×10^{-6} dilution. A loopful of the ACE-B was streaked onto PSE and incubated at 35 C for 48 h. No P. aeruginosa was isolated.

Samples F7, F8, F9 and F10 were held at room temperature for 24 h after picking, then plated with KMB and KMN using a dilution series of 2×10^{-1} to 2×10^{-5}. One set was incubated at 38 C, the second at 42 C. Three tubes of ASP were inoculated with dilutions 2×10^{-3} to 2×10^{-5} and incubated at 35 C. Results are shown in Table 8. Plates incubated at 42 C gave lower colony counts than those incubated at 38 C, but this difference was not analyzed statistically. Three to five fluorescent and non-fluorescent colonies were picked from plates from each sample and identification procedures for P. aeruginosa were performed. Two colonies isolated from lettuce sample F8 on KMB and one colony for F10 in ACE-B were confirmed as P. aeruginosa. A summary of colonies isolated from head lettuce is given in Table 9. Forty-six colonies were picked as presumptively P. aeruginosa, but only eight were confirmed. The potential pathogenicity of these strains isolated from lettuce was not studied.

Samples F11, F12 and F13 represent one-half of an unchilled head of lettuce plated within 5 h after being cut from the field. Dilutions 2×10^{-3} to 2×10^{-6} of these samples were each placed in 5 tubes ASP, one set incubated at the regular 35 C and a second set at 42 C. Dilutions 2×10^{-1} to 2×10^{-5} were plated with KMB, KMN (0.02) and KMN (0.2) then incubated at 38 and 42 C. Samples were plated with PCA and

TABLE 8. EFFECT OF INCUBATION TEMPERATURE ON ESTIMATED NUMBERS OF MICROORGANISMS THAT GREW UNDER CONDITIONS SELECTIVE FOR P. AERUGINOSA

Lettuce Sample	Medium	Count /ml When Using Incubation Temperature of: 35 C	38 C	42 C
F7	KMB		1,400,000	6,600
F7	KMN (0.02)		1,000,000	8,000
F7	MPN	230		
F8	KMB		540,000	640
F8	KMN (0.02)		500,000	400
F8	MPN	23,000		
F9	KMB		280,000	420
F9	KMN (0.02)		360,000	1,060
F9	MPN	2,300		
F10	KMB		5,400,000	1,500
F10	KMN (0.02)		3,600,000	1,800
F10	MPN	230,000		

TABLE 9. P. AERUGINOSA ISOLATED FROM FIELD LETTUCE

Sample	Temp.	Media	Dilution	Method of Confirm.
F8	42	KMB	10^1 A	OXI/FERM
F8	42	KMB	10^1 B	OXI/FERM
F10	35	ACE-B	10^5	OXI/FERM
F12	35	ASP/ACE-B	10^3	OXI/FERM
F11R[a]	35	ASP/ACE-B	10^3	OXI/FERM

[a]Held at 1 C for 48 h prior to sampling.

incubated at 32 C to obtain a total plate count. The other half head of lettuce (samples 11R, 12R and 13R) was placed in a plastic bag and held at 1 C for 48 h prior to sampling. The same methods were used as outlined for the unchilled half-head samples.

Results for both halves of one head of lettuce (samples 12, 12R) are given in Table 10. Lower estimated counts were obtained when the selective temperature of 42 C was utilized. Chilling at 1 C for 48 h also lowered the colony count, except on KMN (0.02) using 38 C incubation. Yeasts naturally present on lettuce were isolated from KMN (0.02) at 38 C and ASP/ACE-B when incubated at 35 or 42 C. Pure culture study of one isolated yeast showed that it was fluorescent, grew on PCA at 42 C, gave positive reactions in ASP/ACE-B at 35 and 42 C, oxidase-negative reaction, and no growth on KMN (0.2) at 35 C. This yeast reacts the same as P. aeruginosa when using the MPN Method and could possibly give false positives. Molds were isolated from lettuce sample F11; they grew on KMN (0.02 and 0.2) at 42 C in 48 h.

Identification of microorganisms other than P. aeruginosa was not completed. Since yeasts, molds and bacteria other than P. aeruginosa grew when using both selective media and selective temperature of 42 C, it was not possible to just count the colonies and report as P. aeruginosa. It was not feasible to pick every colony that grows on a plate and determine if it was P. aeruginosa or not in order to obtain a routine count. Neither was it practical to streak each positive tube of ACE.

Thom et al. (1971) recommended the use of King's Medium B with 0.005% nitrofurantoin incubated at 37 C for the isolation of P. aeruginosa from fecal samples; but in the present study, lettuce samples

TABLE 10. COMPARISON OF ESTIMATED COUNTS OBTAINED UNDER CONDITIONS SELECTIVE FOR *P. AERUGINOSA* FROM UNCHILLED AND CHILLED LETTUCE

Media & Temp	Unchilled (F12)	1 C, 48 h (F12R)
	Colonies/ml	
PCA, 32 C	78,000	50,000
38 C		
NAC	1,600	60
KMN (0.02)	11,000	12,000
KMN (0.2)	140	< 50
MPN, 35 C	50,000	< 5,000
42 C		
NAC	< 50	< 50
KMN (0.02)	180	< 50
KMN (0.2)	< 50	< 50
MPN, 35 C	< 50	< 50

plated with KMN (0.02%) and incubated at 38 C produced a number of colonies which were not P. aeruginosa. KMN (0.02%) plates which were incubated at 42 C gave fewer false colonies; but mold and bacteria other than P. aeruginosa were also found when using this cultural procedure.

KMN (0.2%) was not studied with heat- and cold-stressed pure cultures of P. aeruginosa. Other experiments using this formulation could not be found in the literature; but in the present study, the feeling was that a more selective medium was needed for isolation from field lettuce which contains many pseudomonads which are not normally found in medical samples. Selective media developed for medical applications do not inhibit several yeasts, molds and bacteria that are found in lettuce.

The use of a selective medium containing a higher concentration of nutrients (TSA) or incubation at a temperature lower than 42 C may obtain counts of temperature-stressed cells of P. aeruginosa closer to those found on nonselective PCA, but these changes decrease the selectivity of the procedure for isolation from mixed populations of bacteria. Development of a medium containing a high concentration of nutrients while still maintaining selectivity may be the answer.

The use of spread plate method rather than pour plate may be a superior way of examining samples, since surface colonies are larger, fluorescence is easier to detect, colony morphology is more distinctive and the oxidase reaction can be readily checked. The colonies of P. aeruginosa would be easier to differentiate from any other contaminating colonies.

More work needs to be done in order to establish more selective procedures of isolating P. aeruginosa from a mixed population in food; but procedures must be those which do not inhibit the growth of stressed cells of P. aeruginosa to a significant degree.

CONCLUSION

Stationary phase cells of P. aeruginosa stressed by heat or exponential phase cells stressed by cold were definitely more sensitive to selective conditions employed for enumeration. Media containing cetrimide, naladixic acid, nitrofurantoin or acetamide can reduce the number of stressed cells which will form countable colonies on these selective media, while having no comparable effect on unstressed control cells. Cells stressed by sublethal heat form fewer colonies when plates were incubated at 41 C than at the usual 35 or 37 C. Incubation at 41 C had no significant effect on cells stressed at 5 C.

Until better procedures are developed for the selective isolation of P. aeruginosa from food products, the possible reduction in count obtained when using selective procedures for the enumeration of these bacteria should be taken into account when interpreting results on samples which may have been subjected to temperature stress.

P. aeruginosa can be isolated in low numbers from Arizona lettuce taken from the field and protected from contamination. Whether the strains found possess any degree of virulence for humans was not determined.

LITERATURE CITED

American Public Health Association. 1971. Standard Methods for the examination of water and wastewater. (13th Ed.). American Public Helath Association, Inc., New York. 375 pp.

Bennett, J. V. 1974. Nosocomial infections due to Pseudomonas. In Symposium on Pseudomonas aeruginosa. J. Inf. Diseases, Vol. 130 (suppl.), p. S4-S7.

Blackwood, A. C. and A. C. Neish. 1957. Pyocyanine formation from labelled substrates by Pseudomonas aeruginosa. Can. J. Microbiol. 3:165-169.

Brown, V. I. and E. J. Lowbury. 1965. Use of an improved cetrimide agar medium and other culture methods for Pseudomonas aeruginosa. J. Clin. Pathol. 18:752-756.

Buck, A. C. and E. M. Cooke. 1969. The fate of ingested Pseudomonas aeruginosa in normal persons. J. Med. Microbiol. 2:521-525.

Buchanan, R. E. and N. E. Gibbons (Eds.). 1974. Bergey's Manual of Determinative Bacteriology. (8th Ed.). Williams and Wilkins Co., Baltimore. 1246 pp.

Bühlmann, X., W. A. Vischer and H. Bruhin. 1961. Identification of apyocyanogenic strains of Pseudomonas aeruginosa for non-producing pyocyanin. J. Bacteriol. 82:787-788.

Burton, M. O., J. J. R. Campbell and B. A. Eagles. 1948. The mineral requirements for pyocyanin production. Can. J, Research, C, 26:15-22.

Burton, M. O., B. A. Eagles and J. J. R. Campbell. 1947. The amino acid requirements for pyocyanin production. Can. J. Research, C, 25:121-128.

Burzynska, H. and K. Maciezska. 1974. Comparison of suitability of different media for isolation of Pseudomonas aeruginosa from foods. Roczniki Panstwowego Zaklada Higieny 25:229-235. (Food Science and Technology Abstracts).

Christensen, W. B. 1946. Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J. Bacteriol. 52:461-466.

Drake, C. H. 1966. Evaluation of culture media for the isolation and enumeration of Pseudomonas aeruginosa. Health Lab Sci. 3:10-19.

Farrell, J. and A. H. Rose. 1968. Cold shock in a mesophilic and psychrophilic pseudomonad. J. Gen. Microbiol. 50:429-439.

Favero, M. S., L. A. Carson, W. W. Bond, N. J. Peterson. 1971. Pseudomonas aeruginosa: Growth in distilled water from hospitals. Science 173:836-838.

Frank, L. H. and R. D. DeMoss. 1959. On the biosynthesis of pyocyanine J. Bacteriol. 77:776-782.

Fried, M. A. and K. W. Vosti. 1968. Importance of underlying disease in patients with gram negative bacteremia. Arch. Intern. Med. 121: 418-420.

Georgia, F. R. and C. F. Poe. 1932. Study of bacterial fluorescence in various media. II. The production of fluorescence in media made from peptones. J. Bacteriol. 23:135-145.

Gilardi, G. L. 1971. Characterization of Pseudomonas species isolated from clinical specimens. Appl. Microbiol. 21:414-419.

Gorrill, R. H. and E. M. McNeil. 1960. The effect of cold diluent on the viable count of Pseudomonas pyocyanea (aeruginosa). J. Gen. Microbiol. 22:437-442.

Goto, S. and S. Enomoto. 1970. Nalidixic acid cetrimide agar: A new selective plating medium for the selective isolation of Pseudomonas aeruginosa. Jap. J. Microbiol. 14:65-72.

Green, S. K., M. N. Schroth, J. J. Cho, S. D. Kominos and V. B. Vitanza-Jack. 1974. Agricultural plants and soil as a reservoir for Pseudomonas aeruginosa. Appl. Microbiol. 28:987-991.

Hausler, W. J. (Ed.). 1972. Standard Methods for Examination of Dairy Products. (13th Ed.). American Public Health Association, Washington, D. C. 345 pp.

Haynes, W. C. 1951. Pseudomonas aeruginosa - its characterization and identification. J. Gen. Microbiol. 5:939-950.

Hedberg, M. 1969. Acetamide agar medium selective for Pseudomonas aeruginosa. Appl. Microbiol. 17:481.

Highsmith, A. K. and R. L. Abshire. 1975. Evaluation of a Most-Probable-Number Technique for the enumeration of Pseudomonas aeruginosa. Appl. Microbiol. 30:596-601.

Hood, A. M. 1948. Selective media for Pseudomonas pyocyanea. Monthly Bull. Min. Health and Pub. Health Lab. Serv. 7:248-251.

Jackson, H. 1974. Loss of viability and metabolic injury of Staphylococcus aureus resulting from storage at 5 C. J. Appl. Bacteriol. 37:59-64.

Jensen, J. P. and W. J. Hausler, Jr. 1975. Productivity of boiled and autoclaved violet red bile agar. J. Milk and Food Technol. 38: 279-280.

Kantor, L. T., S. D. Kominos and R. B. Yee. 1975. Identification of nonfermentative gram-negative bacteria in the clinical laboratory. Amer. J. Med. Technol. 41:3-9.

King, E. O., M. K. Ward and D. E. Raney. 1954. Two simple media for demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.

King, J. V., J. J. R. Campbell and B. A. Eagles. 1948. The mineral requirements for fluorescin production. Can. J. Research, C, 26:514-515.

Kominos, S. D., C. E. Copeland and B. Grosiak. 1972. Mode of transmission of Pseudomonas aeruginosa in a burn unit and an intensive care unit in a general hospital. Appl. Microbiol. 23:309-312.

Kominos, S. D., C. E. Copeland, B. Grosiak and B. Postic. 1972. Introduction of Pseudomonas aeruginosa into a hospital via vegetables. Appl. Microbiol. 24:567-570.

Lambe, D. W. and P. Stewart. 1972. Evaluation of pseudosel agar as an aid in the identification of Pseudomonas aeruginosa. Appl. Microbiol. 23:377-381.

Lilly, H. A. and E. J. Lowbury. 1972. Cetrimide-nalidixic acid agar as a selective medium for Pseudomonas aeruginosa. J. Med. Microbiol. 5:151-153.

Lindberg, R. B. 1974. Culture and identification of commonly encountered gram-negative bacilli: Pseudomonas, Klebsiella-Enterobacter, Serratia, Proteus and Providencia. In Opportunistic Pathogens. (J. E. Prier and H. Friedman, Eds.). University Park Press, Baltimore. pp. 19-35.

Lowbury, E. J. L. and A. G. Collins. 1955. The use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. J. Clin. Path. 8:47-48.

Lowbury, E. J. L. and J. Fox. 1954. Epidemiology of infection with Pseudomonas aeruginosa in burn units. J. Hyg. 52:403-416.

Maxcy, R. B. 1970. Non-lethal injury and limitations of coliform organisms on selective media. J. Milk Food Technol. 33:445-448.

Myrvik, Q. N., N. N. Pearsall and R. S. Weiser. 1974. Fundamentals of Medical Bacteriology and Mycology for Students of Medicine and Related Sciences. Lea and Febiger, Philadelphia. 510 pp.

Nelson, F. E. 1943. Factors which influence the growth of heat-treated bacteria: 1. A comparison of four agar media. J. Bacteriol. 45:395-403.

Nelson, F. E. 1971. The combined effects of medium pH and salt concentration on heat-stressed coliform bacteria. Bacteriol. Proc. 1971:20. (Abstr. A116).

Ordal, Z. J. 1970. Current developments in detection of microorganisms in foods: Influence of environmental factors on detection methods. J. Milk Food Technol. 33:1-5.

Ringen, L. M. and C. H. Drake. 1952. A study of the incidence of Pseudomonas aeruginosa from various natural sources. J. Bacteriol. 64:841-845.

Rogers, D. E. 1959. The changing pattern of life-threatening microbial disease. N. Engl. J. Med. 261:677.

Scheusner, D. L., F. Busta, M. L. Speck. 1971. Inhibition of injured E. coli by several selective agents. Appl. Microbiol. 21:46-49.

Seleen, W. A. and C. N. Stark. 1943. Some characteristics of green fluorescent pigment-producing bacteria. J. Bacteriol. 46:491-500.

Shooter, R. A., E. M. Cooke, M. C. Faiers, A. L. Breaden and S. M. O'Farrell. 1971. Isolation of E. coli, Pseudomonas aeruginosa and Klebsiella from food in hospitals, canteens and schools. Lancet II:390-392.

Shooter, R. A., E. M. Cooke, N. Gaya, P. Kumer, N. Patel, M. T. Parker, B. T. Thom, Diana R. France. 1969. Food and medicants as possible sources of hospital strains of Pseudomonas aeruginosa. Lancet I:1227-1229.

Skaliy, P. and R. G. Eagon. 1972. Effect of physiological age and state on survival of desiccated Pseudomonas aeruginosa. Appl. Microbiol. 24:763-767.

Smith, R. F. and S. L. Dayton. 1972. Use of acetamide broth in the isolation of Pseudomonas aeruginosa from rectal swabs. Appl. Microbiol. 24:143-145.

Smolka, R., F. E. Nelson and L. M. Kelley. 1974. Interaction of pH and NaCl on enumeration of heat-stressed S. aureus. Appl. Microbiol. 27:443-447.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. 776 pp.

Speck, M. L., B. Ray and R. B. Read, Jr. 1975. Repair and enumeration of injured coliforms by a plating procedure. Appl. Microbiol. 29:549-550.

Stanier, R. Y., N. J. Palleroni and M. Doudoroff. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:159-271.

Straka, R. P. and J. L. Stokes. 1959. Metabolic injury to bacteria at low temperatures. J. Bacteriol. 78:181-185.

Strange, R. E. and L. G. Ness. 1963. Effects of chilling bacteria in aqueous suspension. Nature 197:819.

Strange, R. E. and J. R. Postgate. 1964. Penetration of substances into cold-shocked bacteria. J. Gen. Microbiol. 36:393-403.

Thom, A. R., Mary E. Stephens, W. A. Gillespie and V. G. Alder. 1971. Nitrofurantoin media for the isolation of Pseudomonas aeruginosa. J. Appl. Bacteriol. 34:611-614.

Traci, P. A. and C. L. Duncan. 1974. Cold shock lethality and injury in Clostridium perfringens. Appl. Microbiol. 28:815-821.

Trust, T. J. and K. H. Bartlett. 1976. Isolation of Pseudomonas aeruginosa and other bacterial species from ornamental aquarium plants. Appl. Environ. Microbiol. 31:992-994.

Wright, C., S. D. Kominos and R. B. Yee. 1976. Enterobacteriaceae and Pseudomonas aeruginosa recovered from vegetable salads. Appl. Environ. Microbiol. 31:453-454.

Young, L. S. and D. Armstrong. 1972. Pseudomonas aeruginosa infections. Crit. Rev. Clin. Lab. Sci. 3:291-347.