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Isolation of *Yersinia enterocolitica* and *Listeria monocytogenes* from fresh vegetables in Saudi Arabia and their growth behavior in some vegetable juices

A. A. SALAMAH

Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT

The occurrence of *Yersinia enterocolitica* and *Listeria monocytogenes* in fresh market vegetables in Saudi Arabia was investigated. The two organisms were isolated from cabbage, carrot, cucumber, lettuce, potato, and radish. The incidence of *Y. enterocolitica* on each of these vegetables was higher than that of *L. monocytogenes*. The isolation rate of both genera was higher on underground crops than on the other crops. The *Y. enterocolitica* isolates were sensitive to chloramphenicol, streptomycin and tetracycline but resistant to erythromycin, ampicillin, penicillin, and vancomycin, whereas the *L. monocytogenes* isolates were sensitive to all of the above antibiotics with the exception of tetracycline to which it experienced some resistance.

The ability of the above two organisms to survive and/or grow in raw juices of potato, carrot and lettuce and in potassium phosphate buffer was investigated. The viable counts were always higher in samples incubated at 26°C than at 4°C. *Y. enterocolitica* was able to grow both in the buffer and juices; best growth occurred in lettuce juice and poorest growth occurred in carrot juice. *L. monocytogenes* grew in lettuce juice and to a lesser degree in potato juice but its number decreased in carrot juice and in the buffer.

INTRODUCTION

Listeria monocytogenes is a Gram-positive rod-shaped bacterium causing listeriosis in humans and animals (Gray & Killinger 1966; Mclaughlin 1987). This organism has been implicated as the causative agent in several large outbreaks of food-borne diseases in many countries (Schlech *et al.* 1983; Heisick *et al.* 1989; Wesley & Ashton 1991). The suspected vehicles of the above outbreaks were various vegetables, sea foods and dairy products.

Yersinia enterocolitica is a Gram-negative rod-shaped bacterium causing various clinical conditions in humans, including acute gastroenteritis, mesenteric adenitis, septicemia, arthritis, and erythema nodosum (Swaminathan *et al.* 1982). It has been implicated in several outbreaks of infections, including episodes in which food items have been incriminated as the vehicle of infection (Greenwood & Hooper 1985).

The isolation of these two pathogenic bacteria from fresh market produce in various countries (Swaminathan *et al.* 1982; Heisick *et al.* 1989) suggested the possibility of their presence in the fresh market produce of Saudi Arabia. The present study was conducted to examine such possibility, and to determine and compare the ability of these bacteria to grow on the juices of some of these vegetables.

MATERIALS AND METHODS

VEGETABLE SAMPLES

A total of 535 fresh local vegetable samples were obtained from the markets in Riyadh. The samples were examined for *Yersinia enterocolitica* and *Listeria monocytogenes* without any rinsing or cleaning.

ENRICHMENT, ISOLATION, AND IDENTIFICATION

(i) *L. monocytogenes.* Five g of each vegetable sample were transferred to 45 ml of *Listeria* enrichment broth (Merck) and incubated at 30°C for 7 days. The *Listeria* broth was streaked on *Listeria* selective agar (Merck) plates and incubated at 30°C for 48 h. The Henry oblique transillumination (SHOT) technique (Lachica 1990) was used for the initial recognition of *Listeria* colonies. The colonies tentatively recognized as *Listeria* were then characterized biochemically as described by Mclaughlin (1987).

(ii) *Y. enterocolitica.* Five g of each vegetable sample were transformed to 45 ml of *Yersinia* enrichment broth (Merck) and incubated at 26°C for 72 h. Alkali treatment was done by transferring 0·5 ml of the *Yersinia* enrichment medium to 4·5 ml of 0·5% KOH in 0·5% NaCl and allowing to stand for 3 min (Aulisio *et al.* 1980). *Yersinia* selective agar (Merck) plates were then inoculated by streaking them with one loop of KOH-treated enrichment. The plates were incubated at 26°C for 48 h. Colonies typical of *Y. enterocolitica* were characterized biochemically (Agbonlahor *et al.* 1982) for the selective and differential deoxycholate-sodium chloride (DYS) medium; the other biochemical tests follow Bercovier *et al.* (1980) and Kapperud (1981).

ANTIBIOTIC SENSITIVITY

The susceptibility of *Y. enterocolitica* and *L. monocytogenes* isolates to antibiotics was determined by the single-disk diffusion method of Bauer *et al.* (1966) using Mueller-Hinton agar (Merck) plates. The response to the following antibiotics was studied: ampicillin, chloramphenicol, erythromycin, gentamicin, penicillin, streptomycin, tetracycline and vancomycin. They were obtained from Oxoid, stored and used according to the manufacturer's suggestions.

VEGETABLE JUICES

Fresh potato, carrot, and lettuce samples were purchased from vegetable market of Riyadh and washed with sterile distilled water. Under aseptic conditions, 60 g of each vegetable were suspended in 600 ml of potassium phosphate buffer (0·1 M, pH

7) and blended for five min. The homogenate was passed through two layers of sterile cheese cloth. The resulting juice was then dispensed into 250 ml sterile flasks (50 ml/flask). The dispensed juices were further sterilized by passing chloroform fumes through them for 2 min, with shaking. The chloroform traces were then removed by passing sterile air with shaking for 5 min, then by placing the treated flasks in a 65°C shaking water-bath for 2 h. Potassium phosphate buffer (0·1 M, pH 7) was treated as above and used as a control.

Growth in the vegetable juices

One set of the potato, carrot and lettuce juices along with the phosphate buffer was inoculated with *L. monocytogenes* and another set was inoculated with *Y. enterocolitica*. Half of the flasks of each set were incubated at 26°C, whereas the other half were incubated at 4°C. Samples were removed at zero time and then at 40 h intervals for a period of 280 h, diluted with phosphate buffer and surface-spread on blood agar base (Merck) plates. The plates were counted after 24 h incubation at 26°C.

RESULTS

The biochemical profiles of the *Y. enterocolitica* and *L. monocytogenes* isolates are shown in Table 1 and Table 2, respectively. The frequency of their isolation from individual vegetables is shown in Table 3. More isolates of *Y. enterocolitica* were obtained from each kind of vegetable tested than *L. monocytogenes*. Cucumber, cabbage and lettuce showed less contamination with both microorganisms than the other vegetables. The susceptibility of all isolates to selected antibiotics is shown in Table 4. The *Y. enterocolitica* isolates were susceptible to chloramphenicol, gentamicin, streptomycin, and tetracycline, less susceptible to erythromycin, and resistant to

Table 1. Biochemical reactions for *Yersinia enterocolitica* isolates obtained from raw vegetables*

Biochemical test	No. of positive isolates (%)
Citrate (Simmons)	0 (0)
DYS medium	127 (100)
Dnase	122 (96)
Glucose	127 (100)
Indole	101 (79·5)
Lactose	61 (48)
Lysine-arginine-iron agar	127 (100)
Melibiose	0 (0)
Motility at 37°C	0 (0)
Motility at 25°C	127 (100)
Nitrate reduction	127 (100)
Ornithin decarboxylase	127 (100)
Sucrose	125 (98·4)
Trehalose	121 (95·3)
Triple-sugar-iron agar	127 (100)
Urease	127 (100)
Xylose	99 (78)

* Total number of isolates tested: 127.

Table 2. Biochemical reactions for *Listeria monocytogenes* isolates obtained from raw vegetables*.

Biochemical test	No. of positive isolates (%)
Catalase	44 (100)
Esculin	44 (100)
Glucose	44 (100)
β-hemolysis	41 (93·2)
Henry oblique transillumination	44 (100)
Mannitol	0 (0)
α-methyl-D-manoside	43 (97·7)
Nitrite	0 (0)
Rhamnose	42 (95·5)
Salicin	44 (100)
Trehalose	44 (100)
Umbrella motility	44 (100)
Voges-Proskauer	44 (100)

* Total number of isolates tested: 44.

Table 3. Incidence of *Listeria monocytogenes* (L.m.) and *Yersinia enterocolitica* (Y.e.) in various raw vegetables

Vegetables	No. of samples examined	No. of positive samples		Incidence (%)	
		L.m.	Y.e.	L.m.	Y.e.
Cabbage	70	2	11	2·8	15·7
Carrot	120	16	33	13·3	27·5
Cucumber	110	4	19	3·6	17·3
Lettuce	80	1	15	1·3	18·8
Potato	80	13	22	16·3	27·5
Radish	75	8	27	10·7	36·0

Table 4. Antibiotic susceptibility of *Listeria monocytogenes* (L.m.) and *Yersinia enterocolitica* (Y.e.) isolates obtained from raw vegetables

Antibiotic	μg/disk	No. of isolates (%) that were					
		Resistant		Intermediate		Sensitive	
		L.m.	Y.e.	L.m.	Y.e.	L.m.	Y.e.
Ampicillin	10	2 (4·5)	125 (98·4)	1 (2·25)	2 (1·6)	41 (93·25)	0 (0)
Chloramphenicol	30	0 (0)	0 (0)	2 (4·5)	4 (3·1)	42 (95·5)	123 (96·9)
Erythromycin	15	1 (2·25)	56 (44·1)	1 (2·25)	10 (7·9)	42 (95·5)	61 (48)
Gentamicin	10	0 (0)	0 (0)	2 (4·5)	0 (0)	42 (95·5)	127 (100)
Penicillin	10	1 (2·25)	124 (97·6)	0 (0)	3 (2·4)	43 (97·7)	0 (0)
Streptomycin	10	2 (4·5)	0 (0)	1 (2·25)	1 (0·8)	41 (93·2)	126 (99·2)
Tetracycline	30	7 (15·9)	0 (0)	1 (2·25)	0 (0)	36 (81·8)	127 (100)
Vancomycin	30	0 (0)	127 (100)	0 (0)	0 (0)	44 (100)	0 (0)

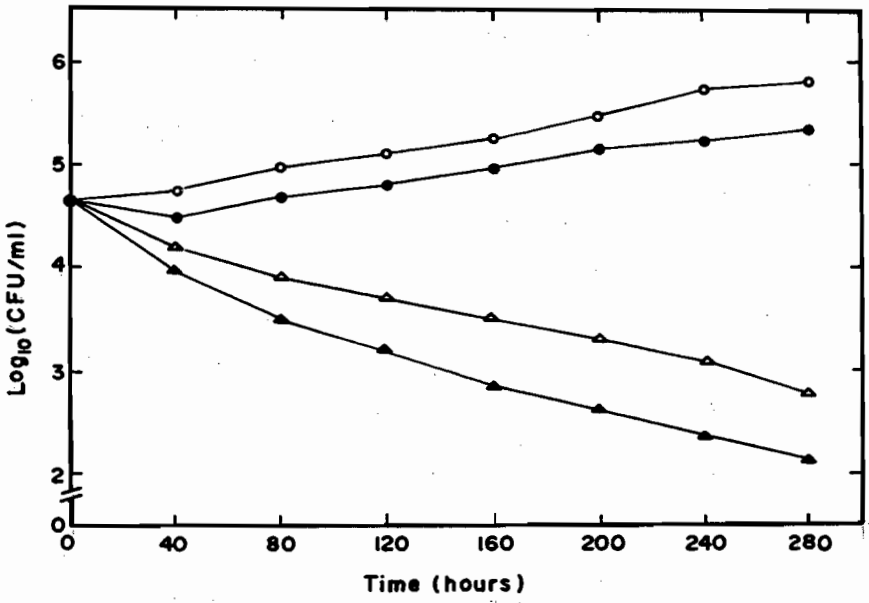

Fig. 1. Changes in populations of *Y. enterocolitica* (circles) and *L. monocytogenes* (triangles) in potassium phosphate buffer. Open symbols represent cells incubated at 26°C, whereas closed symbols represent cells incubated at 4°C.

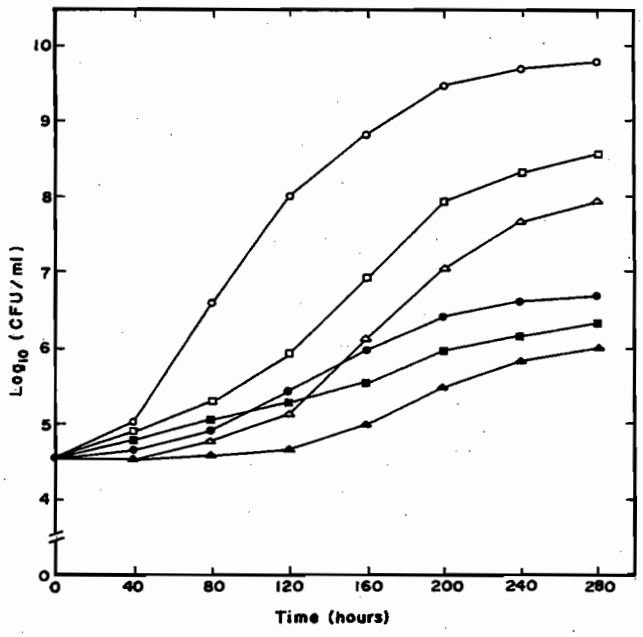

Fig. 2. Changes in populations of *Y. enterocolitica* in the juices of lettuce (circles), potato (squares), and carrot (triangles). Open symbols represent cells incubated at 26°C, whereas closed symbols represent cells incubated at 4°C.

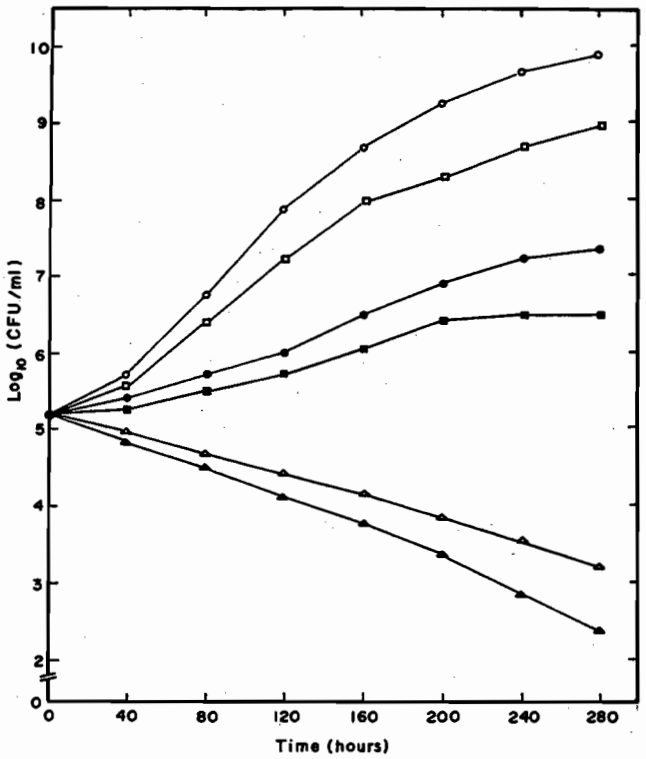

Fig. 3. Changes in populations of *L. monocytogenes* in the juices of lettuce (circles), potato (squares), and carrot (triangles). Open symbols represent cells incubated at 26°C, whereas closed symbols represent cells incubated at 4°C.

ampicillin, penicillin and vancomycin. The *L. monocytogenes* isolates were susceptible to all the antibiotics tested, with the exception of tetracycline; only 15·9% of the isolates were resistant to this antibiotic.

Results from preliminary experiments designed to determine populations of *Y. enterocolitica* and *L. monocytogenes* in potassium phospate buffer are shown in Fig. 1. The viable count in potassium phosphate buffer at 4° and 26°C increased gradually with time for *Y. enterocolitica*, whereas it decreased gradually for *L. monocytogenes*, independent of the growth temperature. For both organisms, however, the viable count of the 26°C grown cells was higher than that of 4°C.

The growth patterns of *Y. enterocolitica* and *L. monocytogenes* in carrot, lettuce, and potato are shown in Figs 2 and 3, respectively. As shown for the phosphate buffer, their viable counts at 26°C were also higher than those at 4°C. *Y. enterocolitica* was able to grow in the tested juices with the growth in lettuce being the highest and in carrot being the lowest. *L. monocytogenes* grew in lettuce juice and to a lesser degree in potato juice, but its viable count decreased in carrot juice.

DISCUSSION

Several studies (e.g. Greenwood & Hooper 1985; Brackett 1988; Bissett *et al.* 1990) have demonstrated the occurrence of *L. monocytogenes* and *Y. enterocolitica* in

related environments on a variety of foods including vegetables. The results shown in Table 3 indicate that *Y. enterocolitica* and *L. monocytogenes* were recorded in all vegetable types tested in Saudi Arabia. These bacteria probably came from the soil in which the vegetables grew. Further, it can be speculated that cabbage, cucumber and lettuce showed less contamination, simply because they normally have less contact with the soil than do underground crops such as potato, radish, and carrot.

Delmas & Vidon (1985) isolated *Y. enterocolitica* from nonspecified vegetables in France with an average incidence percentage of 32·3 which is higher than that recorded in the present study (23·8). Heisick *et al.* (1989) isolated *L. monocytogenes* from raw potato, radish, cabbage and cucumber in USA at higher rates than those recorded in the present study for underground crops and at lower rates for the other crops. However, the latter authors did not study carrot and they found lettuce to be free of *L. monocytogenes*. Further, Wong *et al.* (1990) isolated *L. monocytogenes* from raw vegetables in China at an average rate of 12·2% which is higher than the average rate (8%) recorded in this study.

The antimicrobial sensitivity patterns obtained in the present study are similar to those obtained for *L. monocytogenes* by Wong *et al.* (1990) and for *Y. enterocolitica* by Umoh *et al.* (1984).

The common occurrence of *Y. enterocolitica* and *L. monocytogenes* in raw vegetables, as demonstrated in the present and other reports, raises the question whether or not the two genera may have the ability to grow in the juices of vegetables. The results reported in the present paper reveal that lettuce and potato juices are good substrates for *Y. enterocolitica* and *L. monocytogenes*, whereas carrot could support the growth of *Y. enterocolitica* only. Naturally-occurring and inducible phenolic compounds have been identified in carrot juices (Sarkar & Phan 1979) and these may be the cause of the weak growth of *Y. enterocolitica* and the growth failure of *L. monocytogenes* in the carrot juice.

An epidemic of listeriosis in 1981 was linked to raw cabbage (Schlech *et al.* 1983) and tomatoes were suspected vehicles of transmission of *L. monocytogenes* in another series of cases (Ho *et al.* 1986). Cases of *Y. enterocolitica* infection with vegetables being the vehicle have not been reported.

Knowing that the consumption of fresh vegetables has increased dramatically in recent years, the above findings, coupled with the ability of the two organisms to grow at refrigerator temperature (Greenwood & Hooper 1985; Gellin & Broome 1989), give rise to public health concern. Further precautions, therefore, should be taken upon serving such vegetables particularly to the individuals with impaired immunity.

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عزل البكتيريا يرسينيا إنتيروكوليتيكا ولستيريا مونوسيتوجنس من خضروات طازجة في المملكة العربية السعودية، مع دراسة إمكانية نموها في عصير بعض الخضروات

علي عبدالله السلامه
وحدة الميكروبيولوجيا،
قسم النبات والميكروبيولوجيا بكلية العلوم، جامعة الملك سعود،
ص.ب ٢٤٥٥، الرياض ١١٤٥١، المملكة العربية السعودية

خلاصة

تمت دراسة مدى وجود كل من يرسينيا إنتيروكوليتيكا ولستيريا مونوسيتوجنس في بعض الخضروات الطازجة في المملكة العربية السعودية. تم عزل جنسي البكتريا من الملفوف والجزر والخيار والخس والبطاطس والفجل. وكان معدل عزل يرسينيا إنتيروكوليتيكا من جميع الخضروات المختبرة أعلى من معدل عزل لستيريا مونوسيتوجنس، كما كان معدل عزل الجنسين من المحاصيل الجذرية أكثر من معدل عزلهما من المحاصيل غير الجذرية.

وكانت عزلات يرسينيا إنتيروكوليتيكا حساسة للكلورامفنيكول والستربتوميسين والتتراسيكلين، ومقاومة للإرثروميسين والأمبسيلين والبنسيلين والڤانكوميسين، بينما كانت عزلات لستيريا مونوسيتوجنس حساسة لجميع المضادات المذكورة ما عدا التتراسيكلين الذي واجه بعض المقاومة.

تمت دراسة قدرة الجنسين على الصمود و/أو النمو في البطاطس والجزر والخس الطازجة وفي محلول فوسفات البوتاسيوم المنظم. وكان عدد الخلايا الحية للجنسين بصفة عامة أكثر عند ٢٦°م منه عند درجة ٤°م. وقد نمت يرسينيا إنتيروكوليتيكا في كل من المحلول المنظم وفي عصير الخضروات، وكان أفضل النمو في الخس وأقله في الجزر. أما لستيريا مونوسيتوجنس فقد نمت في الخس وإلى حد ما في البطاطس، بينما تناقص عددها في الجزر وفي المحلول المنظم.