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I am submitting herewith a thesis written by Laure Lynn Larkin entitled "The Influence of pH on the Cellular Composition of Yersinia enterocolitica." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

Frances A. Draughon
Frances Ann Draughon, Major Professor

We have read this dissertation
and recommend its acceptance:

Sharon L. Melton

PMichael Dando —

Accepted for the Council:

Cawmikel
Vice Provost
and Dean of the Graduate School

THE INFLUENCE OF pH ON THE CELLULAR
COMPOSITION OF YERSINIA ENTEROCOLITICA

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ABSTRACT

Six strains of Yersinia enterocolitica were grown at pH 5.0, 7.0 and 9.0. Variations in the pH of the environment altered the amount of total lipid present, as well as the relative percentages of the individual fatty acids and phospholipids.

A higher amount of total lipid was observed at acidic pH's with a lesser amount at alkaline pH's. The fatty acid analysis of strains E752, IP383 and 190-34/1B showed a decrease in the relative percentage of unsaturated fatty acids in acidic environments. All strains tested except E752 and IP383 showed a decrease in the relative percentage of the branched forms when the organism was grown in acidic environments. Cultures grown at pH 5.0 also tended to have a higher relative percentages of cyclopropane fatty acids. The phospholipid analysis showed large increases in the relative percentage of phosphatidyl ethanolamine at higher pH's and increases in the relative percentage of phosphatidyl serine at low pH's. (It is not known if the amount of unsaturated hydrocarbons effected the relative amounts of phospholipid detected due to the nature of the HPLC-UV analysis.) Assuming that unsaturated fatty acids were equitably incorporated into the hydrocarbon tails of the phospholipids, it would appear that the cell envelope can adapt the biosynthesis of phospholipids to environmental pH.

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CHAPTER I

INTRODUCTION

The ability of foodborne pathogens to survive conditions of high acidity, alkalinity or cold storage is important because all of these techniques are sometimes used in foods as methods of pathogen control. The pathogenic organism Yersinia enterocolitica has the ability to survive and multiply under all of these conditions creating a unique problem for food processors. Y. enterocolitica has been recognized as causing outbreaks of foodborne gastroenteritis since 1966 (Highsmith et al., 1977). Reported outbreaks have been increasing steadily for the past few years.

Several studies have documented the survival of Yersinia enterocolitica at pH extremes. Growth has been reported at low pH levels such as 4.0 (Brackett, 1986), 4.2 (Kendall and Gilbert, 1980), and 4.6 (Stern et al., 1980). It is possible to recover Y. enterocolitica from foods such as sauerkraut, tartar sauce and pickles which may have a pH as low as 3.5 (Stern et al., 1980).

Yersinia enterocolitica has also been recovered from shelled eggs which may have a pH of 9.4-9.7 (Amin and Draughon, 1987). The ability of Y. enterocolitica to survive highly alkaline conditions forms the basis for its isolation from foods. Most other foodborne enteric organisms are destroyed by the pH of 12.67 that is typical of the KOH treatment to which Yersinia isolates are subjected (Schiemann, 1983).

One study has even reported an increase in viable cells at pH 9.6 (Kendall and Gilbert, 1980).

This research was undertaken in an attempt to determine the compositional changes related to the pH tolerance of Yersinia enterocolitica. Specifically, the study evaluated the effect of pH extremes on the total lipid, fatty acids and phospholipids in the cellular composition for six different strains of pathogenic Y. enterocolitica.

CHAPTER II

REVIEW OF THE LITERATURE

1. The Genus Yersinia

Characteristics and Classification

Yersinia enterocolitica is a gram negative, nonsporeforming, facultatively anaerobic bacillus with an average length of 1.0 to 3.5 μ m (Feeley et al., 1976). The organism produces an endotoxin and is motile at 25° C by means of peritrichous flagella, but not at 36° C (Stern and Pierson, 1979). Y. enterocolitica is able to grow at 4° C while the optimum temperature is 25° C. The organism is able to survive a pH range of 4.0 to 12.67 for short periods of time (Schiemann, 1985; Brackett, 1986). Yersinia appears to be more sensitive to curing agents than other enteropathogens (Genigeorgis, 1986). It has been shown to grown in the presence of 5% salt but not at 7%. Yersinia is sensitive to heat, freezing and irradiation. Heating to 60° C for 1 to 3 minutes inactivates levels of $10^{6.5}$ cells/g (Hanna et al., 1977).

The classification for the organism Yersinia enterocolitica was changed from the family Pasteurellae to the family Enterobacteriaceae in 1970. This change was made because the organism was antigenically related to the other organisms in the family Enterobacteriaceae.

Yersinia is considered separately from other enteric bacteria because it is not a prominent member of the gastrointestinal tract. The organism has been referred to by a variety of names including Pasteurella "x",

Pasteurella pseudotuberculosis b, Pasteurella pseudotuberculosis-like and Bacterium enterocoliticum, (Feeley et al., 1976). The name Bacterium enterocoliticum was designated for a group of cultures collected by the New York State Health Department starting in the 1930s from children with enteritis (Schiemann, 1985). The name Yersinia was chosen to honor the French microbiologist Yersin who first isolated the plague bacterium (Schiemann, 1985).

There are three Yersinia species known to cause human illness: Y. pestis, the plague bacillus; Y. pseudotuberculosis; and Y. enterocolitica. The organism Y. enterocolitica was further divided in 1980 into four species: enterocolitica, frederiksenii, kristensenii and intermedia. A fifth species has also been proposed for a biotype isolated from water, Yersinia aldovae (Schiemann, 1985). Yersinia enterocolitica has been reported in sporadic outbreaks since 1939 but has not received much attention until recently (Lee, 1977).

Yersinia enterocolitica occurs as environmental or pathogenic strains. The organism is differentiated into 5 biotypes designated 1-5. These groups are based on the various biotype's reactions in seven tests. These tests consist of the following; the presence of lipase and deoxyribonuclease, production of indole, nitrate reduction, and the ability to produce acid from D-xylose, sucrose and D-trehalose (Krieg and Holt, 1984). These biotypes are further divided into serotypes based on somatic antigens and phage typing (Kendall and Gilbert, 1980). Thirty four serotypes have been identified. Biotype 1 strains are

usually environmental strains while biotype 4 strains are usually pathogenic (Schiemann, 1985). The latter biotype is characterized by serotype 0:3 which is most commonly associated with human infections in Europe.

Isolation and Identification Techniques

The methods used to recover Y. enterocolitica were originally designed for clinical use and adapted to foods (Lee, 1977). The use of a cold enrichment was first recommended for Yersinia pseudotuberculosis isolation (Lee, 1977). Cold enrichment is the primary step for any sample containing appreciable numbers of competing microflora. Y. enterocolitica is slow growing and is easily outcompeted by other gram negative bacteria at higher temperatures (Schiemann, 1985; Feeley et al., 1976). Low temperatures allow Yersinia to multiply enough to be easily isolated on plate media (Schiemann, 1985). Many different cold enrichment formulas are available but no single formula is able to recover all of the biotypes and serotypes (Stern and Pierson, 1979; Lee, 1977). For this reason, two or more enrichment formulas are recommended for isolation from foods (Stern and Pierson, 1979). Schiemann (1985) suggested that Bile - Oxalate - Sorbose broth (BOS) incubated at 22 - 25° C for 3 - 5 days gave adequate recovery. Many researchers enrich in Phosphate Buffered Saline (Doyle and Hugdahl, 1983; Stern and Pierson, 1979). Other cold enrichment techniques used to recover Y. enterocolitica include selenite F, saline, 1/15M disodium phosphate

(Feeley et al., 1976) Butterfield's phosphate, phosphate, and Rappaport broth (Stern and Pierson, 1978).

Alkaline treatments are sometimes used to further reduce the background bacteria before plating (Schiemann 1985, 1983; Lee, 1977; Stern and Pierson, 1979; Doyle and Hugdahl, 1982). The technique was first described by Aulisio et al. (1979). Yersinia is much more alkalotolerant than other gram negative bacteria found in foods (Schiemann, 1985, 1983). A portion of the enrichment media is subjected to a 0.5% KOH - 0.5% NaCl solution for a short period before plating on agar (Schiemann, 1985, 1983; Stern and Pierson 1979).

After the KOH treatment, the organism is usually plated on media such as Yersinia Selective Agar (YSA) or MacConkeys Agar (Feeley et al., 1976; Stern and Pierson, 1979; Schiemann, 1985, 1983; Lee, 1977). Other enteric plating media can also be used with variable success (Feeley et al., 1976; Stern and Pierson, 1979; Lee, 1977). Some strains and serotypes are sensitive to these media and often it is difficult to differentiate Yersinia from other lactose negative colonies on these agars (Stern and Pierson, 1979; Lee, 1977). Enteric media used includes Deoxycholate Citrate, Eosin Methylene Blue, Lysine Sucrose Urea, Salmonella Shigella, YL agar, Bismuth Sulfite, DNase and YM agar (Stern and Pierson, 1979; Feeley et al., 1976; Lee, 1977). Due to the nondifferential properties of the enteric media, YSA tends to be the medium of choice. Yersinia colonies are unique on YSA which makes for fast presumptive identification (Schiemann, 1985). The colonies are bright red "bullseyes" with transparent borders.

Presumptive colonies from YSA can be confirmed as belonging to the genus Yersinia by two tests, Kliger Iron Agar (KIA) and Christensen's Urea Agar (Schiemann, 1985). Confirmation of the species Y. enterocolitica can be completed in six additional tests including: sucrose, rhamnose, Simmon's citrate, raffinose and alpha methylglucoside (Schiemann, 1985).

Confirmed isolates are then tested for pathogenicity. This can be done by using animal models such as: Sereny Test, fatal infections in mice or gerbils, diarrhea in mice, monkeys, rabbits, or rabbit ileal loops (Schiemann, 1985; Lee, 1977). Non-animal virulence testing systems include HeLa Cell invasiveness (Lee, 1977), positive agglutination in tissue culture media at 36° C (Laird and Cavanaugh, 1980), calcium dependency at 35° C, uptake of Congo red dye and the synthesis of a unique outer membrane antigen at 35° C (Schiemann, 1985). Pathogenic Y. enterocolitica can also be distinguished by their inability to ferment salicin and hydrolyze esculin (Schiemann, 1985).

The methodology for the isolation of Yersinia enterocolitica falls far short of what is necessary. The length of time required for cold enrichment is not acceptable for perishable foods, while isolation on selective agars does not differentiate pathogenic forms from environmental ones (Schiemann, 1985). Several companies and regulatory agencies are attempting to develop rapid detection methods based on DNA Hybridization or Monoclonal Antibody Technology.

2. Implications for Human Health

Environmental strains are indistinguishable from their pathogenic counterparts on plate media. For this reason the only assessment of the incidence comes from foodborne illness outbreaks. This is not very accurate because Y. enterocolitica infections are not notifiable diseases in the U.S. (Lee, 1977). The reported recovery rate for fecal specimens worldwide ranges from 0 to 1-2%, with some hospitals reporting higher rates than for Shigella (Lee, 1977). Experts suggest that this is accurate and that Yersinia is now second to Salmonella as the cause of gastroenteritis (Stern and Pierson, 1979). Yersinia infections appear to be seasonal, peaking in the fall and winter (Swaminathan et al., 1982).

Pathogenicity appears to be dependent on several aspects including the strain of Y. enterocolitica. Many studies have shown increased virulence in cultures grown below 26° C (Lee, 1977; Stern and Pierson, 1979). Excess dietary iron also aggravates Yersinia infections by decreasing the ability of human serum to fight off the infection (Swaminathan et al., 1982). Experts speculate that the ability of Y. enterocolitica to invade human epithelial cells in the gut plus the ability to produce toxin determines the extent of pathogenicity (Stern and Pierson, 1979). Researchers have found that invasiveness correlates with the presence of a 41 Mdal plasmid (Stern and Pierson, 1979).

The production of toxin however, is not plasmid mediated (Swaminathan et al., 1982). The enterotoxin has molecular weight of 10,000 - 50,000 and is heat stable (Stern and Pierson, 1979; Swaminathan et al., 1982). It is able to survive heating to 121° C for 30 minutes, storage at 4° C for 7 months and a pH of 1 - 11 (Swaminathan et al.,

1982). This means that Yersinia toxin can survive most food processing and storage techniques as well as gastric juices. Since the toxin is not produced at 37° C, it is doubtful that the organism can produce toxin in vivo (Swaminathan et al., 1982). Serotypes 0:3, 0:8 and 0:9 are almost always enterotoxigenic while environmental isolates rarely produce toxin. Toxin production is not a good indicator of pathogenicity, however, because some avirulent strains also produce toxin (Swaminathan et al., 1982).

The pathogenic strains of Yersinia enterocolitica are responsible for a variety of human ailments including gastrointestinal diseases such as enteritis, mesenteric lymphadenitis and terminal ileitis (Schiemann, 1985). Yersinia is also responsible for infections of the skin, eye, muscle, wound abscesses, endocarditis, pharyngitis and post-infection immunologic sequelae such as arthritis, erythema nodosum and thyroid disorders (Schiemann, 1985).

The symptoms of Yersinia infections vary with the age of the afflicted. Infants primarily manifest symptoms of fever and diarrhea, while older children are subjected to mesenteric lymphadenitis and ileitis that tends to mimic acute appendicitis (Lee, 1977). Adults usually show symptoms of acute abdominal pains, acute enteritis, arthritis, erythema nodosum and in the elderly or immune suppressed patients septicemia may result (Lee, 1977). The disease can be reproduced experimentally in mice, gerbils and monkeys (Krieg and Holt, 1984).

Results from surveys of developed countries indicate that Y. enterocolitica is responsible for 1 - 3 % of all human enterities cases

(Genigeorgis, 1986). The estimated threshold level for foodborne illness from Yersinia is 10^6 cells (Snyder, 1986).

There are a variety of sources for this organism. Yersinia is frequently isolated from several animals, pigs being the most common (Silliker, 1986). Healthy humans and animals have been found to be carriers (Krieg and Holt, 1984). Water also may be a reservoir for Y. enterocolitica. Some strains are reported to proliferate in sterile water at 4, 25 and 37° C. (Swaminathan et al., 1982). It is possible to isolate Yersinia from a wide variety of food sources including raw milk, meat, poultry, shellfish, fruits and vegetables. Research has shown that small levels of the organism are able to reach counts in the millions in fresh meats after storage at 4 - 7° C, and in milk stored at 0 - 2° C (Genigeorgis, 1986). Yersinia has also been shown to increase to 10^8 cells per gram in less than 2 weeks in pork refrigerated to 4° C (Stern and Pierson, 1979). Low levels in cooked beef increased to 10^{10} cells per gram in 10 days (Stern and Pierson, 1979). The organism was consistently found in high numbers on meat derived from dark cutters (Swaminathan et al., 1982).

Yersinia is heat sensitive and is easily destroyed by adequate cooking or pasteurization. The organism is also very sensitive to radiation treatment (Stern and Pierson, 1979). Post processing contamination is the main problem for pasteurized and irradiated foods.

There are few documented outbreaks of Yersiniosis in the United States. In general the vehicle of transmission is thought to be food or water. However, one outbreak of transmission directly from an infected animal traced to an infected bitch and her litter was reported.

Subsequent infection of the owners and their immediate family resulted in 16 illnesses and 2 deaths (Gutman et al., 1973). In 1976 New York experienced an outbreak in which the implicated food was chocolate milk and 222 people became ill. Post pasteurization contamination was determined to be the probable source (Black et al., 1978). Reconstituted dry milk and chow mein were implicated in a 1981 outbreak which caused 239 people to become ill (Shayegani et al., 1983). This outbreak also occurred in New York. The probable source was a cook who carried the organism. Tofu was implicated in 1981 and 1982 outbreaks in Washington where 87 cases were reported (Aulisio et al., 1983). The water the tofu was packaged in was deemed the source of contamination. Milk was again responsible for another outbreak of Yersiniosis in 1982. This occurred in the Memphis - interstate area and 172 cases of illness were confirmed although the actual number of infections was thought to be much higher. Contamination was traced to milk crates returned from hog farms (CDC, 1986). The only outbreak traced to meats occurred in 1984. Thirteen people were made ill following consumption of precooked turkey breasts. Sampling showed the product contaminated with Yersinia (Genigeorgis, 1986).

3. Growth Characteristics of *Yersinia enterocolitica*

Yersinia enterocolitica has been isolated from such high acid foods as sauerkraut, pickles and tartar sauce (Stern et al., 1980), and from highly alkaline foods such as shelled eggs which may have a pH as high as 9.7 (Amin and Draughon, 1987). Several studies have documented a wide growth range for this organism. Hanna and coworkers (1977) found

the organism was able to grow up to pH 9.0 with an optimum at neutral pH. However, they observed no increase in cell numbers at pH 5.0 (Hanna et al., 1977). While optimum growth was achieved at neutral pH, Y. enterocolitica was able to grow at pH 4.6 and 9.6 in a study completed by Stern and coworkers (1980). Kendall and Gilbert (1980) were able to demonstrate growth of serotypes 0:3 and 0:9 at a pH as low as 4.4. In another study, viable cells were recovered after 21 days at pH 4.0 (Brackett, 1986). Alkalotolerance of the organisms was investigated by Schiemann (1983) as a basis for selective isolation. He showed that cells could survive exposure to a pH of 12.67 for short periods (Schiemann, 1983). Many external conditions were found to effect the alkalotolerance including the medium, temperature and phase of growth (Schiemann, 1983).

The pH range for growth of most foodborne bacteria is between 4.5 and 9.0 (Banwart, 1981). Escherichia coli has a pH range for growth similar to Y. enterocolitica (<4.4 - 10) while other gram negative bacteria such as Pseudomonas, Salmonella and Serratia have narrower ranges (Banwart, 1981). The study conducted by Schiemann (1983) found the destruction rate for all of the gram negative bacteria tested was much higher than the rate for Y. enterocolitica. The mean $\Delta \log N$ per minute for Y. enterocolitica was 0.94 while the other bacteria had a value of >4.0 (Schiemann, 1983).

Very little is known about bacteria that are resistant to pH extremes (Langworthy, 1978; Schieman, 1983). The internal pH of the cells is known to be near 7.0 regardless of the pH of the external environment (Banwart, 1981; Langworthy, 1978). Maintenance of a neutral

internal pH is essential. Molecules such as ATP and DNA are acid labile (Langworthy, 1978). At basic pH's, amino acid transferase RNA is inhibited and protein synthesis is stopped (Banwart, 1981). Life would not exist if the internal pH were allowed to fluctuate with that of the environment. Since the internal pH is not effected by the environment, the effects of pH must be external (Langworthy, 1978).

Extreme pH levels are toxic for several reasons. Undissociated molecules of acid or basic substances readily penetrate the cells (Banwart, 1981). Low pH (3 - 5) environments allow hydrogen ions to absorb to particulate matter and displace cations which can cause them to be leached from the microenvironment (Langworthy, 1978). Other ions such as Al^{3+} , Mn^{2+} , Cu^{2+} and Mo^{3+} become more soluble, possibly reaching toxic levels (Langworthy, 1978). Toxicity at low pH's also results from the increased ability of normally nontoxic organic acids to enter the cell in the protonated state (Langworthy, 1978). Essential elements like Fe^{2+} , Ca^{2+} , Mg^{2+} and Mn^{2+} become insoluble and precipitate at high pH levels of 8 to 11 (Langworthy, 1978). The environmental pH might also effect the electrical charge of the cell surface by increasing the net positive charge at low pH, and increasing the net negative charge at high pH (Langworthy, 1978). The ability to survive in extreme pH environments is thus limited by the state and availability of nutrients as well as the presence of toxic metabolities.

4. Cell Wall Structure of *Yersinia enterocolitica*

The ability of a cell to withstand environmental stress such as toxic metabolites or pH extremes is largely dependent on the structure of its cell wall. The cell wall of gram negative organisms consists of

a multilayered complex containing varied chemical constituents. The function of each layer is determined by the composition of the layer, the molecular arrangement and the interaction of other adjacent layers (Costerton et al., 1974). The outer membrane consists of phospholipids, proteins and lipopolysaccharides (Stryer, 1981). The phospholipids and proteins form a bilayer while the lipopolysaccharides are associated between the bilayers (Costerton et al., 1974). The lipopolysaccharides in the outer membrane consist of three regions: lipid A, core oligosaccharides, and O side chain. The lipid A fraction is the hydrophobic portion while the core oligosaccharides and the O side chain are hydrophilic (Stryer, 1981). The lipopolysaccharides are synthesized in the cytoplasmic membrane and transferred to the outer membrane (Stryer, 1981). This lipopolysaccharide assists in the ability of the membrane to act as a barrier and increases rigidity. Lipopolysaccharide also reduces the susceptibility of the cells to phagocytosis and increases the susceptibility to phage conversions (Stryer, 1981). The lipoprotein that is present in this layer may also be associated with binding the inner layer of the outer membrane to the peptidoglycan layer just below it by hydrophobic interactions (Costerton et al., 1974). This lipoprotein fraction also forms porins in the outer membrane that assist in the transport of small polar molecules (Stryer, 1981). The main function of the entire layer is likely to act as a "molecular seive" (Costerton et al., 1974).

The adjoining layer of peptidoglycan probably functions as a stabilizer of cell shape. The peptidoglycan is also synthesized in the cytoplasmic membrane (Costerton et al., 1974). This layer does not

have barrier capability but it is able to bind specific enzymes (Costerton et al., 1974).

The innermost cytoplasmic membrane of the cell is separated from the peptidoglycan layer by periplasmic space. This zone contains enzymes that are released when the outer membrane is damaged or stressed by such things as antibiotics or pH extremes (Costerton et al., 1974). The cytoplasmic membrane is where the other cell wall components are synthesized and transported to the other membrane locales (Costerton et al., 1974).

CHAPTER III

MATERIALS AND METHODS

1. Test Organisms

The test organisms included 6 strains of Yersinia enterocolitica. Y. enterocolitica strains Y7P and IP383 were obtained from M. P. Doyle at the Food Research Institute, Madison, WI. Strains OH-18020-2-1, 190-34/1B, WA(+), 0:13,7 and E752 were obtained from J. S. Cholensky of the FDA Minneapolis, MN. The stock cultures were stored at 4° C on tryptose soy agar (BBL: Cockeysville, MD) slants supplemented with 0.2% yeast extract (BBL) and transferred monthly. The cultures were revived 18 hr before use in 100 ml of Brain Heart Infusion Broth (Difco, Detroit, MI).

2. Media and Buffers

Glycine buffer consisted of filter sterilized 0.2 M Glycine (Mallinckrodt; Paris, KY) to which autoclaved 0.2 M NaOH (Fisher Scientific; Fair Lawn, NJ) was added to achieve a final pH of 10.0, or 0.2 M HCl (Fisher Scientific) was added to achieve a final pH of 3.0. The pH was determined using a Fisher Accumet pH Meter Model 600.

Buffered Brain Heart Infusion Broth (BBHIB) was prepared by reconstituting Brain Heart Infusion Broth (BHI) double strength according to manufacturer's instructions and autoclaving. An equal volume of 0.2 M Glycine-HCL, 0.2 M Glycine or 0.2 M Glycine NaOH was aseptically added to the BHI to reach pH levels of 5.0, 7.0 and 9.0, consecutively.

3. Preparation of Cultures

The organisms were transferred to 250 ml screw capped flasks containing 100 ml of BBHIB at pH 5.0, 7.0, and 9.0. The flasks were incubated at 25° C for 24 hr for two consecutive transfers. Duplicate Fernbach flasks containing 2000 ml of BBHIB at the three pH levels were then inoculated with 25 ml of each culture. The pH 5.0 and 9.0 cultures were incubated at 25° C for 24 hr while the pH 7.0 flask was incubated for only 12 hr to insure that all cells were in log phase.

4. Total Lipid Determination

A modification of the method of Bligh and Dyer (1959) was used to determine the total lipid present in the cells. A 1500 ml portion of the BBHIB was centrifuged at 14,000 X g for 20 min at 4° C using a refrigerated IEC centrifuge (Needham Heights, MA). The pellet was washed twice in 200 ml distilled water. The cells were transferred to preweighed, acid washed, oven dried 25 ml beakers. The pellet was then dried for 18 hr at 83° C under a vacuum. The weight of the pellet was determined on an analytical balance and 10 ml of methanol (Mallinckrodt, Address) and 10 ml of chloroform (American Burdick & Jackson; Muskegon, MI) were added to the beaker. The cells were resuspended and poured into a 125 ml separatory flask containing 12 ml of 0.2 M Glycine-HCl buffered pH 5.0 water, 20 ml methanol and 5 ml chloroform. The flask was agitated and allowed to stand for 15 minutes. Equal portions of chloroform and pH 5.0 water amounting to 30 ml were added and the flask was reagitated. The layers were allowed to separate overnight. The

chloroform extract was filtered through a 0.2 μ m filter into a pre-weighed, acid washed 125 ml roundbottom flask. The flask was dried for 18 hr at 83° C under a vacuum, weighed, and the total lipid was calculated on a dry weight basis.

5. Fatty Acid Composition

The lyophilized pellet obtained from 500 ml of broth was converted to methyl esters by the procedure of Supelco Inc. (Bellefonte, PA). The pellet was placed in a 16 X 150 mm screw capped test tube containing 5 ml of 5% NaOH in 50% aqueous methanol. The cells were heated at 100° C for 30 minutes and cooled. The pH was lowered to 2.0 with 6N HCl. Methyl esters were formed by adding 5 ml of boron trifluoride reagent (Eastman Kodak; Rochester, NY) and heating the mixture for 5 minutes at 80° C. The mixture was again cooled and 10 ml chloroform/hexane (1:4) was added and the tube was agitated. A few drops of a saturated NaCl solution were added and the layers were allowed to separate. The chloroform/hexane layer was pipetted into a clean test tube. The original tube received another 5 ml of chloroform/hexane. This was siphoned off and added to the tube containing the 10 ml chloroform/hexane fatty acid methyl esters. The chloroform/hexane was evaporated to a volume of 0.4 ml under nitrogen. A 2 μ l sample of the fatty acid methyl esters was injected into a Shimadzu GC - 6AM with an SP-2100 (Supelco; Bellefonte, PA) 30 meter, 0.25 mm ID, 0.2 μ m df capillary column. Detection was by flame ionization. The temperature was programmed to increase from 150 to 200° C at a rate of 4° C/min with an initial 4 min isocratic hold. The flow

rate of the helium carrier gas was 30 ml/min. The range was 10^8 X and the sensitivity was 10^4 . The chromatograms were analyzed against a bacterial fatty acid methyl ester standard (Supelco 4-5436) and generally confirmed by mass spectroscopy on a Shimadzu QP-1000 GC/MS, (Kyoto, Japan) fitted with a GC-9A on the same column using the above conditions.

6. Phospholipid Procedure

Phospholipids were analyzed using a modified procedure developed by Chen and Kou (1982). The lyophilized pellet from 1000 ml of broth was suspended in 15 ml of HPLC grade chloroform - methanol (2:1 v/v). The lipid extract was separated into two phases by adding 3 ml water. The lower chloroform phase was then extracted and evaporated to dryness under a stream of nitrogen. The extract was redissolved in HPLC grade chloroform and analyzed against known standards including; phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylglycerol (Sigma; St. Luis, MO) by HPLC on a Waters Assoc. (Millford, MA) Model 6000A Solvent Delivery System. A Porasil column (Waters Assoc.) with a 120 um particle size was used in a solvent mixture of HPLC grade acetonitrile (Fisher Scientific) - methanol - 85% phosphoric acid (Fisher Scientific) (130:5:1.5 v/v) at a flow of 1 ml/min. Detection was accomplished using a Waters Lambda-Max Model 480 LC Spectrophotometer set at 203 nm.

7. Statistical Analysis

Data was analyzed using Analysis of Variance (ANOVA) to determine if there were significant differences among strains and cultures at the

three pH levels and/or replications. Any significant differences ($p < 0.05$) detected in the ANOVA were separated by Tukey's Multiple-Range Test.

CHAPTER IV

RESULTS AND DISCUSSION

1. Total Lipid Determination

The total lipid content of the six strains of Yersinia enterocolitica was determined per gram on a dry weight basis. The objective of this experiment was to determine if the quantity of total lipid within the cell was changing at the pH extremes. Total membrane lipid values for gram-negative bacteria have been reported as high as 25% (Salton, 1964).

Statistical analysis of the values obtained at the three pH levels showed that the amount of lipid present in the cells was significantly higher ($P < 0.05$) at pH 5.0 (Table 1). The total lipid tended to decrease as the pH increased, but the decrease between pH 7.0 and pH 9.0 was not significant at $P < 0.05$. The high lipid content at pH 5.0 possibly thickens one or more of the membrane leaflets and acts as a barrier; however, having elevated lipid contents in the membrane at alkaline pH would likely increase membrane instability due to the tendency for saponification. High lipid contents have also been reported to assist in antimicrobial resistance, this is likely true for acidic pH as well (Russell and Furr, 1986). The results seem to indicate that the acidic pH was more stressful to Y. enterocolitica than the basic pH. This was also evidenced by the organisms increased growth at alkaline pH's.

Table 1. Percent Total Lipid Per Gram on a Dry Weight Basis of Yersinia enterocolitica at Three pH's Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Strain	Total Lipid Percentages			
	pH	5	7	9
E752		10.19 ^a	5.83 ^b	4.57 ^b
IP383		10.05 ^a	4.70 ^b	4.16 ^b
Y7P		7.55 ^a	5.18 ^b	4.82 ^b
O:13,7		12.62 ^a	6.72 ^b	5.10 ^b
190-31/1B		12.87 ^a	7.94 ^b	6.08 ^b
WA(+)		11.81 ^a	7.06 ^b	7.13 ^b

^{a, b}Values in rows with different superscripts are significantly different (P<0.05).

2. Analysis of Fatty Acid Composition

The fatty acid composition of the six strains of Y. enterocolitica grown at different pH levels, was determined using gas liquid chromatography. The objective of this phase of the experiment was to determine if there was a relationship between the types and content of the fatty acid components present at the various pH's. The fatty acids found in the six strains comprising more than 50% of the total were C16:1, C16:0, i-C16:0 and C17:0 Δ (Table 2). Strains 190-34/1B and WA(+) contained 20H-16:0 instead of C17:0 Δ at all of the pH levels (Appendix 5, 6). The results obtained at pH 7.0 compare well with those found by Jantzen and Lassen (1980) for 31 strains of Y. enterocolitica. They found C16:0, C16:1 and C17:0 Δ to be the major fatty acid constituents of Y. enterocolitica. Their method did not analyze for i-C16:0 or 20H-16:0. Y. pseudotuberculosis and Y. pestis also had fatty acid profiles which were very similar to Y. enterocolitica (Jantzen and Lassen; 1980).

Statistical analysis showed that only the type of fatty acid and the interaction of pH and strain with the type of fatty acid were significant ($P < 0.0001$). It appears that only specific fatty acids within the cell change in response to environmental pH. The most significant difference was observed at pH 5.0, indicating the increased stress provided by the acidic environment in relation to neutral or alkaline environments. No trends across species were observed in the degree of saturation in relation to pH, however, strain differences were observed. Saturation of the fatty acids increases the stability of the membrane and could offer a possible means of pH adaptation (Tanford, 1973;

Table 2. Mean Percent of the Major Fatty Acids Across all pH Levels in Y. enterocolitica Grown on Brain Heart Infusion Broth at 25° C, all Cells in Log Phase. (Mean values by strain can be found in Appendix 1-6.)

Fatty Acid	Relative Percent - Fatty Acid
16:1	26.66 ^a
17:0 Δ	13.46 ^b
16:0	12.48 ^{bc}
16:0	10.98 ^c
20H-16:0	8.93 ^d
18:1 (trans)	5.85 ^e
12:0	5.19 ^{ef}
14:0	3.79 ^{fg}
19:0 Δ	2.40 ^g
# 4	2.14 ^g

^{a-g}Means in column with different superscripts are significantly different (P<0.05).

= Unknown component.

Longworthy, 1978). A comparison of saturated and unsaturated fatty acids by strain at pH 5.0, 7.0, and 9.0 is shown in Table 3. No significant ($P < 0.05$) genus or serotype specific trends in the relative percent of saturated fatty acids were observed (Table 3). Three of the strains; E752, IP383 190-34/1B, had a significantly ($P < 0.05$) lower concentration of saturated fatty acids at pH 9.0 than at pH 5.0 (Table 3). The remaining strains; WA+, Y7P, 0:13,7, had no significant differences ($P < 0.05$) in the relative percentages of saturated fatty acids.

Significant differences ($P < 0.05$) were noted for the levels of cyclopropane fatty acids, branched forms as well as cis versus trans fatty acids (Table 4). Several trends could be observed from the data (Figure 1). The quantity of cyclopropane fatty acids present in the strains did signific. Two of the strains; WA+, 190-34/1B, had small amounts of the cyclopropane forms at all of the pH levels. No significant ($P < 0.05$) variations in cyclopropane fatty acids with pH were observed for these strains (Figure 1). The 9:13,7, IP383, Y7P, all had significantly ($P < 0.05$) more cyclopropane fatty acids present at pH 5.0 (Figure 1). The increased quantity of cyclopropane fatty acids would probably assist in maintaining the fluidity of the membrane at the lower pH (Stryer, 1981).

The quantity of branched fatty acids present at the three pH levels again varied by strain (Figure 2). Two strains; E752 and IP383, had low levels of branched fatty acids at all pH levels (Figure 2). The remaining strains; WA+, 0:13,7, Y7P, 190-34/1B, had significantly more ($P < 0.05$) branched fatty acids at pH 9.0 than pH 5.0 (Figure 2). At pH

Table 3. Mean Relative Percentages of Saturated Fatty Acids* at pH 5, 7 and 9 of *Yersinia enterocolitica* grown in Brain Heart Infusion Broth at 25 C. All Cells Were in the Log Phase of Growth.

Strain	Serotype	pH	Mean Relative Percent		
			5.0	7.0	9.0
E752	0:3		74.02b	72.35b	50.03ab*
IP383	0:9		94.53c*	52.86a	47.32a
WA+	0:8		65.19b	71.66ab	66.27bc
Y7P	0:8		58.88b	53.44a	69.03c
137	0:13,7		47.55a	67.38b	59.59bc
190-34/1B	0:8		94.06b*	58.38b	61.64abc

abc Means followed by different letters in columns are significantly different ($p < 0.05$).

*y Means followed by different letters in rows are significantly different ($p < 0.05$).

* = Saturated fatty acids are a summation of the following relative percentages; 12:0, 13:0, 30H-12:0, 14:0, 114:0, 15:0, 20H14:0, 30H14:0, 116:0, 16:0, 117:0, 17:0, 17:0, 20H-16:0, 18:0, 19:0, 19:0, 20:0.

Table 4. Mean Relative Percentages of Fatty Acids Found in *Yersinia enterocolitica* at three pH's Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase. Actual Values for Each Strain are Located in Appendix.

	Mean Relative Percentage Fatty Acid		
	5	7	9
12:0	3.60	4.96	6.81
#1	0.01	0.22	0.12
13:0	0.00	0.13	0.09
#2	0.03 ^a	0.08 ^{ab}	0.14 ^b
30H-12:0	0.25	0.43	0.27
14:0	2.73 ^a	2.97 ^a	5.53 ^b
i15:0	0.69	0.60	0.69
a15:0	0.34 ^a	0.46 ^a	1.00 ^b
15:0	0.18	0.40	0.64
20H-14:0	0.27	0.26	0.80
30H-14:0	0.44	0.23	0.44
#3	0.05	0.02	0.02
i16:0	3.50 ^a	9.18 ^a	23.64 ^b
16:1	17.86 ^a	23.65 ^{ab}	28.72 ^b
16:0	17.17 ^a	7.21 ^b	9.32 ^b
#4	0.23 ^a	4.61 ^b	1.34 ^b
i17:0	0.00	0.00	0.23
17:0 Δ	24.39 ^a	13.80 ^b	3.55 ^c
17:0	0.01 ^a	0.07 ^a	0.92 ^b
20H-16:0	6.00 ^a	14.07 ^b	6.35 ^a
#5	0.00	3.25	0.05
18:2	1.24	0.10	1.93
18:1cis	3.95	1.29	0.86
18:1trans	3.32 ^a	6.83 ^b	7.10 ^b
18:0	1.27 ^a	0.57 ^b	0.38 ^b
#6	0.00	1.86	0.02
19:0 Δ	5.33 ^a	2.03 ^b	0.21 ^c
19:0	0.00	0.02	0.03
20:0	0.00	0.37	0.37
# 2*	0.26 ^a	0.54 ^b	0.46 ^b

abc Values in rows with different superscripts are significantly different (P<0.05).

= Unknown compound.

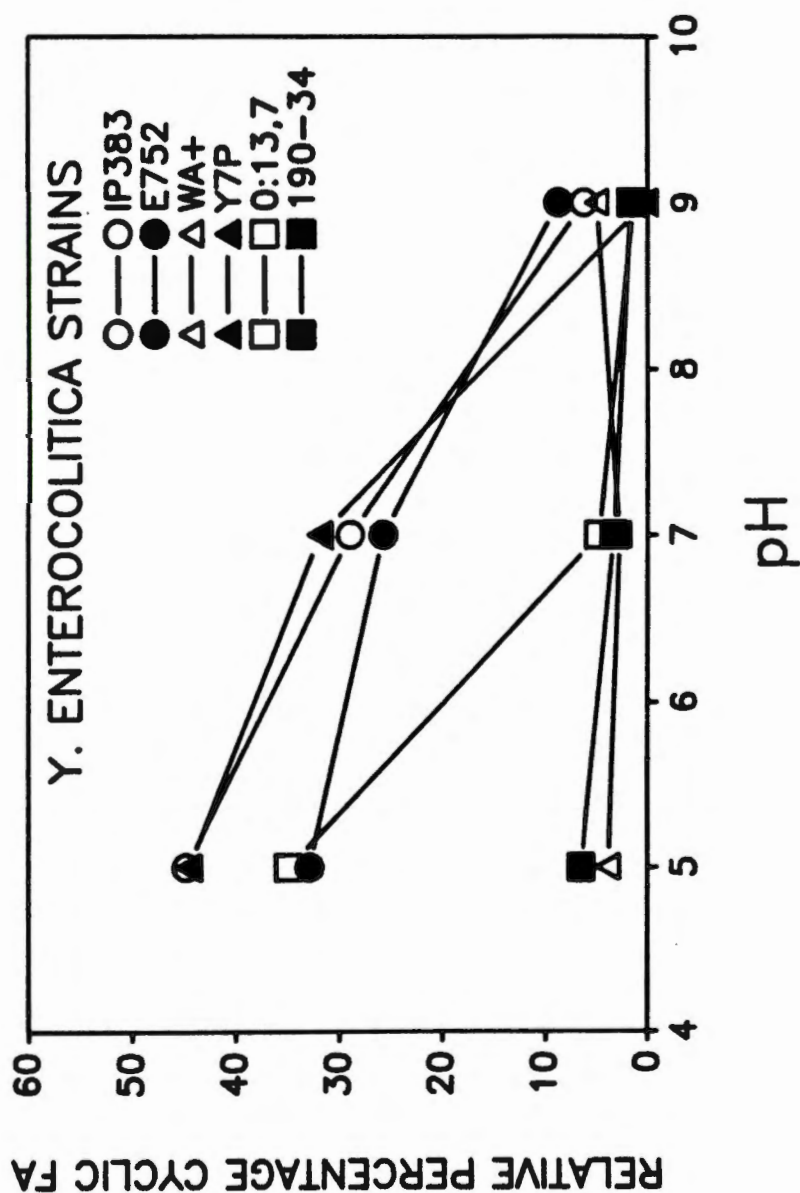


Figure 1. Influence of pH on the relative percent of cyclopropane fatty acids isolated from six strains of *Yersinia enterocolitica* during log phase growth at 25° C in Brain Heart Infusion Broth.

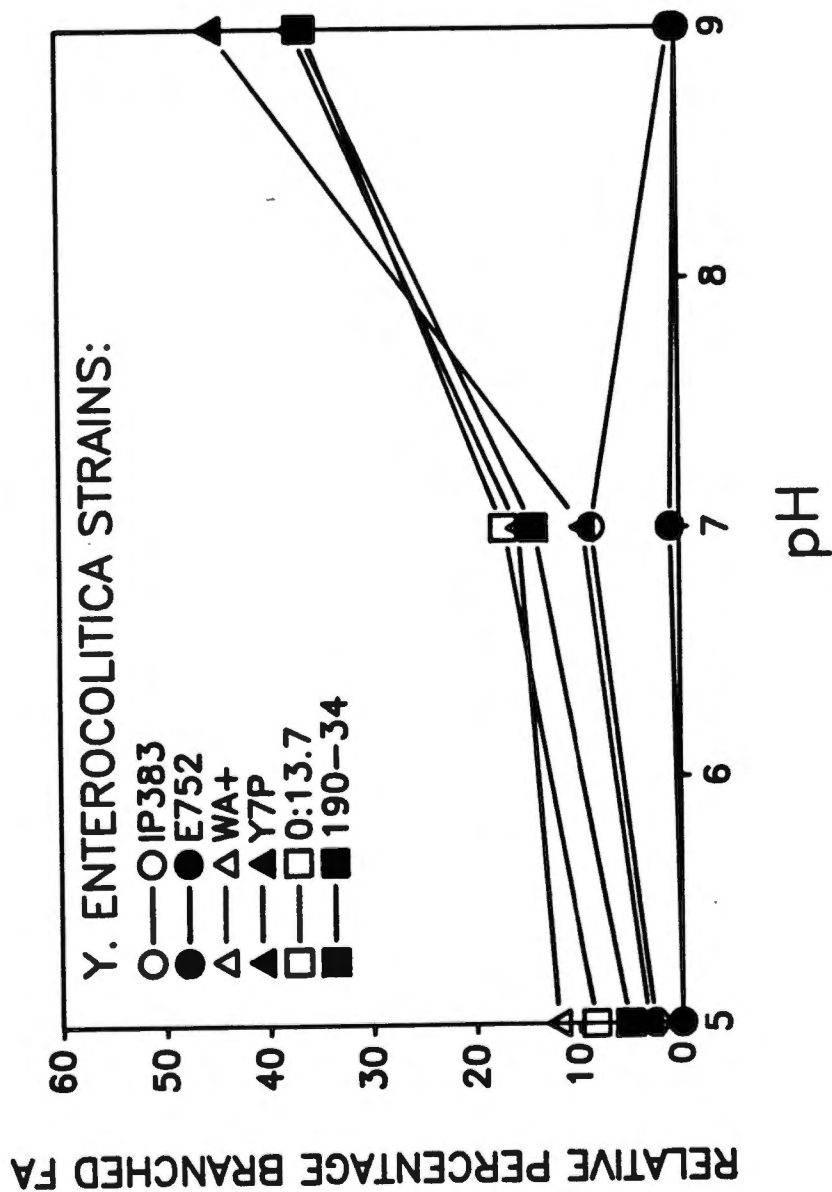


Figure 2. Influence of pH on the relative percent of branched fatty acids isolated from six strains of Yersinia enterocolitica.

9.0, all strains showed increased amounts of branched forms of C15:0 and C16:0. The combination of branched forms with the lower total lipid values obtained at this pH made the total relative percentage of the branched forms even higher. A possible effect of the branching is to increase stability of preventing saponification at alkaline pH (Longworthy, 1978). The amount of C18:1 in the trans form also increased at pH 9.0. The increased levels of trans form might have the effect of increasing the rigidity of the cell envelope which would prevent the introduction of OH^- into the cell envelope structure (Stryer, 1981; Langworthy, 1978). This effect may have been offset by large increase in C16:1 at this pH. The analysis did not show whether the structure was a cis or trans form. Saponification of the cellular lipid would result in eventual breakup of the cell envelope.

Three of the six strains tested; E752, Y7P, IP383 exhibited no significant variation ($P < 0.05$) in the total relative percentages of hydroxy fatty acids in response to pH (Figure 3). Both WA+ and 190-34/1B had significantly lower ($P < 0.05$) quantities of hydroxy fatty acids in the form of 2OH-16:0 at pH 9.0 (Figure 3) (Appendix 5, 6). The serotype 0:13, 7 strain had significantly less hydroxy fatty acid at both pH extremes (Figure 3). All of the strains had a relatively low level of hydroxy fatty acids at pH 9.0 (Figure 3). The possibility exists that this may be related to the high concentration of OH^- present in the environment and the corresponding potential for saponification of the fatty acids in the membrane structure.

The trends observed at pH 5.0 consisted of an increase in the relative percentage of cyclopropane acids and cis C18:1 with a decrease

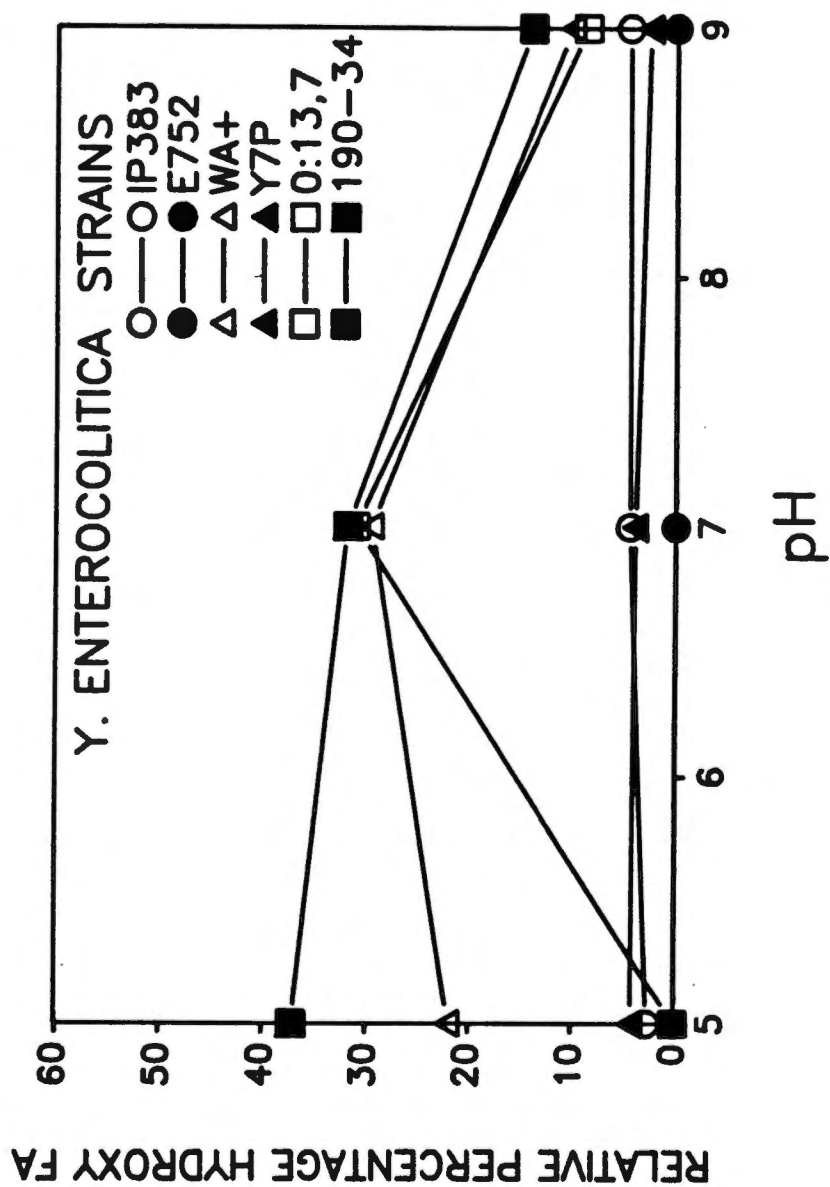


Figure 3. Influence of pH on the relative percent of Hydroxy fatty acids isolated from six strains of Yersinia enterocolitica.

in the quantity of branched and trans forms. Both the increased amount of cis C18:1 and cyclopropane forms serve to increase membrane fluidity by the initiation of "kinks" in the hydrocarbon tails (Styer, 1981).

Nutrient uptake is probably the responsibility of porin proteins. Levels of porin proteins have been shown to be pH independent (Bielecki and Hrebenda, 1982). Maintaining the membrane at both pH extremes appears to be the cells primary problem followed by nutrient uptake. At high pH val. many essential elements become insoluble, while at low pH the solubility of some ions is increased to the point that they may become toxic (Langworthy, 1978).

It is also important to note the significant variation of the fatty acids in relation to identification of bacteria. Other researchers have noticed variation in they cyclopropane fatty acids in relation to temperature, media, atmosphere and growth phase (Marr and Ingraham, 1962). With this in mind, it becomes clear that a good deal of caution should be exercised in using fatty acid profiles to identify bacteria. Growth conditions prior to the analysis need to be strictly controlled.

3. Phopholipid Determination

The relative percentage phospholipid was determined by high performance liquid chromatography. The objective of this experiment was to determine if any correlation existed between pH and the type and quantity of phospholipid.

Five phospholipids were identified in the cell of Y. enterocolitica: phosphatidyl inositol, phosphatidyl serine,

phosphatidyl ethanolamine, phosphatidyl glycerol and a compound with the same Rf as phosphatidyl choline are rarely found in bacteria.

Langworthy (1978) reported finding trace amounts of phosphatidyl choline in acidophiles. Toirabene (1973) did report finding a compound with the same Rf value as phosphatidyl inositol in Y. pseudotuberculosis.

Further testing yielded inositol as a constituent, so it is possible that Y. enterocolitica and Y. pseudotuberculosis possess low levels of phosphatidyl inositol. Since the analytical technique used in this study only detects the presence of double bonds it is possible that other phospholipids are present but not detected. Comparison of the values to those obtained by Tornabene (1973) for Yersinia pestis and Yersinia pseudotuberculosis showed a similar phospholipid composition with the primary constituent being phosphatidyl ethanolamine.

Phosphatidyl ethanolamine in a basic form is likely to be a more base stable form. Since phosphatidyl ethanolamine was the main phospholipid in Y. enterocolitica at pH 9.0, this would suggest that the cell can adjust its phospholipid biosynthesis to a form which has a greater affinity towards basic pH. At pH 5.0 however, the amount of phosphatidyl ethanolamine decreased while the amount of phosphatidyl serine increased (Table 5). Phosphatidyl serine is an acid form, and is thus more acid stable. Apparently, Y. enterocolitica may be able to alter biosynthesis of phospholipids to achieve a more acid stable form.

This trend was not immediately observable from the mean values for all strains because of skewing resulting from strain 0:13,7 (Appendix 7). Therefore, data from 0:13,7 is separated from other strains and presented separately in Appendix 7. This strain manifested

Table 5. Mean Relative Percentages of Phospholipids of Log Phase *Yersinia enterocolitica* Grown in Brain Heart Infusion Broth at 25° C at Three pH's. Data presented are the means of five strains. (Actual values by strain can be found in Appendix 7-12.)

Phospholipid	Mean Relative Percentage Phospholipid		
	5	7	9
Phosphatidyl inositol	4.26 ^a	4.68 ^a	1.01 ^a
Phosphatidyl serine	32.93 ^a	3.18 ^b	6.74 ^b
Phosphatidyl ethanolamine	42.67 ^a	82.81 ^b	87.78 ^b
Phosphatidyl glyderol	6.95 ^a	4.90 ^a	1.56 ^a
Phosphatidyl choline	12.96 ^a	4.44 ^b	2.91 ^b

^{a, b} Means in rows with different superscripts are significantly different (P<0.05).

an opposing trend for phosphatidyl ethanolamine and phosphatidyl serine, possibly due to enzyme differences for this strain.

Statistical analysis of the values showed significant differences ($P < 0.05$) occurring for phosphatidyl serine, phosphatidyl ethanolamine and phosphatidyl glycerol. The percent phosphatidyl glycerol tended to be higher in most strains at pH 5.0 (Appendix 7). Phosphatidyl glycerol can be converted into acid-stable amino acid esters or base-stable cardiolipin (Vaczi, 1973) providing another possible pH defense mechanism. The amounts of the phosphatidyl choline-like compound tended to increase at the lower pH's except for strain 0:13,7 where it decreased and strain 190-34/1B where it was not found (Appendix 7). The low levels of the phosphatidyl choline-like compound or its absence would indicate that it is of minor importance in coping with environmental pH changes. Changes in the degree of saturation would affect the relative percentage of phospholipids observed by this method. Further studies using fatty acids labeled with radioisotopes may revolve which phospholipids contain the unsaturated fatty acids.

The effect of pH on the lipopolysaccharide component may be even more important for survival at pH extremes. Lack of lipopolysaccharide has been observed in acid sensitive mutant strains of E. coli (Bielecki et al., 1982; Russell and Furr, 1986). The lipopolysaccharide of Y. enterocolitica has been characterized (Rische et al., 1973). Studies need to be undertaken to evaluate the effects of pH on lipopolysaccharides and changes in enzyme systems regulating the cell envelope. Both of these could be affected by extremely acidic or alkaline environments. Langworthy (1978) reported that organisms

requiring extreme pH values do not have peculiarities in the functioning of their enzyme systems. He suggests that the internal pH is maintained by the cell membrane or by metabolically active pumping.

CHAPTER V

CONCLUSIONS

The six strains of Yersinia enterocolitica (E752, Y7P, IP383, 190-34/1B, WA(+), and O:13,7) used in this experiment manifested specific changes in the levels of total lipids, fatty acids and phospholipids when subjected to different pH extremes.

The amount of total lipid in the cell tended to increase as pH decreased. This seems logical from the perspective that the cell's primary problem in survival and growth at pH extremes is most likely maintaining an intact cell envelope followed by nutrient uptake. The high lipid content plus the increased levels of cyclopropane and cis forms of the fatty acids at pH 5.0 may have served to assist in the maintenance of the membrane by increasing the fluidity (Stryer, 1981; Langworthy, 1978). Cells subjected to pH 5.0 also had elevated amounts of phosphatidyl serine, an acid stable compound.

An opposing trend was observed for the cells subjected to alkaline environments. Lower total lipid percentages were observed for the cells subjected to alkaline environments. A possible benefit of the lower lipid levels, when high levels of OH^- were present in the environment, is that less substrate was available for saponification which would result in eventual membrane breakdown. The cells subjected to pH 9.0 would most likely have more rigid membranes due to the presence of branched and trans forms of fatty acids (Stryer, 1981;

Langworthy, 1978). In addition, almost all of the phospholipid was in the form of phosphatidyl ethanolamine which is base stable.

Phospholipids may also play a role in anion transport; therefore the ability of the cell to adapt its total lipid and phospholipid biosynthesis to the pH of its environment may be one reason that Y. enterocolitica shows increased tolerance to wide pH ranges and extremely alkaline environments.

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APPENDIX

Table A-1. Mean Relative Percentage of Fatty Acids Found in Yersinia enterocolitica Strain E752 at Three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Fatty Acid	pH	E752 Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
12:0		5.96	6.70	6.02
#1		0.00	0.43	0.13
13:0		0.00	0.00	0.08
#2		0.00	0.06	0.08
30H12:0		0.00	0.22	0.27
14:0		4.58	4.67	5.42
i15:0		0.00	0.61	0.23
a15:0		0.00	0.56	0.22
15:0		0.00	0.33	2.38
20H14:0		0.00	0.04	0.85
30H14:0		0.00	0.22	0.36
#3		0.00	0.00	0.10
i16:0		0.00	1.17	11.76
16:1		5.13 ^a	15.83 ^b	35.04 ^b
16:0		29.94	20.21	16.81
#4		0.00	5.92	2.90
i17:0		0.00	0.00	0.02
17:0Δ		29.64 ^a	23.09 ^a	6.49 ^b
17:0		0.00	0.00	0.00
20H16:0		0.00	0.01	0.04
#5		0.00	5.66	0.29
18:2		0.00	0.02	0.06
18:1cis		15.44 ^a	5.43 ^b	2.27 ^b
18:1trans		5.42	4.58	5.77
18:0		0.69	0.34	0.08
#6		0.00	9.74	0.09
19:0		3.24	0.90	0.00
20:0		0.00	1.98	1.97
#2*		0.00	0.00	0.00

^{ab}Values in rows with different superscripts are significantly different (P<0.05).

i or a = Branched forms of fatty acids.

= Unknown compound.

Table A-2. Mean Relative Percentage of Fatty Acids Found in Yersinia enterocolitica Strains IP383 at three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Fatty Acid	pH	IP383		
		Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
12:0		4.64	4.19	4.33
#1		0.00 ^a	0.50 ^b	0.15 ^{ab}
13:0		0.00	0.00	0.19
#2		0.00 ^a	0.15 ^a	0.42 ^b
3OH-12:0		0.57	0.39	0.38
14:0		3.90	3.16	7.61
i15:0		0.00	0.49	0.25
a15:0		0.57	0.51	0.35
15:0		0.00	0.61	0.25
20H-14:0		0.07	0.21	2.74
3OH-14:0		2.05 ^a	1.01 ^a	1.10 ^b
#3		0.00	0.00	0.00
i16:0		2.09	4.79	0.00
16:1		0.14 ^a	22.28 ^b	38.34 ^b
16:0		34.25 ^a	1.44 ^b	22.51 ^c
#4		0.00	15.65	1.68
i17:0		0.00	0.00	1.21
17:0Δ		37.09 ^a	18.15 ^{ab}	6.38 ^b
17:0		0.00	0.00	0.11
20H-16:0		0.00	2.03	0.59
#5		0.00	11.70	0.00
18:2		0.00	0.00	0.53
18:1cis		0.00	0.00	0.81 ^b
18:1trans		5.33	10.23	8.91
18:0		1.62	0.93	0.82
#6		0.00	0.16	0.00
19:0Δ		7.68 ^a	1.43 ^b	0.13 ^b
19:0		0.00	0.00	0.00
20:0		0.00	0.00	0.00

^{a-c}Values in rows with different superscripts are significantly different (P>0.05).

i or a = Branched forms of fatty acids.

= Unknown compound.

Table A-3. Mean Relative Percentage of Fatty Acids Found in Yersinia enterocolitica Strain Y7P at three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Fatty Acid	pH	Y7P Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
12:0		4.57 ^a	4.09 ^{ab}	3.76 ^b
#1		0.05	0.26	0.35
13:0		0.00	0.00	0.03
#2		0.14	0.23	0.25
3OH-12:0		0.58	0.70	0.61
14:0		2.23 ^a	2.25 ^a	7.39 ^b
i15:0		0.08	0.18	0.79
a15:0		0.87	0.85	0.90
15:0		0.28 ^a	0.52 ^b	0.35 ^{ab}
20H-14:0		1.20	1.14	0.69
3OH-14:0		0.00	0.00	0.91
#3		0.21	0.31	0.00
i16:0		2.27 ^a	8.42 ^a	43.51 ^b
16:1		35.89 ^a	29.07 ^{ab}	21.35 ^b
16:0		0.00	0.62	4.31
#4		1.07	3.03	2.56
i17:0		0.00	0.00	0.00
17:0Δ		37.31 ^a	29.29 ^b	0.00 ^c
17:0		0.06 ^a	0.40 ^a	4.79 ^b
20H-16:0		1.02	2.07	0.33
#5		0.00	0.00	0.00
18:2		1.73	0.21	0.96
18:1cis		1.05	0.40	0.94
18:1trans		2.12	6.03	4.48
18:0		1.25 ^a	0.44 ^b	0.43 ^b
#6		0.00	0.00	0.00
19:0Δ		7.17 ^a	2.47 ^b	0.06 ^b
19:0		0.00	0.00	0.17
20:0		0.00	0.00	0.00
#2		0.00	0.00	0.00

a-c Values in rows with different superscripts are significantly different (P<0.05).

i or a = Branched forms of fatty acids.

= Unknown compound.

Table A-4. Mean Relative Percentage of Fatty Acids Found in Yersinia enterocolitica Strain 0:13,7 at three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Fatty Acid	pH	0:13,7 Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
12:0		0.00	4.08	15.86
#1		0.00	0.00	0.00
13:0		0.00	0.00	0.16
#2		0.00	0.00	0.00
3OH-12:0		0.00	0.00	0.17
14:0		1.34	2.33	3.85
i15:0		0.00	0.29	0.55
a15:0		0.12	0.19	2.30
15:0		0.09	0.24	0.18
2OH-14:0		0.00	0.00	0.00
3OH-14:0		0.00	0.00	0.91
#3		0.00	0.00	0.00
i16:0		8.22	16.59	24.94
16:1		32.10	25.09	23.38
16:0		0.00	6.89	2.69
#4		0.00	0.00	0.00
i17:0		0.00	0.00	0.00
17:0Δ		28.53 ^a	1.53 ^b	1.62 ^b
17:0		0.00	0.00	0.00
2OH-16:0		0.00	31.00	16.85
#5		0.00	0.00	0.00
18:2		5.80	0.12	8.38
18:1cis		9.26	0.66	0.42
18:1trans		4.82	5.93	7.78
18:0		2.93 ^a	0.69	0.39
#6		0.00	0.00	0.00
19:0Δ		6.31	3.18	0.91
19:0		0.00	0.00	0.00
20:0		0.00	0.00	0.00
#2		0.46	1.17	0.86

^{a-c}Values in rows with different superscripts are significantly different (P<0.05).

i or a = Branched forms of fatty acids.

= Unknown compound.

Table A-5. Mean Relative Percentage of Fatty Acids Found in Yersinia enterocolitica Strain 190-34/1B at three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Fatty Acid	pH	190-34/1B Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
12:0		4.44	5.13	3.27
#1		0.00	0.00	0.00
13:0		0.00	0.00	0.00
#2		0.00	0.00	0.00
3OH-12:0		0.00	0.00	0.00
14:0		3.15	2.62	3.89
i15:0		0.00	1.00	1.58
a15:0		0.00	0.11	0.45
15:0		0.00	0.31	0.19
2OH-14:0		0.00	0.00	0.00
3OH-14:0		0.00	0.00	0.00
#3		0.00	0.00	0.00
i16:0		5.08	13.09	34.55
16:1		2.24	32.46	29.04
16:0		37.08 ^a	0.65 ^b	1.87 ^b
#4		0.00	0.00	0.00
i17:0		0.00	0.00	0.00
17:0Δ		1.49	0.95	1.63
17:0		0.00	0.00	0.00
2OH-16:0		37.19 ^a	31.78 ^a	13.99 ^b
#5		0.00	0.00	0.00
18:2		0.00	0.08	0.22
18:1cis		1.19	0.54	0.19
18:1trans		1.36 ^a	6.63 ^b	7.74 ^b
18:0		0.76 ^a	0.39 ^{ab}	0.22 ^b
#6		0.00	0.00	0.00
19:0Δ		4.96 ^a	2.33 ^b	0.00 ^c
19:0		0.00	0.00	0.00
20:0		0.00	0.00	0.00
#2		1.16	1.39	1.15

^{a-c}Values in rows with different superscripts are significantly different (P<0.05).

i or a = Branched forms of fatty acids.

= Unknown compound.

Table A-6. Mean Relative Percentage of Fatty Acids Found in Yersinia enterocolitica Strain WA(+) at three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Fatty Acid	pH	WA(+)		
		Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
12:0		3.23	6.00	6.21
#1		0.00	0.00	0.00
13:0		0.00	1.08	0.00
#2		0.00	0.00	0.00
30H-12:0		0.00	1.43	0.00
14:0		2.38	2.56	3.93
i15:0		0.10 ^a	0.39 ^a	1.89 ^b
15:0		0.73	0.36	0.16
20H-14:0		0.00	0.00	0.00
30H-14:0		0.00	0.00	0.00
#3		0.00	0.00	0.00
i16:0		7.19 ^a	13.86 ^{ab}	34.25 ^b
16:1		32.65	16.35	23.57
16:0		20.31	13.28	3.25
#4		0.00	0.00	0.00
i17:0		0.00	0.00	0.00
17:0Δ		0.20	1.39	5.01
17:0		0.00	0.00	0.00
20H-16:0		21.91	28.09	10.12 ^b
#5		0.00	0.00	0.00
18:2		0.29	0.16	0.28
18:1cis		0.76	0.00	0.00
18:1trans		1.11 ^a	7.86 ^b	8.61 ^b
18:0		0.57	0.56	0.25
#6		0.00	0.00	0.00
19:0Δ		3.01	1.97	0.00
19:0		0.00	0.00	0.00
20:0		0.00	0.00	0.00
#2		0.78	1.18	1.27

^{a-c}Values in rows with different superscripts are significantly different (P<0.05).

i or a = Branched forms of fatty acids.

= Unknown compound.

Table A-7. Mean Relative Percentage of Phospholipids Found in *Yersinia enterocolitica* at three pH's, Grown in Brain Heart Infusion Broth at 25° C, all Cells in Log Phase

Strain	Phospholipid	Mean Relative Percentage Fatty Acid		
		5.0	7.0	9.0
E752	PI	0.93	6.09	1.22
	PS	40.68 ^a	2.28 ^b	9.94 ^b
	PE	24.31 ^a	74.43 ^b	84.43 ^b
	PG	8.16	11.85	0.86
	PC	24.91	5.35	3.55
Y7P	PI	2.39	0.66	0.58
	PS	45.61 ^a	4.46 ^b	2.59 ^b
	PE	41.48 ^a	88.41 ^b	92.97 ^b
	PG	0.00	1.77	1.94
	PC	10.53	4.69	1.91
IP383	PI	10.38	9.38	1.41
	PS	20.39	1.53	7.63
	PE	54.99	80.35	84.63
	PG	7.53	5.56	0.28
	PC	6.72	3.18	6.05
O:13,7	PI	24.73	9.74	5.28
	PS	37.03 ^a	75.16 ^b	90.79 ^b
	PE	24.50 ^a	5.98 ^b	0.75 ^b
	PG	13.75	7.78	1.56
	PC	0.00	1.34	1.62
190 34/1B	PI	0.00	3.59	1.72
	PS	20.69	2.19	6.32
	PE	57.68	91.44	86.46
	PG	21.63 ^a	2.77 ^b	5.49 ^b
	PC	0.00	0.00	0.00
WA(+)	PI	7.16	2.63	0.00
	PS	33.35 ^a	6.07 ^b	7.23 ^b
	PE	38.48 ^a	82.00 ^b	91.09 ^b
	PG	0.00	0.30	0.00
	PC	20.99 ^a	8.99 ^{ab}	1.67 ^b

a-b Values in rows with different superscripts are significantly different ($P < 0.05$).

PI = phosphatidyl inositol
 PS = phosphatidyl serine
 PE = phosphatidyl ethanolamine
 PG = phosphatidyl glycerol
 PC = phosphatidyl choline

VITA

Laure Lynn Larkin was born on July 28, 1963, in Milwaukee, Wisconsin. Upon graduation from Aquinas High School in LaCrosse, Wisconsin in June 1981, she entered the University of Wisconsin at River Falls. In May 1985 she received a Bachelor of Science degree in Conservation. While at the University of Wisconsin-River Falls she began experimenting with Yersinia enterocolitica and Campylobacter jejuni in relation to their incidence in raw milk.

She entered the University of Tennessee at Knoxville in January 1986 and began work towards a Master of Science degree in Food Technology and Science. At the University of Tennessee-Knoxville she conducted her thesis research on Y. enterocolitica and continued experimenting with C. jejuni in an attempt to achieve growth of this organism under aerobic conditions using an Oxygen Reducing Membrane Fraction developed at ORNL. Upon completion of her course work she took a position in microbiological methods development for emerging pathogenic organisms.