CHEMICAL AND BIOLOGICAL STUDIES ON LIPIDS OF *LISTERIA* SPECIES

THESIS SUBMITTED

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In the name of Allah, the Beneficent, the Merciful.

Read ! and thy Lord is Most Honourable, and Most Benevolent,
Who taught (to write) by pen,
He taught man that which he knew not.
(Sura Al-Alaq 30: 3-5)
AL-Quran

Dedicated

to

my beloved Parents

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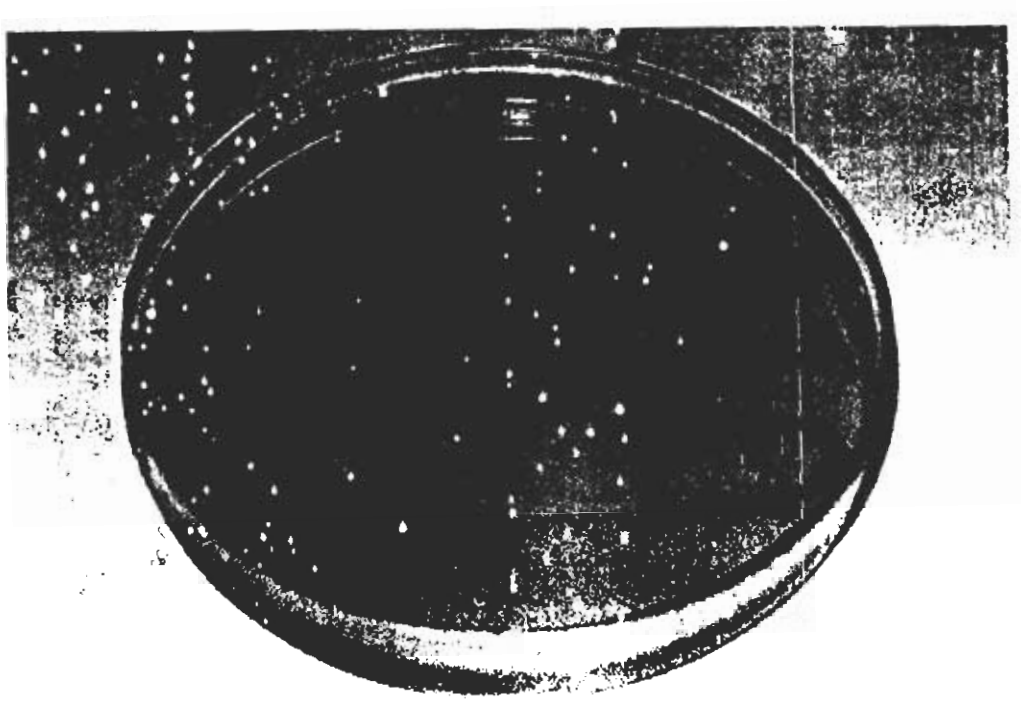

Growth of *L. monocytogenes* NCTC 7973 on TSA plate containing 1% 2, 3, 5 triphenyl tetrazolium chloride (tetrazolium salt was reduced to red formazon).

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LIST OF ABBREVIATIONS

BSA	Bovine serum albumin
CAMP	Christie Atkins Peterson
CFU	Colony forming unit
CLE	Crude lipid extract
DLC	Differential leukocyte count
DPX	Distrene- plasticizer -xylol
EC_{50}	50 percent effective dose
FA	Fatty acids
FAME	Fatty acid methyl ester
FITC	Fluorescein isothiocyanate
GC-MS	Gas chromatography-Mass spectrometry
H & E	Haemtoxylin and Eosin
HPLC	High pressure liquid chromatography
HREIM	High Resolution Electron Impact Mass
i.p	intra peritoneal
i.v	intravenous
K_2HPO_4	Dipotassium hydrogen phosphate
LD_{50}	50 percent lethal dose
MF	Mixture of (VLC) fractions
mg/kg	milligram / killogram
µl	Micro-litre
MPA	Monocytosis producing activity / ability
nm	Nanometer
NCTC	National culture type collection
O.D	Optical density
PBMN	Peripheral blood monocyte number
PBS	Phosphate buffer saline
PIH	Post inoculation hour
RSA	Rapid slide agglutination
SE	Saline extract
SLCC	Special *Listeria* culture collection
SLS	Sodium lauryl sulfate
Spp.	Species
SRBC	Sheep red blood cell
TLC	Thin layer chromatography
TPA	Tryptose phosphate agar
TPB	Tryptone phosphate broth
TSA	Tryptone soya broth
TTPTA	Tryptone Thallous Acetate Potassium Tellurite agar
VLC	Vacuum liquid chromatography
VMF-1	Mixture of (VLC) fractions 1-5
VMF-11	Mixture of (VLC) fractions 6-11
W/V	Weight / Volume

SUMMARY

Virulent and avirulent species of *Listeria* were studied and characterized on the basis of their morphological, cultural and biochemical characters. In addition CAMP test was also performed for species differentiation. The growth of *L. monocytogenes* NCTC 7973 on TSB and TPB incubated at different temperatures was investigated. The maximum production of biomass was observed in TSB medium at 20°C or 4°C. However low temperature substantially reduced the growth response in both the media. The LD_{50} of *Listeria* species was determined in white mice by intraperitoneal route and was found to be between $0.544x10^5$ — $2.01x10^8$. Animal passage modestly enhanced the virulence of *L. monocytogenes* NCTC 7973, as indicated by the lower LD_{50} value for passaged culture then unpassaged culture. This study further addresses above findings, in details, utilizing histopathological studies to portray the lesions pathogenesis with the help of microscopy.

5-7.8% (dry weight) of lipid was yielded in different *Listeria* species depending upon the degree of virulence of the species. The cellular fatty acid composition determined by gas chromatography-mass spectrometry, was found not to differ among *L. monocytogenes* NCTC 7973, *L. ivanovii* SLCC 7842, *L. seeligeri* SLCC 3954 by far the most common members are C_{15} and C_{16} chain length fatty acids. This pattern is rather similar in all species, whereas C_{19} and C_{22} carbon chain fatty acid is characteristic of lipid present in *L. monocytogenes*. At low temperature, *L. monocytogenes* is able to change the relative composition, which could increase the fluidity of the bacterial membrane. A key player in this connection is anteiso C_{15} fatty acid as more C_{15} fatty acid is produced at low temperature.

A marked monocytic reaction was observed after the injection of virulent *L. monocytogenes*, partly virulent *L. ivanovii*. There was no change after injection of *L. seeligeri*. Monocytosis producing activity is present not only in *L. monocytogenes* cells (live/killed) but was also present in lipid. On further fractionation of lipid by vacuum liquid chromatography the active fractions were found to contain high numbered carbon chain fatty acids along with the phosphorus, protein and carbohydrate.

The listerial lipids significnatly increased mouse resistnace toward the *Listeria* infection. Killed *L. monocytogenes* cells as well as their lipids exhibit a very high adjuvant activity. Experiments conducted for determining the effect of sustained monocytosis on serum antibody levels, suggests an association between monocytes and humoral immune response. MPA was also found to be present in saline extractable material (SE) from *L. monocytogenes*. The minimum dose of SE capable of producing a monocytosis was 50 mg/kg but it was found to be non-toxic when tested on *Aretemia salina* leach larvae.

Both MPA and immunostimulating activity were readily extracted with aqueous solvents and found as two interdependent activities by high performance liquid chromatography. As low as 50 mg/kg of the SE fractions SE-A and SE-C caused an elevation in the level of circulating monocytes and was found to be an effective promotor of immune response as indicated by the high serum antibody titre in mice and rabbits. Chemical analysis of SE and its active fractions showed the presence of phosphorus, carbohydrate and trace amount of protein. Furthermore SE and its fractions were found to stimulate the cell mediated and humoral components of the immune system in experimental animals. SE and its active fraction elicidated a dose-related increase in SRBC, induced by 4 hours (early) and 24 hours (delayed) hypersensitivity reactions in rats.