



UNIVERSITI PUTRA MALAYSIA

**EFFECT OF EPIPHYTIC LACTIC ACID BACTERIA ISOLATED FROM
GUINEA GRASS ON NUTRITIONAL VALUE OF THE SILAGES**

MAHDI PASEBANI

FP 2010 21



**EFFECT OF EPIPHYTIC LACTIC ACID
BACTERIA ISOLATED FROM GUINEA GRASS ON
NUTRITIONAL VALUE OF THE SILAGES**

MAHDI PASEBANI

MASTER OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

2010



**EFFECT OF EPIPHYTIC LACTIC ACID BACTERIA ISOLATED FROM
GUINEA GRASS ON NUTRITIONAL VALUE OF THE SILAGES**

By

MAHDI PASEBANI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

October 2010



DEDICATION

To my Father and my Mother who are the entire world to me



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFECT OF EPIPHYTIC LACTIC ACID BACTERIA ISOLATED FROM GUINEA GRASS ON NUTRITIONAL VALUE OF THE SILAGES

By

MAHDI PASEBANI

October 2010

Chairman : Associate Professor Halimatun Bt Yaakub, PhD

Faculty : Agriculture

An investigation was carried out to isolate and identify predominant indigenous and epiphytic lactic acid bacteria from guinea grass. The effects of epiphytic lactic acid bacteria on the nutritional value and aerobic quality of the silages were also studied. The standard plate counts of naturally occurring bacteria in guinea grass were estimated to 2.65×10^5 CFU/g in fresh grass while the amount for epiphytic lactic acid bacteria were only 8.3×10^3 CFU/g. Isolations were carried out by 10 fold serial dilution which resulted in 18 purified bacteria. Three indigenous bacterial species comprised of *Flavimonas oryzihabitans*, *Enerobacter cloacae*, *Sphingomonas paucimobilis* B and four epiphytic lactic acid bacteria included of *Weissella confusa*, *Weissella paramesenteroides*, *Leuconostoc mesenteroides* ssp. *dextranicum*, and *Lactococcus lactis* ssp. *hordniae* were identified by BIOLOG identification system. The four lactic acid bacteria were individually applied in inoculation of guinea grass silage at the rate of $1 \times$



10^5 CFU/g in fresh grass. Fixed amount of MRS broth was considered as a carrier for inoculation of these lactic acid bacteria and thereby, two controls (with and without broth) were proposed to evaluate the effect of carrier separately. Six treatments of silages were opened on days 14, 21 and 28 in three replications. Temperature, pH value, proximate and van soest analysis and ammonia nitrogen were measured for each silage sample. Epiphytic lactic acid bacteria were effective to increase crude protein and crude fat while acid detergent fiber, neutral detergent fiber and ammonia nitrogen declined significantly. Decreasing of pH value was speeded up in all inoculated silages and hence, the silage reached to stable phase on day 14 and remained stable up to day 21 and 28. The inoculated silages were remained in the accepted range of pH value up to 48 h when they exposed to the air.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN EPIFITIK BAKTERIA ASID LAKTIK ISOLASI DARIPADA RUMPUT
GUINEA PADA NILAI NUTRISI DALAM SILASE**

Oleh

MAHDI PASEBANI

Oktober 2010

Pengerusi: Associate Profesor Halimatun Yaakub, PhD

Fakulti: Pertanian

Suatu penyelidikan telah dijalankan untuk mengira bakteria hasilan secara semulajadi dan mengenalpasti kedua-dua bakteria asli utama dan epifitik bakteria asid laktik. Selanjutnya, kesan epifitik bakteria asid laktik pada nilai nutrisi dalam silase telah dipelajari. Perhitungan bakteria dengan plat piawai standard dalam rumput guinea segar menunjukkan bahawa jumlah bakteria hasilan secara semulajadi adalah setara dengan 2.65×10^5 CFU/g sementara jumlah ini untuk epifitik bakteria asid laktik dengan hanya 8.3×10^3 CFU/g. Oleh kerana itu, keperluan tambahan bakteria asid laktik sebagai aditif adalah disahkan hasil silase rumput guinea. Selain itu, dengan kejadian pembusukan aerobik. Tiga spesies bakteria asli terdiri daripada *Flavimonas oryzihabitans*, *Enerobacter cloacae*, *Sphingomonas paucimobilis* B dan empat epifitik

bakteria asid laktik termasuk *Weissella confusa*, *Weissella paramesenteroides*, *Leuconostoc mesenteroides* ssp. *dextranicum*, dan *Lactococcus lactis* ssp. *hordniae* telah dikenalpasti oleh sistem pengenalan BIOLOG. Empat bakteria asid laktik telah dilaksanakan secara individu dalam inokulasi silase rumput guinea pada kadar 1×10^5 CFU/g rumput segar. Jumlah pati MRS yang tetap telah dianggap sebagai pembawa semasa inokulasi daripada bakteria asid laktik dan dengan demikian, dua kawalan, iaitu kawalan dan kawalan dengan pati yang telah ditentukan untuk menilai kesan pembawa secara individunya. Enam rawatan silase telah dibuka pada hari 14, 21 dan 28 dalam tiga replikasi. Suhu, nilai pH, analisis proksimat dan van Soest dan nitrogen ammonia telah diukur pada setiap sampel silase. Epifitik bakteria asid laktik adalah berkesan untuk meningkatkan protein kasar dan lemak kasar sementara asid detergen serat dan serat detergen neutral adalah menurun secara signifikansi. Penurunan nilai pH telah dipercepat dan oleh itu, silase dapat mencapai ke tahap stabil pada hari ke-14 dan tetap stabil selama 48 jam semasa dikemukakan kepada udara.

ACKNOWLEDGEMENTS

First and foremost, my sincere gratitude goes to my Supervisory Committee Members. Associate Professor Dr. Halimatun Bt Yaakub, the Chairman who support me throughout of my studies. I was always receiving precise assistance from Professor Abdul Razak Alimon in the nutritional analysis. Associate Professor Dr. Kamaruzaman Sijam who gave me valuable advice during my microbiology laboratory work.

I would like to thank Universiti Putra Malaysia who accepted me as a graduate student. This study was supported by Research University Grant Scheme (RUGS), Universiti Putra Malaysia. I would also like to thank staff members of the Microbiology Laboratory of Department of Plant Protection, and Nutrition Laboratory of Department of Animal Science, Universiti Putra Malaysia because of their technical assistance.

I cherished the honest and kind help from fellow post graduate students, Mr. Saeid Nikbin, Mr. Shahram Shakiba, Mr. Seyed Reza Hashemi, Ms. Aida binti Zakaria and Ms. Tang Siew Ching.



I certify that an Examination Committee has met on 2010 to conduct the final examination of Mahdi Pasebani on his Master of Science thesis entitled “Effect of Epiphytic Lactic Acid Bacteria Isolated from Guinea Grass on Nutritional Value of the Silages” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Master of Science degree.

Members of the Examination Committee are as follows:

Loh Teck Chwen, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia

Zainal Aznam Mohd Jelan, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Radziah Othman, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Wan Zahari Mohamed, PhD

Livestock Strategic Research Centre
Malaysian Agricultural Research and Development Institute
(External Examiner)

BUJANG KIM HUAT, PhD
Professor/Deputy Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date:



This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The Members of the Supervisory Committee were as follows:

Halimatun Yaakub, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Abdul Razak Alimon, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Kamaruzaman Sijam, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies,
Universiti Putra Malaysia.

Date:



DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and not currently submitted for any other degree at Universiti Putra Malaysia or other institutions.

MAHDI PASEBANI

Date: 26 October 2010



TABLE OF CONTENTS

	Page
DEDICATION	iii
ABSTRACT	iv
ABSTRAK	vii
ACKNOWLEDGEMENTS	x
APPROVAL	xi
DECLARATION	xiii
TABLE OF CONTENTS	xiv
LIST OF FIGURES	xvi
LIST OF TABLES	xxv
LIST OF PLATES	xvii
LIST OF ABBREVIATIONS	xviii
 CHAPTER	
 1 GENERAL INTRODUCTION	 1
 2 LITERTAURE REVIEW	 3
2.1 Forages	3
2.1.1 Guinea Grass (<i>Panicum – maximum</i>)	5
2.2 Lactic Acid Bacteria	6
2.2.1 Classification of LAB at the Genus Level	6
2.2.2 Classification of LAB at the Species Level	8
2.2.3 Fermentation Pathways	11
2.3 Silage	13
2.3.1 Ensilage Process and Mechanisms	14
2.3.2 Crop Factors Influencing Ensiling	16
2.3.3 Harvest Management	17
2.3.4 Types of Silo	18
2.3.5 the Chemistry of Silage	19
2.3.6 Bacterial Inoculants	22
2.3.7 Aerobic Stability of the Silage	23
 3 ISOLATION AND IDENTIFICATION OF INDIGENOUS BACTERIA AND EPIPHYTIC LACTIC ACID BACTERIA FROM GUINEA GRASS	 25
3.1 Introduction	25
3.2 Methods and Materials	26
3.2.1 Grass Sampling for Bacteria Isolation	26
3.2.2 Media Preparation	27
3.2.3 Streaking Technique	27
3.2.4 Isolation of Bacteria	28



3.2.5	Bacteria Purification	29
3.2.6	Gram Staining Test and KOH Test	29
3.2.7	Oxidise Test	30
3.2.8	Identification	30
3.3	Results	32
3.3.1	Isolation and Identification	32
3.4	Discussion	37
3.5	Conclusion	39
4	EFFECT OF EPIPHYTIC LACTIC ACID BACTERIA INOCULANTS ON NUTRITIONAL VALUE AND AEROBIC STABILITY OF GUINEA GRASS SILAGE	40
4.1	Introduction	40
4.2	Materials and Methods	41
4.2.1	Experimental Design	41
4.2.2	Harvesting and Silage Making	42
4.2.3	Preparation of Lactic Acid Bacteria Inoculants	43
4.2.4	Sampling	45
4.2.5	Determination of Temperature and pH Value	46
4.2.6	Determination of Nutritional Value of Silages	46
4.2.7	Determination of Ammonium Nitrogen	47
4.2.8	Determination of Aerobic Stability	47
4.3	Data Analysis	48
4.4	Results	49
4.4.1	Population of Bacteria	49
4.4.2	Temperature at Day 14, 21 and 28 of Ensiling	50
4.4.3	The pH Value at Day 14, 21 and 28 of Ensiling	52
4.4.4	Nutritional Value of the Fresh Guinea Grass and Silages	53
4.4.5	The Aerobic Stability of the Silages	57
4.5	Discussion	59
5	CONCLUSION	63
	REFRENCES	65
	APPENDIX A	72
	APPENDIX B	86
	BIODATA OF STUDENT	91
	LIST OF PUBLICATIONS	92

