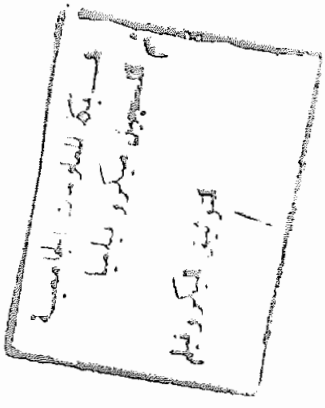
AN ATTEMPT TO PRODUCE RENNIN-ENZYME FROM STREPTOCOCCI



By

ASHRAF GAMIL ATTALLAH

A thesis submitted in partial fulfillment

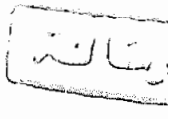
of

the requirements for the degree of

Master of Science

in

Agriculture
(Microbiology)



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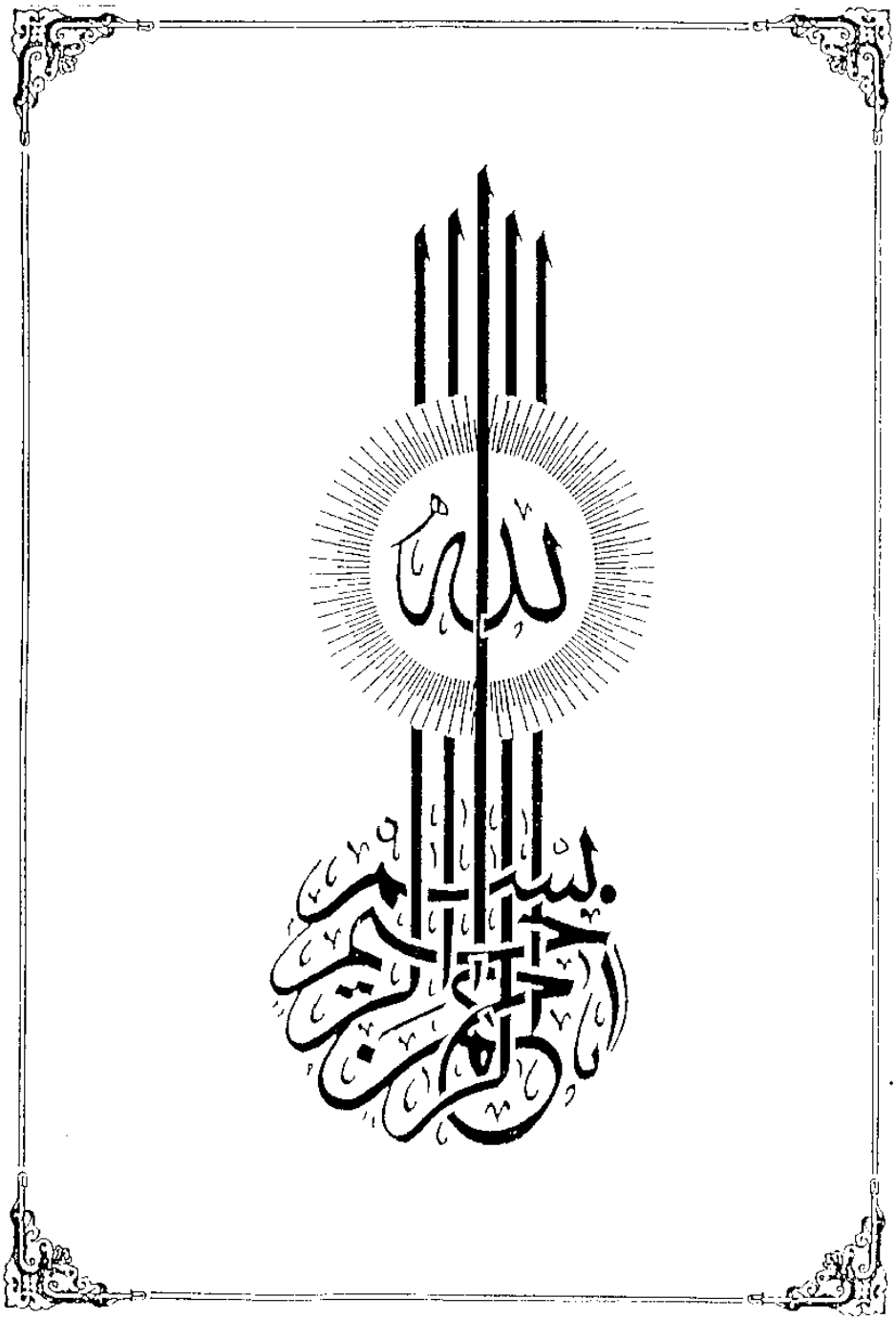

APPROVAL SHEET

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AN ATTEMPT TO PRODUCE RENNIN-ENZYME FROM STREPTOCOCCI

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Abstract

Different *Escherichia coli* and *Streptococcus lactis* rennin-producer transformants were obtained when buffalo brain, cow brain, buffalo maw or *Bacillus cereus* DNAs were used. Different genetic stability and rennin activity were found. Rennin gene was located at recipient chromosome.

Key words:

Streptococci
Rennin-enzyme.

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INTRODUCTION

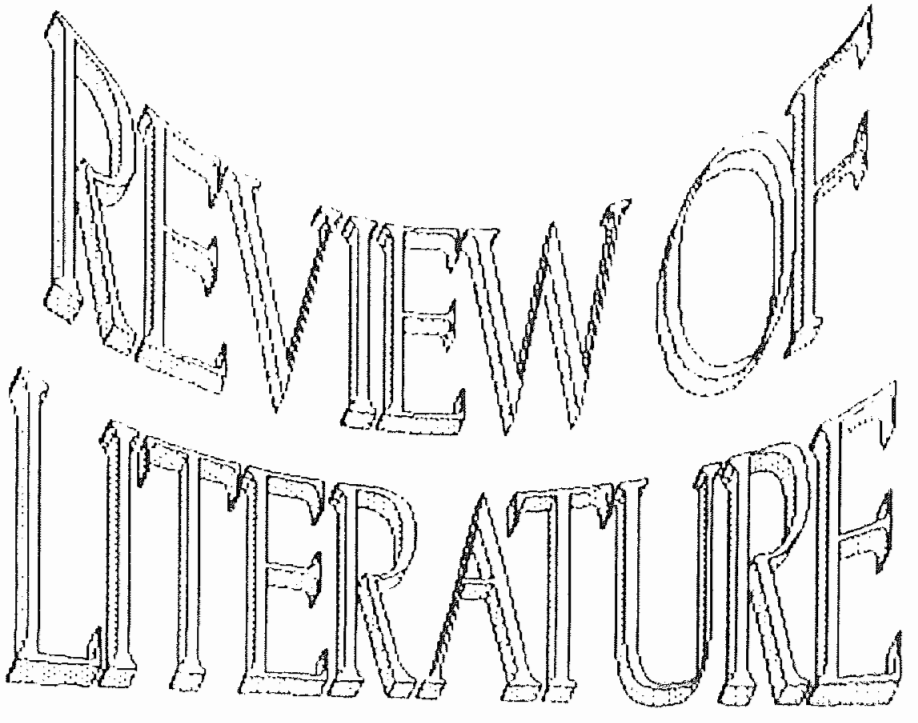
INTRODUCTION

Calf rennet , a crude extract of gastric enzyme containing 85 to 95% chymosin and 10 to 15% bovine pepsin (Hicks, et al., 1988) has been the traditional and preferred milk-clotting enzyme preparation for cheese making. Between 1962 and 1972, world cheese production was more than doubled, causing calf stomach, the raw material from which calf rennet is extracted, to become scarce. As a result, the price of calf rennet raised to over $ 65/gal (Nelson, 1979). Research was directed toward finding suitable substitutes for calf rennet, patents have been issued for the production of milk-clotting enzymes from some bacteria and Fungi. These enzymes have been approved from manufacturing all standard varieties of cheese. Recent developments in genetic engineering technology provided recombination chymosin as another alternative to the shortage of calf rennet. Contrary to commercial calf rennet, recombinant chymosin contains only the protein resulting from the expression of one gene sequence, so it contains only a single variant (Hicks et al., 1988).

Due to the increasing production of cheese several million tons, world wide, and a decline in the number of slaughtered calves, intensive research has been underway since 1960th to develop enzymatic product of microbial origin; calf rennet substituent or

rennin-like enzymes.

Therefore, this investigation is an attempt to obtain rennin enzyme streptococci producers. *S. lactis* was used as a target host, while cow and buffalo calf different tissues were used as rennin gene donors in addition to *B. cereus* which used as a donor for rennin-like gene.


REVIEW OF
LITERATURE

REVIEW OF LITERATURE

1. Rennin and microbial rennin like-enzyme:

Chymosin (EC.3.4.23.4), also known as rennin or chymase is the major proteolytic enzyme constituent of the rennet, an extract from the fourth stomach of 3 to 4 weeks old calves which have been raised on milk (Foltmann, 1970). Rennin is initially formed as pre-prochymosin which consist of pro-chymosin (PC), i.e., pro-rennin or the zymogen with 16 amino acids signal peptide at it's amino terminal end. Calf chymosin is secreted as inactive precursor, pro-chymosin (PC), consisting of single peptide chain with 365 amino acids residues it's molecular weight is 40,777 daltons. Prochymosin irreversably converted into active enzyme (rennin), i.e., 323 amino-acids it's molecular weight is 35, 652 daltons and is essential for desired enzymatic curdling, by limited proteolysis during which a total of 42 amino-acids residues are released from the amino-terminal part of the peptide chain (Pederson, Foltmann, 1975 and Pederson et al., 1979.

Farah and Bachmann (1987) stated that, variation of rennet action was found to be associated with milk kind diversity in terms of coagulation time. Cow milk coagulation was 2-3 times faster than that of camel milk. With both camel and cow milk, cogulation time was reduced with decreasing pH, increasing temperature and adding Ca^{++}. Isolation of chymosin from it's natural source, rennet, actually yields a mixture of prochymosin and chymosin each existing as two types called