THE PHYSIOLOGY OF STAPHYLOCOCCAL ENTEROTOXIN PRODUCTION

A thesis presented in partial fulfilment of the requirements for the degree of Ph.D in Microbiology at Massey University.

Audrey Winifred Jarvis

1974

ABSTRACT

The aims of this investigation were to study the environmental factors which determine enterotoxin production by staphylococci. Effects of pH, oxygen, medium composition, and in particular of added carbohydrates in a defined medium were investigated under controlled conditions, using several strains of each enterotoxin type.

The production of staphylococcal enterotoxins A, B and C in shake-flasks was studied throughout the growth cycle for nine strains of staphylococci. Enterotoxins were first detected in the exponential phase, and except for strain S-6, no appreciable increase in enterotoxin occurred during the stationary phase of growth. Enterotoxins were however produced in relatively small quantities by non-replicating cells from late exponential or stationary phases of growth. Chloramphenicol inhibited enterotoxin production by non-replicating cells. A small quantity of enterotoxin B was produced in the presence of actinomycin D, suggesting the accumulation of a small pool of mRNA for enterotoxin production.

The production of enterotoxins was investigated in a fermenter under controlled conditions of pH and aeration, using a casein hydrolysate medium and a defined amino acid medium. Enterotoxin production in the fermenter was considerably less than in shake-flasks for eight out of nine strains of staphylococci. The reasons for these differences between fermenter and shake-flask were investigated, particular attention being given to pH, aeration and antifoam.

Changes in the environment caused changes in the growth patterns of staphylococci as shown by alterations in the specific growth rate, the duration of the transition period from exponential to stationary phases of growth and the final cell yield. Attempts were made to determine how far the changes in the final yields of enterotoxin were due to effects on the specific rates of enterotoxin production, and which differences were consequences of altered growth patterns.

The optimum pH for enterotoxin A, B and C production in casein hydrolysate and amino acid media was determined. Growth of staphylococci under conditions of controlled pH resulted in a higher final yield of enterotoxin than growth without pH control. The implications of the effect of pH on enterotoxin A production in food is discussed.

The repression of enterotoxins when glucose or glycerol were added to the growth medium was studied under controlled conditions of pH, and with constantly maintained glucose or glycerol. Since enterotoxins A, B and C were repressed by both compounds in growing cells when the pH was held constant, repression was not due to the fall in pH which occurred when the pH was not controlled. The repression of enterotoxin by glucose or glycerol was always accompanied by an increase in growth rate. It has been suggested by other workers that there is an inverse relationship between growth rate and extracellular protein production (Coleman, 1967, Stormonth and Coleman, 1973). However, when S.aureus S-6 was grown in continuous culture, it was found that an increase in growth rate caused an increase in enterotoxin production. Also the addition of glucose to the medium repressed enterotoxin production when the growth rate was held constant.

Although glucose and glycerol repressed enterotoxin production by growing cells, neither compound inhibited enterotoxin production by non-replicating cells which had been grown without glucose. However, experiments with strain S-6 showed that cells grown in the presence of glucose and resuspended without glucose lacked the ability to produce enterotoxin B. This suggested that glucose inhibited the production of an essential pre-requisite for the synthesis or release of enterotoxin, such as an enzyme for the final conversion of an enterotoxin precursor to enterotoxin. Experiments with chloramphenicol had shown that there was no appreciable build-up of protein precursor for enterotoxin in non-replicating cells. However, very small accumulations of a precursor could repress enterotoxin synthesis by a feedback mechanism.

There were often marked differences in the effect of environmental changes on enterotoxin production by different strains of the same enterotoxin type. One of the aims of this investigation was to examine differences which have been reported to exist between mechanisms of production of the various enterotoxins, particularly enterotoxins A and B. The differences between strains were often greater than supposed differences between enterotoxins, and throughout this investigation several strains were used for each experiment wherever possible.

Staphylococci produce a large number of extracellular proteins. Throughout this study the production of lipase, deoxyribonuclease, lysozyme and total extracellular protein was followed to determine the extent to which the effects of environmental changes were specific to enterotoxin as distinct from a general effect on extracellular protein production.

ACKNOWLEDGEMENTS

I am grateful to Dr W.A. McGillivray, Director of the Dairy Research Institute for the opportunity to undertake this research.

I am also grateful to my supervisors, Drs G.G. Pritchard and R.C. Lawrence for their advice and encouragement throughout this investigation.

I am greatly indebted to Dr M.S. Bergdoll, University of Wisconsin, for supplying strains 100, S-6 and 361, and for the gift of enterotoxins A, B and C, and their corresponding antisera, also to Dr R.W. Bennett, for supplying strain 743.

My thanks are due to Mrs B. Hodren for excellent technical assistance, and to Dr Lawrence and Mrs Hodren for lipase, deoxyribonuclease and lysozyme assays. I would like to thank Dr K. Pearce for Mg^{++} determinations, and Dr K.R. Marshall for helpful discussion concerning the continuous culture experiments. I would also like to express my appreciation of the patience shown by my family during this study.

Part of the findings from Section 3 have been published in Infection and Immunity, 1973. 7: 874-854, under the title 'Production of staphylococcal enterotoxins A, B and C under conditions of controlled pH and aeration', by Audrey W. Jarvis, R.C. Lawrence and G.G. Pritchard.

CONTENTS

FIGURES

TABLES