Purification, characterization and utilization of bacterial rennet from *Bacillus subtilis* K-35 for cheese manufacture
INTRODUCTION

The milk coagulant traditionally used for cheese making is the rennet extract from the abomas of 10 to 30-day-old milk-fed calves. There has been a chronic world shortage of animal rennet during the last 20 years (FAO Report, 1968) and this has stimulated interest in the search for suitable rennet substitutes for use as coagulants in cheese manufacture.

India has to depend on imports to meet its entire rennet requirements, since slaughter of calves is not permitted in most of the States due to religious sentiments. Cheese made with calf rennet is also not acceptable to a large section of the Indian population who have such considerations. Development of rennet substitutes from non-animal sources is therefore, the natural step towards overcoming these difficulties and pave the way for speedy development of cheese industry in India.

Several plant and microbial sources have been investigated for production of milk clotting enzymes in different parts of the world with varied successes. The quality of cheese made with the use of plant enzymes have not been entirely satisfactory. Among a variety of microorganisms, a few species of aerobic spore forming bacteria and a number of mould genera are potential sources of milk clotting enzymes. The main advantage of exploitation of microbial source lies in its easy adoptability to production on unlimited scale at low cost.
Further, the microbial enzymes have been known to exhibit a wide range of activity apart from differing in substrate specificity and mode of action.

If milk clotting enzyme from a microbial source is to be used as a substitute for rennet for cheese making, it has to possess certain major characteristics similar to animal rennet. A brief narration of the nature and properties of rennet and its function in cheese manufacture would exemplify the basic requirements of the substitute. Rennet is a general term applied to the enzyme extract obtained from the fourth stomach of the suckling calves which contains mainly a milk clotting enzyme, rennin, and a small amount of another enzyme, pepsin. The pure enzyme, rennin is an acid protease and is listed as EC 3.4.4.3 according to the present system of classification. The zymogen and the enzyme are also designated as prochymosin and chymosin (EC 3.4.23.4) to avoid confusion with another enzyme present in kidneys, which is known as renin.

Most of the coagulant mixtures used in cheese manufacture contain a small amount of rennet. The rationale appears to be to simultaneously conserve rennet and also to avoid some of the problems arising from the use of substitutes alone. The above approach has been successful when a mixture of rennet with pig pepsin (50:50) is used, since this combination has been found suitable as a milk coagulant for cheese manufacture (Martens and Naudts, 1973). Mixtures of coagulants can also be used with the object of adjusting the rate of proteolysis during cheese ripening to the desired level.
All commercial preparations of rennet substitutes available at present, have been extensively purified and characterized. Very little information is, however, available in regard to purification and characterization of bacterial rennet. In view of the above considerations, the following aspects of study on rennet produced by a strain of Bacillus subtilis K-25 have been taken up.

(i) Purification of bacterial rennet using standard biochemical techniques.
(ii) Determination of the homogeneity of purified milk clotting enzyme.
(iii) Study of the properties of purified milk clotting enzyme.
(iv) Use of the above enzyme singly or in combination with calf rennet/swine pepsin for the preparation of Cheddar cheese.