Summary

HMGR inhibitors (statins) are one of the most important products of biological origin and are in ever increasing demand because of their various clinical applications apart from lipid lowering ability. Hence, in India, demand for statins and its analogues currently exceed their production capacity. The most important/viable route of synthesis of statin is by the way of fermentation of microorganism rather than chemical synthesis. However from the extensive literature on this subject it is evident that there are only few microorganisms used for statin production. This survey of literature has stimulated the exploration of statin production using microorganisms from various natural sources, which is cost effective with a promise for developing newer technology for meeting the gap is demand and supply. The microorganisms reported in the present study are the first attempt to use as a novel, but equally competitive source for the production of HMGR inhibitors.

With the demand for cholesterol lowering drugs increasing significantly, efforts are on the anvil to search for newer and cheaper sources that can be potential candidates for commercial/industrial production of statins. Hence, the present study has been focused on the use of novel and unexplored microorganisms for the production of HMGR inhibitors. The most common microorganism that has been employed in the statin production is *Aspergillus terreus*, which has always been described and accepted as the culture of choice for commercial production of statins. The aim of the present study is to produce statins by employing microorganisms other than *A. terreus* strain.

Statins hold a significant investment opportunity as the healthcare sector projects immense demand for statins both in India and international market. This

scenario is likely to intensify as more statins go off patent. Hence, this work would immensely benefit the industries coping to meet the prevailing demand for statins in the market.

The summary of the results obtained and the conclusions arrived at on the basis of the results presented in the present thesis are briefly outlined as below:

1. The Chapter I includes a brief introduction to the research undertaken, wherein the necessity of undertaking the work is justified and the aims and objectives of the study are specified.
2. In Chapter II, an exhaustive review of the relevant aspects of statins, chemistry and historical aspects, mechanism of action, the characteristics of statins and their producers, etc., is presented and also an attempt has been made to evaluate the different microorganisms used in fermentation for production of statins.
3. Chapter III deals with the materials used and methodology adopted in the present study. The aspects covered are mainly – isolation, screening and rapid confirmation of statin producing microbial strains, characterization of statins through UV, IR, HPLC, inhibition of yeast and human HMGR gene, in silico tools to explore structure-function relationship between fungal and bacterial enzymes involved in the semi-synthesis of Simvastatin.
4. In Chapter IV, the results obtained during the study are presented. The important conclusions are indicated in brief as below:
    i. The natural microbial isolates were initially subjected for statin production and evaluated for their HMGR inhibitor production potential based on UV spectra, TLC and their zone of inhibition in yeast growth inhibition bioassay. Out of 81 isolates screened, 3 isolates (FG 7, BG 17 and BG 188) exhibited zone of inhibition of more than 12 mm. The

same were considered as promising strains for statin production and of these three also, FG 7 and BG 17 were found to be the best producers.

ii. Based on their biochemical properties, and 16S rRNA sequencing the three strains FG 7, BG 17 and BG 188 were found to be *Aspergillus cervinus* (isolated from marine source), *Enterococcus faecalis* and *Bacillus anthracis* (isolated from biological fluid) respectively- (First study to report bacteria isolated from biological fluid to produce semi synthetic statins).

iii. SK-02, SK-03 and SK-04 were the products formed by FG 7, BG 17 and BG 188 respectively. Analytical and biological characterization of all the products confirmed the production of Simvastatin by these three new microbial strains comparable to the industrially used fungal strain.

iv. Molecular biology studies revealed the presence of LovD gene in the marine fungal strain *A. cervinus* (FG 7) which catalyzed the production of semi synthetic Simvastatin. However similar gene sequences could not be found in the bacterial genome by PCR amplification using primers designed based on fungal LovD gene nucleotide sequence suggesting that the fungal and the bacterial enzymes that catalyze this reaction are quite unrelated but responsible for similar bioactivity in both bacteria and fungi.

v. In- silico studies showed that in *Enterococcus faecalis* beta-lactamase gene of transpeptidase family might be responsible for the bio-catalytic property.

vi. Having a bacterial source available for production of statins will provide a considerable advantage and benefits over fungal sources of statin

production. Bacterial sources can prove to be an important industrial tool for semi-synthetic production of statins at industrial scale.

5. In Chapter V, a detailed discussion of the findings of the present work is made with reference to the work done by earlier investigators in this field.
6. The bibliography referred in the present thesis is included in the References section at the end of the thesis.