# Abstract

Cardiovascular diseases (one of the most common consequences of high cholesterol levels) are the most prevalent cause of death and disability in both developed as well as developing countries. The World Health Organization estimates that almost 20% of all strokes and over 50% of all heart attacks can be linked to high cholesterol. Most people around the globe with high cholesterol are not getting the treatment they need, claims the largest study of 147 million people, as only few people are put on cholesterol lowering drugs. Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyzes the rate-limiting step in cholesterol biosynthesis and is most promising class of cholesterol lowering drugs. These HMGR inhibitors can reduce death induced by coronary heart disease and heart attack rates by 20 to 50 percent. Natural statins are produced as fungal secondary metabolites. Till date some terrestrial fungal strains (mainly *Aspergillus terreus*) is used for industrial scale production of statin molecules. Finding a novel source with a potential to synthesize HMGR inhibitor molecules will represent an important milestone in bio-catalysis of statin or their analogs and successful engineering of a biosynthetic enzyme into an industrially useful manufacturing tool would lead to substantial cost reduction and efficient synthesis of potential derivatives for therapeutic applications. This necessitates the invention of novel sources/production strategies of existing drugs to manage the increasing incidences of higher cholesterol levels. With the emerging multiple therapeutic effects (like anti inflammatory, anti cancer, bone regeneration, cognitive disorders) which are beyond just lipid lowering, these wonder drugs “statins” are proving to be much more powerful drugs than ever thought. The majority of fungi inhabiting the world (especially the marine resources of Indian subcontinent) and the bacterial population

from unique natural sources have not yet been explored and exploited for production of natural and semi synthetic statins. Given this background, in this thesis we screened unexplored natural microbial population from different ecological niches to find a novel source for efficient synthesis of HMGR inhibitor molecule.

In the present thesis, we report a novel marine fungal source and two bacteria capable of producing HMGR inhibitors. The results obtained in the present thesis indicate that these new sources can be a good alternative to the available limited natural sources and other expensive chemical methods for production of statins. Of the different soil, water and biological fluids, eighty one microorganisms, irrespective of its type, were used in rapid screening for HMGR inhibitor production. Upon subjecting the isolates to rapid screening for production of simvastatin (most common semi-synthetic HMGR inhibitor) by thin layer chromatography and yeast inhibition bioassay, only three isolates (i.e. only about 4% of total isolates) have exhibited the corresponding Rf and inhibition zone on bioassay plate. Of these three isolates, two were bacterial isolates (BG 17 and BG 188) obtained from biological fluid and one was fungal isolate (FG 7) obtained from marine water source. These exhibited inhibition zone of 16 mm and 14 mm (BG 17 and FG 7 respectively) and hence were considered as good HMGR inhibitor producers while BG 188 showed a moderate zone so was included for further studies. To confirm the results obtained in bioassay studies, the extract were subjected to thin layer chromatography. A TLC separation method was used for rapid screening which effectively separated the statins from other major co-metabolites. The compounds (SK-02, SK-03 and SK-04) produced by the strains (FG-7, BG-17 and BG-188) exhibited similar Rf values (0.71) to that of standard simvastatin. Further, in addition to confirmation of simvastatin production by the isolates on TLC plates,

quantitative estimation was also carried out using UV-VIS spectrophotometer. The spectral studies of standard simvastatin revealed the maximum absorption peak λ max at 238 nm. Therefore, 238 nm was taken as constant for all culture samples and was used for measuring the optical densities for estimation of simvastatin concentrations for further studies. Thereafter, on the basis of results observed in the preliminary screening based on yeast inhibition bioassay, thin layer chromatography, UV-VIS absorption spectra, it was considered that the strains FG7, BG 17 and BG 188 are potential HMGR producing candidates for further de tailed studies. The present study evaluated *Aspergillus cervinus* (FG 7) from a marine source for the enzyme mediated production of semi-synthetic simvastatin. The successful ability of this marine fungus to produce simvastatin emphasizes further investigation of fungal isolates from different/unexplored ecological niches for useful applications. A systematic search should be initiated for fungi from extreme marine environment such as deep-sea, hypoxic zones and hydrothermal vents for isolation of industrially important strains. This study first time reports the potential of two bacteria *Enterococcus faecalis (BG 17)* and *Bacillus anthracis* (BG 188) isolated from human biological fluid for production of semi synthetic HMGR inhibitor, simvastatin. So far, as per best of our knowledge, there has been no reports of any a microorganism belonging to class of natural bacteria isolated from any biological fluid that has the bioconversion ability for production of semi-synthetic statins, like those presented in this study.