Masters in Biotechnology

A Bioprocess for Production and Purification of Rifamycin B

Tarek Dishisha

ABSTRACT

Tuberculosis (TB) remains one of the worldwide leading infectious diseases causing 3 million deaths/year, 8 million new cases/year and 1/3 of world population are expected to harbour latent TB infection. *Mycobacterium tuberculosis* is the causative agent and the poor hygienic conditions stands behind its wide spreading. A combined antibiotic regime for almost 6 months is required for its treatment. Rifampicin, a semi-synthetic antibiotic, selectively inhibits *M. tuberculosis* growth and proliferation through inhibition of DNA-dependent RNA polymerase required for protein synthesis. Rifamycin B obtained as a secondary metabolite during fermentation using the Gram positive actinomycete, *Amycolatopsis mediterranei* is considered the precursor of rifampicin as well as a series of derivatives such as rifamide and rifabutine which are active against leprosy and Traverllers' diarrhoea, respectively. The recovery and purification of rifamycin B is done via liquid-liquid extraction using organic solvents such as chloroform, ethyl acetate and/or butyl acetate in repeated steps of acidification, extraction and back-extraction into a buffer system. Solvent extraction, however suffers from many drawbacks such as high toxicity, cost for regeneration, limitation for disposal, flammability, many problems with occupational exposure, and difficulties in integration with fermentation process for *in situ* product capture which is required to avoid product inhibition. In the present study, a process for production of rifamycin B and subsequent recovery and purification from the fermentation broth using chromatographic resin was developed. Among the different resin evaluated, the strong anion exchange resin Amberlite IRA-401 showed the highest rifamycin B binding capacity and was chosen for further investigation. After rifamycin B production by fermentation, solid-liquid separation at pH 4.2 - 4.5 was found optimum to get rid of most negatively charged species that could interfere with the binding of rifamycin B to the resin. Based on pH profile and solubility of rifamycin B, a pH of 6 – 8.6 was optimum for resin binding as more than 90% of the acidic groups are present in ionized form. The binding kinetics of rifamycin B from standard solution as well as cell free fermentation broth to the resin was determined under batch and column processes. The adsorption isotherm was determined and can be described as high affinity binding in a manner similar to that of dyes and ionic solution and did not follow Langmuir or Freudenlich kinetics that are common for most antibiotics. Elution was done using a mixture of ethanol and sodium chloride. Under optimum conditions, column separation process was performed and reusability of the resin was feasible for at least 3 repeated cycles. This approach could have potential for *in situ* rifamycin B removal as well as industrial production process.