FACTORS AFFECTING RAPAMYCIN (SIROLIMUS) PRODUCTION - A REVIEW

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***Abstract*: Immunosuppressants are gaining in popularity in recent years due to their important contribution to the medical and pharma industries and huge demand in the global market. Rapamycin (sirolimus) is an immunosuppressive drug that also has antitumor antifungal, anti-aging, and neuroprotective properties. Many strains of *Streptomyces hygroscopicus* use a submerged fermentation approach to produce rapamycin. Albeit the wild strain's production capacity is extremely low, therefore to enhance the production of rapamycin, a thorough investigation of media optimization, bioprocess engineering parameters is required to address this issue. This review emphasizes the remarkable approaches of media optimization, statistical approach, and studies on gene level to scale up the titer of rapamycin.**

***Keywords:* Sirolimus, Rapamycin, Streptomyces hygroscopicus., Immunosuppressant, Transplantation, Fermentation.**

I. Introduction

Immunosuppressants are prescribed in a wide range of medical situations and enhance the quality of life for persons with various severe illnesses, and can even save many lives across the world [1]. Cyclosporine, Tacrolimus, Ascomycin, Rapamycin etc. are widely using as an immunosuppressant drug. Rapamycin is a analog of ascomycin is [2].

Rapamycin also known as sirolimus first produced by the *Streptomyces hygroscopicus NRRL 5491* bacterium as an antifungal property. It was extracted from mycelium using solvent extraction, purified using silica gel column chromatography, and crystallized as a colorless solid with the empirical formula C56H89NO14 that melts at 183-185°C [3]. Figure 1 shows the structure of rapamycin. It was also disclosed after some time that rapamycin also plays an important role in anti-tumor [4], neurobiological [5, 6, 7], and immunosuppressive [8] activities (Figure 2). As immunosuppressive activity rapamycin has a more efficient method of action and reduced biological toxicity than FK506 and cyclosporine [9,10].

Rapamycin binds to its intracellular receptor FK506-binding protein 12 and inhibits the mammalian target of rapamycin. (FKBP12). The FKBP12-rapamycin complex binds to the FKBP12-rapamycin-binding (FRB) domain of mTOR, a phosphoinositide kinase-related kinase. The FKBP12–rapamycin combination, once loaded onto the FRB domain, suppresses mTOR, a protein that connects with a variety of proteins in mammals and plays an important role in motility and survival cellular activities [11, 12]. Rapamycin also influences T-cell activation in the second phase by inhibiting the growth-promoting cytokine signaling transduction pathway involving mTOR [13,14].

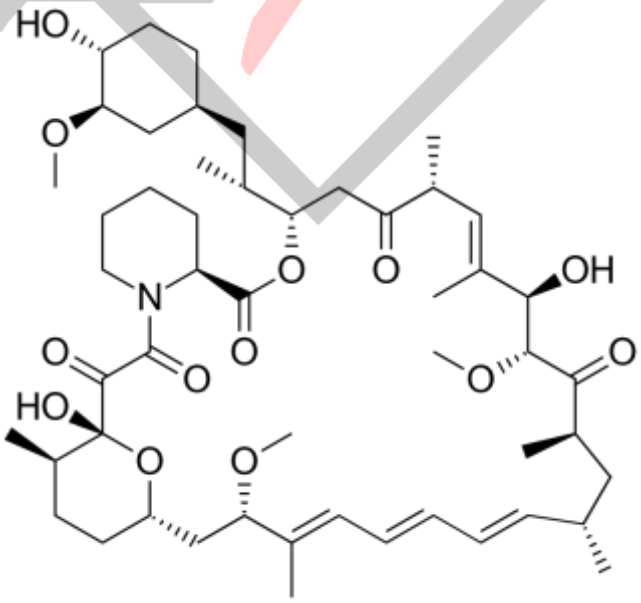

Fig -1 Structure of Rapamycin

Because of its pharmacological importance and wide applicability, rapamycin has recently piqued the interest of numerous scientific researchers. The poor yield of rapamycin is still a problem for commercialization; hence a lot of studies have gone into strain

modification and culture optimization. Many efforts have recently been made to harness the therapeutic effects of rapamycin and its derivatives.

Many researchers have focused on enhancing rapamycin production through screening for potent producer strains, changing biochemical and physical parameters, and growing conditions. On the other hand, some scientists have carried out experiments employing the Plackett-Burman experimental design and Response Surface Methodology (RSM) to confirm the most important variables affecting rapamycin production yield from *Streptomyces hygroscopicus.* Various strain-improving strategies have recently been published in this regard [15]. Rapamycin is also used in the treatment of lung diseases such as Lymphangioleiomyomatosis (LAM) [16] and Vascular Malformation[17]. Previous studies also suggest that kinetic studies of organisms especially Streptomyces *hygroscopicus* in depth is also required to achieve high productivity of rapamycin such as Dissolved O_2 and CO_2, P^H, cell biomass and specific growth rate, etc. [18].

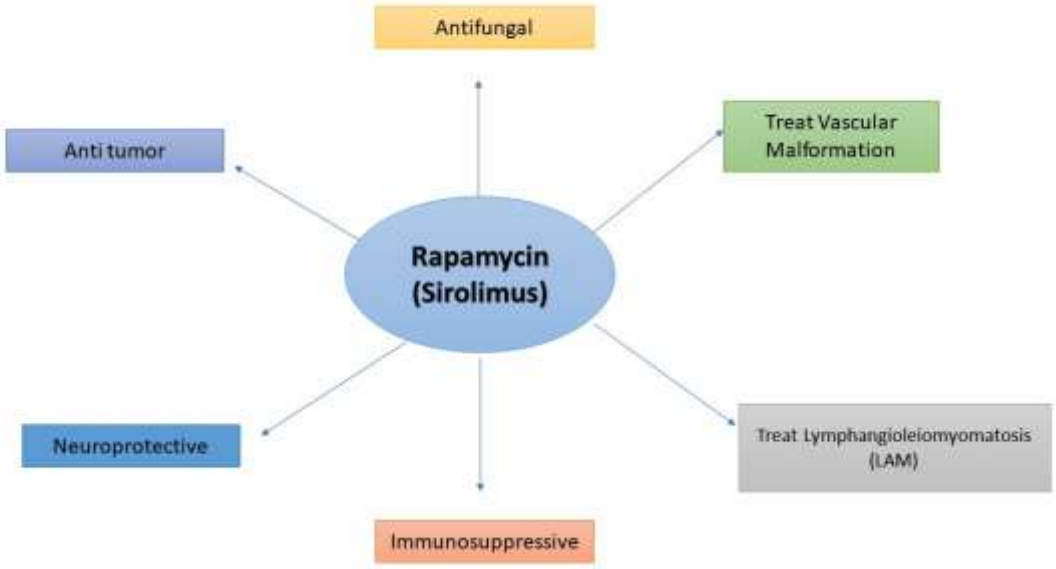

Fig-2 Role of Rapamycin

There are numerous other approaches with great promise, and the exploration of such novel strategies using biotechnological engineering techniques can aid in overcoming the limited availability of rapamycin. The purpose of this review is to provide information on optimization conditions, and yield improvement of rapamycin.

II. Enhancing rapamycin production through traditional methods

Researchers have been carried out several experiments with conventional medium components, nutritional circumstances, and operating parameters such as fermentation run time, and vessel temperature all have a role in achieving a suitable yield to increase rapamycin yield by *Streptomyces hygroscopicus* for the past decade.

To investigate the role of carbon sources on the production of rapamycin, cellobiose, fructose, galactose, inositol, mannose, mannitol, and xylose were among the 35 carbon sources evaluated. The best positive carbon source for rapamycin synthesis was found to be 2 g/l fructose and fructose at 2 and 5 g/l mannose produced the best results as a secondary carbon source [19]. In a separate experiment, the same group also found the role of certain amino acids in connection with rapamycin production. In their experiments, aspartic acid, arginine, and histidine were added to the fermentation, and this combination was found to be beneficial in the construction of a chemically defined medium for rapamycin synthesis [20, 21]. Using a chemically defined media and feeding lysine, a precursor of pipecolic acid, researchers were able to boost rapamycin production by 1.5 times. The addition of ammonium sulfate to the new chemically defined medium increased rapamycin synthesis by more than 30%. Researchers also investigated the effects of phosphate (K_2HPO4), ammonium (NH_4Cl), magnesium ($MgSO_4.7H2O$), and iron ($FeSO_4$) salts on rapamycin production and discovered that adding these nutrients reduced rapamycin production. $FeSO_4$, on the other hand, increased rapamycin synthesis at a concentration of 100 mg/l (0.36 mM) higher than that required for growth [21]. Media components and their physical structure also play an important role. Various particle sizes of soybean meal powder were found to marginally improve rapamycin synthesis [15] due to rapamycin's preventive effect against oxidative stress and reactive oxygen species [22, 23]. Other articles suggested the use of change one variable at a time, Plackett Burman Design (PBD), Response Surface Methodology (RSM) with Central Composite Design (CCD) are being employed for higher production of antimicrobial compounds [24]. Efforts were undertaken to boost rapamycin microbial production by tweaking one or multiple parameters at a time. When D-mannose and soybean meal were used as carbon and nitrogen sources, respectively, rapamycin production was shown to be higher than when other carbon and nitrogen sources were used. On the fourth day of production, *S. hygroscopicus NRRL 5491* was reported to produce 248.71 mg/L of rapamycin. Using the one-factor-at-a-time technique of process optimization, it was discovered that L-lysine, at a concentration of 10 g/L, raised rapamycin synthesis to

268.87 mg/L. Computational technologies were used to better optimize the production medium. Finally, mannose, soybean meal, and L-lysine concentrations were found to be important contributors utilizing the PB design.[25]. A group of researchers investigated a fed-batch bioprocess optimization strategy for improving rapamycin yield. Batch rapamycin production was carried out in a fermenter under the same conditions as their shake flask tests. They found how varied agitation rates, such as 200, 300, 400, and 500 rpm, affected rapamycin production while maintaining the aeration rate at a constant level. They observed that low agitation rates favored early development but resulted in less rapamycin production, while high agitation speeds (500 rpm) resulted in a drop in both growth and rapamycin titer. But at 400 pm, the greatest output of rapamycin was recorded, with the dissolved oxygen being above 10% throughout the process. Rapamycin titer was achieved at 412 mg/L using two-stage fermentation techniques in which lower and greater agitation rates were used in the 12 L fermenter. The highest yield of rapamycin produced in a 120 L fed-batch fermenter with an optimized pH of 4.8 was 812.5 mg/L. They also observed that the biosynthesis of rapamycin was boosted to an acceptable level by a combination feeding of 250 g/L glycerol and 10 g/L K_2HPO4 (30 g/L lysine and pH value set to 4.8 +/-02) [26]. It's been seen in *Streptomyces spp*. have high oxygen uptake demand in the fermentation process, which plays a crucial role in maintaining antibiotic production [27]. Therefore, the amount of dissolved oxygen (DO_2) in the air is also important for filamentous bacteria to change shape and produce rapamycin, according to this study by Yen et al., 2013, lower dissolved oxygen lengthens the lag period but results in higher rapamycin titer, whereas higher dissolved oxygen enhances biomass growth and early rapamycin synthesis, Apart from dissolved oxygen, the same group also observed that rising and falling pattern of P^H is also a factor for higher productivity of rapamycin[28].

III. Increasing rapamycin production through understanding responsible gene and cutting-edge technology

The rapamycin PKS is made up of three multifunctional enzymes (RapA, RapB, and RapC) that each has 14 modules: modules 1–4 in RapA is involved in polyketide chain initiation and extension, modules 5–10 in RapB are responsible for chain elongation up to C16, and modules 11–14 in RapC play a role for the termination of the polyketide [29]. By looking beyond, the PKS area, further genes involved in rapamycin production have been discovered. A specific protein encoded by *rapP* stops the chain production. Rapamycin synthesis was dramatically reduced when *rapP* was removed from the chromosome of *S. hygroscopicus*. [30, 31]. It was also observed that *rapK* deleted strain affects rapamycin production significantly and further rapamycin production was restored by genetic complementation of *rapK* in the deletion mutant, which produced no rapamycin in deleted *rapK* strain [32]. RapG and RapH have DNA-binding sites and are related to transcriptional activator families including the LuxR family's large ATP-binding regulators (LAL) and AraC, respectively. In the wild-type *S. rapamycinicus*, adding an extra copy of rapH and/or rapG resulted in a significant increase in rapamycin production. RapH and RapG are essential positive regulators of rapamycin biosynthesis, according to a recent study by the Biotica group [33]. In *S. hygroscopicus*, overexpression of rapH and rapG under the direction of the ActIIORF4/PactI activator/promoter increased rapamycin synthesis by 27–55% and 20–32%, respectively. To limit the potential self-regulatory interference of RapH and/or RapG and other endogenous regulatory genes, the ActII-ORF4/PactI expression system was utilized, which is a strong activator/promoter expression system that has been widely used for many actinomycetes, including *S. hygroscopicus* [34] When compared to the wild-type strain, adding an extra copy of rapH and rapG under the control of S. hygroscopic native promoter region boosted rapamycin formation by 40% on average. The expression of some rapamycin biosynthesis genes is negatively regulated by rapS and rapY, according to a transcriptional study of wild-type and mutant strains. RapS was also discovered to suppress the expression of RapY, which in turn controls the expression of an ABC-transporter encoded by rapX, demonstrating the importance of RapS and RapY in the biosynthesis of rapamycin. [35]. Apart from this, Ribosome engineering [36] has been utilized to boost the yields of desired metabolites and trigger secondary metabolite production in Streptomyces strains [37]. When compared to the original strain, many rounds of ribosome engineering with streptomycin resulted in the formation of a mutant strain of *Streptomyces gandocaensis* that resumed stable production with higher secondary metabolite yields [38]. Protoplast-related approaches like protoplast mutation, intra-, and interspecies fusion were used in another attempt to develop high-yield rapamycin-producing strains. Progress was not made when utilizing UV, N-methyl-N-nitro-N-nitrosoguanidine, diethyl sulfates, or fusion of various parental protoplasts of S. hygroscopicus. However, a high-yield rapamycin producer with an excellent output of 445 mg was created using a combination of interspecies protoplast fusion and one round of genome shuffling. [39]. It would also be beneficial to develop a rapamycin analog that is both practicable and cost-effective on a large scale. The production of rapalogs can also be aided by genome-editing techniques. CRISPR/CRISPR associated protein (Cas) is a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein (Cas) system that was recently discovered. As an adaptable microbial immune system, CRISPR represents a family of DNA repeats [40]. To produce a library of rapalog, the CRISPR/Cas technique can be used to modify their genomes and the rapamycin biosynthetic gene clusters. Lanqing Dang and colleagues employed a GSMM-guided metabolic engineering technique to boost *S. hygroscopicus* ATCC 29253's rapamycin synthesis. The increased rapamycin production capacity of the transgenic strains with pfk gene deletion and rapK, dahP overexpression were demonstrated during fermentation. The production of rapamycin was increased further by knocking down the target gene pfk and simultaneously expressing the target genes dahP and rapK. In comparison to the parent strain (103.5 mg/L), the engineering strain *S. hygroscopicus-Δk-DR* had a rapamycin titer of 250.8 mg/L. This technique can be used to improve the production of other secondary metabolites [41].

IV Conclusion

Rapamycin has become one of the most usable medications in recent years due to it's multifunctional and potential usage. This drug has made significant advances in the fields of immunosuppression and other medical applications and it has a global commercial demand due to its multifunctional action. Researchers across the world are working to uncover more of its crucial functional aspects to boost its industrial-scale production in terms of cost-effective analysis and broad-spectrum activity. Some of the critical factors

of rapamycin production are explored in this review. Following a review of numerous literatures, it is possible to conclude that the majority of medical research to date has been published on the application part of rapamycin and very little information regarding factors that affect rapamycin productivity at a large scale. Because low titers of rapamycin produced by different strains of *S. hygroscopicus* is one of the major concerns for large-scale industrial production of rapamycin. To address this, a combination of modulating media composition through statistical approach [42], finding critical parameters of fermentation, exploration of responsible genes, and regulatory network in rapamycin production can provide deep insights into uncovering complete rapamycin biosynthesis pathways and enhance productivity in order to meet global market demand with commercially viable processes to reduce production costs globally.

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References:

[1] U. Luthra, A. Tomar, Tacrolimus - Strategies for Enhancing Tacrolimus Production at the Classical and Transcriptional Level, International Journal of Science and Research,10 (2021) 233–237. https://doi.org/10.21275/SR211005095325.

[2] U. Luthra, A. Tomar, Strategies for enhancing productivity of Ascomycin – A review, International Journal of Research and Analytical Reviews, 8 (2021) 954–964. http://doi.one/10.1729/Journal.28689.

[3] S. N. SEHGAL, H. BAKER and Claude VEZINA - RAPAMYCIN (AY-22,989), A new antifungal antibiotic II. Fermentation, isolation and characterization, The journal of antibiotic, VOL. XXVIII NO. 10 (1974) 727.

[4] D.P. Houchens, A.A. Ovejera, S.M. Riblet, D.E. Slagel, Human brain tumor xenografts in nude mice as a chemotherapy model, Eur J Cancer Clin Oncol. 19 (1983) 799–805. https://doi.org/10.1016/0277-5379(83)90012-3.

[5] C. Malagelada, Z.H. Jin, V. Jackson-Lewis, S. Przedborski, L.A. Greene, Rapamycin protects against neuron death in in vitro and in vivo models of Parkinson's disease, J Neurosci. 30 (2010) 1166–1175. https://doi.org/10.1523/JNEUROSCI.3944-09.2010.

[6] T. Pan, S. Kondo, W. Zhu, W. Xie, J. Jankovic, W. Le, Neurobiology of Disease Neuroprotection of rapamycin in lactacystin-induced neurodegeneration via autophagy enhancement, 32 (2008) 16–25. https://doi.org/10.1016/j.nbd.2008.06.003.

[7] L.S. Tain, H. Mortiboys, R.N. Tao, E. Ziviani, O. Bandmann, A.J. Whitworth, Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss, Nat Neurosci. 12 (2009) 1129–1135. https://doi.org/10.1038/nn.2372.

[8] R.R. Martel, J. Klicius, S. Galet, Inhibition of the immune response by rapamycin, a new antifungal antibiotic, Can J Physiol Pharmacol. 55 (1977) 48–51. https://doi.org/10.1139/y77-007.

[9] E. Patsenker, V. Schneider, M. Ledermann, H. Saegesser, C. Dorn, C. Hellerbrand, F. Stickel, Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis, J Hepatol. 55 (2011) 388–398. https://doi.org/10.1016/j.jhep.2010.10.044.

[10] L. Dang, J. Liu, C. Wang, H. Liu, J. Wen, Enhancement of rapamycin production by metabolic engineering in Streptomyces hygroscopicus based on genome-scale metabolic model, J Ind Microbiol Biotechnol. 44 (2017) 259–270. https://doi.org/10.1007/s10295-016-1880-1.

[11] Y.J. Yoo, H. Kim, S.R. Park, Y.J. Yoon, An overview of rapamycin: from discovery to future perspectives, J Ind Microbiol Biotechnol. 44 (2017) 537–553. https://doi.org/10.1007/s10295-016-1834-7.

[12] R. Zoncu, A. Efeyan, D.M. Sabatini, MTOR: From growth signal integration to cancer, diabetes and ageing, Nat Rev Mol Cell Biol. 12 (2011) 21–35. https://doi.org/10.1038/nrm3025.

[13] 0. Halloran PF, Molecular mechanisms of new immunosuppressants. Clin Transplant (1996) 10:118-123. 21.

[14] Hay N, Sonenberg N, Upstream and downstream of mTOR. Genes Dev(2004) ; 18:1926-1945.

[15] M.A. Mohamed, W.A. Elkhateeb, M.A. Taha, G.M. Daba, New Strategies in Optimization of Rapamycin Production by Streptomyces hygroscopicus ATCC 29253, 12 (2019) 4197–4204. https://doi.org/10.5958/0974-360X.2019.00722.4.

[16] E. Pahon (28 May 2015). "FDA approves Rapamune to treat LAM, a very rare lung disease". *FDA.gov*. U.S. Food and Drug Administration. Retrieved 1 August 2016.

[17] V.Dekeuleneer, E. Seront, A. Van Damme, Boon LM, Vikkula M. "Theranostic Advances in Vascular Malformations". The Journal of Investigative Dermatology (2020*)*. 140 (4): 756–763.

[18] S. Dutta, B. Basak, B. Bhunia, A. Dey, Journal of Bioprocess Engineering and Biorefinery, Volume 3, (2014), pp. 243-256(14). https://doi.org/10.1166/jbeb.2014.1105.

[19] I. Kojima, Y.R. Cheng, V. Mohan, A.L. Demain, Carbon source nutrition of rapamycin biosynthesis in Streptomyces hygroscopicus, J Ind Microbiol. 14 (1995) 436–439. https://doi.org/10.1007/BF01573954.

[20] Y.R. Cheng, A. Fang, A.L. Demain, Effect of amino acids on rapamycin biosynthesis by Streptomyces hygroscopicus, Appl Microbiol Biotechnol. 43 (1995) 1096–1098. https://doi.org/10.1007/BF00166931.

[21] M.S. Lee, I. Kojima, A.L. Demain, Effect of nitrogen source on biosynthesis of rapamycin by Streptomyces hygroscopicus, J Ind Microbiol Biotechnol. 19 (1997) 83–86. https://doi.org/10.1038/sj.jim.2900434.

[22] J. Jiang, J. Jiang, Y. Zuo, Z. Gu, Rapamycin protects the mitochondria against oxidative stress and apoptosis in a rat model of Parkinson's disease, (2013) 825–832. https://doi.org/10.3892/ijmm.2013.1280.

[23] D. Qin, R. Ren, C. Jia, Y. Lu, Q. Yang, L. Chen, X. Wu, J. Zhu, Y. Guo, P. Yang, Y. Zhou, N. Zhu, B. Bi, T. Liu, Rapamycin Protects Skin Fibroblasts from Ultraviolet B-Induced Photoaging by Suppressing the Production of Reactive Oxygen Species, Cell Physiol Biochem. 46 (2018) 1849–1860. https://doi.org/10.1159/000489369.

[24] D. Sharma, R.K. Manhas, Application of Plackett-Burman experimental design and Box and Wilson design to improve broad-spectrum antimicrobial compound, Indian J Biotechnol. 12 (2013) 386–394.
[25] R. Sinha, S. Singh, P. Srivastava, Studies on process optimization methods for rapamycin production using Streptomyces hygroscopicus ATCC 29253, Bioprocess Biosyst Eng. 37 (2014) 829–840. https://doi.org/10.1007/s00449-013-1051-y.
[26] X. Zhu, W. Zhang, X. Chen, H. Wu, Y. Duan, Z. Xu, Generation of high rapamycin producing strain via rational metabolic pathway-based mutagenesis and further titer improvement with fed-batch bioprocess optimization, Biotechnol Bioeng. 107 (2010) 506–515. https://doi.org/10.1002/bit.22819.
[27] Y. Chen, J. Krol, V. Sterkin, W. Fan, X. Yan, W. Huang, J. Cino, C. Julien, New process control strategy used in a rapamycin fermentation, 34 (1999) 383–389.
[28] H.W. Yen, H.P. Hsiao, L.J. Chen, the enhancement of rapamycin production using Streptomyces hygroscopicus through a simple pH-shifted control, J Taiwan Inst Chem Eng. 44 (2013) 743–747. https://doi.org/10.1016/j.jtice.2013.01.025.
[29]. S.R. Park, Y.J. Yoo, Y.H. Ban, Y.J. Yoon, Biosynthesis of rapamycin and its regulation: Past achievements and recent progress, J Antibiot (Tokyo). 63 (2010) 434–441. https://doi.org/10.1038/ja.2010.71.
[30]. J.B. Lester, G.A. Bohmt, J. Staunton, P.F. Leadlay, The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin, 92 (1995) 7839–7843.
[31]. A. König, T. Schwecke, I. Molnár, G.A. Böhm, P.A.S. Lowden, J. Staunton, P.F. Leadlay, The pipecolate incorporating enzyme for the biosynthesis of the immunosuppressant rapamycin, Eur J Biochem. 247 (1997) 526–534. https://doi.org/10.1111/j.1432-1033.1997.00526.x.
[32]. M.A. Gregory, S. Gaisser, R.E. Lill, H. Hong, R.M. Sheridan, B. Wilkinson, H. Petkovic, A.J. Weston, I. Carletti, H. Lee, J. Staunton, P.F. Leadlay, Isolation and Characterization of Pre-rapamycin, the First Macrocyclic Intermediate in the Biosynthesis of the Immunosuppressant Rapamycin by S. hygroscopicus **, (2004) 2551–2553. https://doi.org/10.1002/anie.200453764.
[33]. E. Kus, N. Coates, I. Challis, M. Gregory, B. Wilkinson, R. Sheridan, H. Petkovic, Roles of rapH and rapG in Positive Regulation of Rapamycin Biosynthesis in Streptomyces hygroscopicus, 189 (2007) 4756–4763. https://doi.org/10.1128/JB.00129-07.
[34]. Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F. & Hopwood, D. A. Practical Streptomyces Genetics (The John Innes Foundation, Norwich, 2000.
[35]. Y. Ji, Y. Jae, Characterization of negative regulatory genes for the biosynthesis of rapamycin in Streptomyces rapamycinicus and its application for improved production, (2015) 125–135. https://doi.org/10.1007/s10295-014-1546-9.
[36]. Ochi K, Okamoto S, Tozawa Y, Inaoka T, Hosaka T, Xu J, Kurosawa K, Ribosome engineering and secondary metabolite production. Adv Appl Microbiol,(2004) 56:155–184.
[37]. G. Wang, T. Hosaka, K. Ochi, Dramatic Activation of Antibiotic Production in Streptomyces coelicolor by Cumulative Drug Resistance Mutations □ †, 74 (2008) 2834–2840. https://doi.org/10.1128/AEM.02800-07.
[38] S.R. Park, A. Tripathi, J. Wu, P.J. Schultz, I. Yim, T.J. Mcquade, F. Yu, C. Arevang, A.Y. Mensah, G. Tamayo-castillo, C. Xi, D.H. Sherman, Discovery of cahuitamycins as biofilm inhibitors derived from a convergent biosynthetic pathway, Nat Commun. 6 (2016) 1–11. https://doi.org/10.1038/ncomms10710.
[39]. L. Tain, H.J. Mortiboys, E. Ziviani, O. Bandmann, Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss Europe PMC Funders Group Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss, (2009). https://doi.org/10.1038/nn.2372.
[40]. R. Jansen, J.D.A. Van Embden, W. Gaastra, L.M. Schouls, Identification of genes that are associated with DNA repeats in prokaryotes, Mol Microbiol. 43 (2002) 1565–1575. https://doi.org/10.1046/j.1365-2958.2002.02839.x.
[41]. L. Dang, J. Liu, C. Wang, H. Liu, J. Wen, Enhancement of rapamycin production by metabolic engineering in Streptomyces hygroscopicus based on genome-scale metabolic model, J Ind Microbiol Biotechnol. 44 (2017) 259–270. https://doi.org/10.1007/s10295-016-1880-1.
[42]. U.Luthra, N.K.Singh, A.Tripathi, Optimization of Nutrient Components For Rapamycin Production By *Streptomycetes hygroscopicus* Under Submerged Fermentation Using Plackett Burman Design Carried By Response Surface Methodology, 2 (2014) 1619–1631.