2. Review of literature

2.1 Mycophenolic Acid

In recent years, microbial metabolites have become a hotspot of scientific research. Secondary metabolites mainly isolated from a microbial source [Berdy, 2005; Zhou, et al., 2018]. Pharmaceutical industries commonly use several fungal fermentation processes for the large-scale production of drugs. Mycophenolic acid (6-(4-hydroxy-6-methoxy methyl 3oxophthalanyl)-4-methyl-4-hexenoic acid, $C_{17}H_{20}O_6$, MPA) is a fungal secondary metabolite [Bentley, 2000; Sydenstricker, 1958; von Borstel, et al., 2020]. MPA has been produced by many *Penicillium species* [Ismaiel, et al., 2014; Sadhukhan, et al., 1999] as well as other fungi [Mahmoudjanlou, et al., 2020; Puel, et al., 2005]. Mycophenolic Acid and derivatives of mycophenolic acid, such as mycophenolate mofetil and sodium mycophenolate, are used in the treatment of patients with organ transplantation and autoimmune disease by inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH) [Badrick, et al., 2009; Kitchin, et al., 1997]. MPA and its derivatives are commercially used as frontline immunosuppressive agents to prevent the rejection of transplant organs [Arns, et al., 2006; Kitchin, Pomeranz, 1997]. Commercial immunosuppressants based on MPA include CellCept (Mycophenolate mofetil; Roche) and Myfortic (mycophenolate sodium; Novartis). B and T lymphocytes entirely depend on IMPDH for the synthesis of nucleotides, while other human cells use different pathways for this synthesis, and are less affected by the anti-proliferative effect of MPA [Allison and Eugui, 2000]. Due to this reason, MPA is highly selective and have fewer side effects as compared to other immunosuppressants drug. Mycophenolic acid also exhibits several

therapeutic roles and is useful in the treatment of various autoimmune, cancer, fungal, and viral diseases [Rahman, et al., 2013; Shaw, et al., 2007]. MPA is also produced by chemical synthesis by an aromatic annulations strategy described by Ardestani *et al*. [Ardestani, et al., 2010]. Cyclobutanone and alkyl intermediates are interacted with benzene to yield a penta substituted phenol compound, and then further nine-step reaction yields mycophenolic acid. Clutterbuck *et al.* in 1932 produced mycophenolic acid from *Penicillium brevicompactum* [Chen, et al., 2021; Clutterbuck, et al., 1932].

2.2 Discovery of Mycophenolic Acid

In the late nineteenth and early twentieth century, a disease called pellagra roamed among the poor people of southern Europe and the southern U.S.A. The people living in these regions had a diet that was heavily dependent on corn, so naturally many scientists suspected that the disease was caused by the intake of bad corn. A lot of work was put in by the scientific community to come up with a solution to the pellagra problem [Sydenstricker, 1958]. An Italian physician named Bartolomeo Gosio started his work in 1892 [Bentley, 2000]. He studied pure fungal cultures obtained by the Italian maize, that he thought might be the cause of pellagra. Gosio isolated a mouldy strain from spoiled maize and gave the name *Penicillium glaucum*, one of the *brevicompactum*. The species is now called *Penicillium brevicompactum*. From the filtrated cultures of deteriorated corn, he was able to obtain a crystalline product that had a melting point of 143-145 °C and gave an intense blue color with a ferric chloride solution [Gosio, 1893]. That was later concluded that this substance must have been mycophenolic acid [Clutterbuck, Oxford, 1932]. Gosio could also show that this compound had an inhibitory effect on the growth of the *Bacillus anthracis* [Gosio, 1896]. Remember this is years before Fleming´s famous discovery in 1928 [Fleming,

1929], so in a way, this makes mycophenolic acid the first ever described antibiotic. According to Bentley and Clutterbuck *et al*, Gosio published his data in 1893 in the Reale Accademia Medicina di Torino and in 1896 in Rivista d'igiene e Sanitá pubblica [Bentley, 2000].

A couple of decades after Gosio´s work two American scientist named Alsberg and Black in 1913, isolated a substance from the fungus *Penicillium stoloniferum*, which they named mycophenolic acid. They thought that this substance was similar to the one Gosio isolated but not identical since the melting point of this substance was 141 °C and that it mixed with ferric chloride solution turned into green color rather than blue [Alsberg and Black, 1913].

But in 1932 Clutterbuck and colleagues analyses a number of *Penicillium* strains and concluded that the substances that Gosio and Alsberg and Black had isolated must have been one and the same namely mycophenolic acid [Clutterbuck, Oxford, 1932]. The differences observed were likely due to impurities, different concentrations, and inaccurate instrumentation. In the nineteen twenties and thirties the real cause of pellagra was shown to be an insufficiency of niacin (vitamin B_3) [Sydenstricker, 1958], but the work on mycophenolic acid continued.

2.3 Chemical Structure of Mycophenolic Acid

One must remember that when mycophenolic acid was first discovered over one hundred years ago, much of the technology a modern scientist takes for granted when elucidating the structure of a molecule for example nuclear magnetic resonance (NMR), was not available that time. Gosio could through various reactions conclude that mycophenolic

acid contained no nitrogen and through a combustion experiment he came up with the, as I would show, incorrect molecular formula $C_9H_{10}O_3$. Gosio was unable to come up with any molecular structure. According to Clutterbuck *et al* Alsberg and Black in the USDA Bureau of Plant Industry published the correct formula of mycophenolic acid $C_{17}H_{20}O_6$. Clutterbuck and his colleagues were able to confirm much of the earlier work and come up with some new facts, but they were not able to elucidate the complete structure [Clutterbuck and Raistrick, 1933].

In 1948 two possible structures of mycophenolic acid was proposed by Birkinshaw and coworkers [Birkinshaw, et al., 1948]. But it would take to 1952 until Birkinshaw *et al* were able to determine that proposed structure (I) was in fact the correct one and this is to this day the generally accepted structure of mycophenolic acid [Birkinshaw, et al., 1952]. Proposed structures of mycophenolic acid shown in Figure 2.1A and Figure 2.1B.

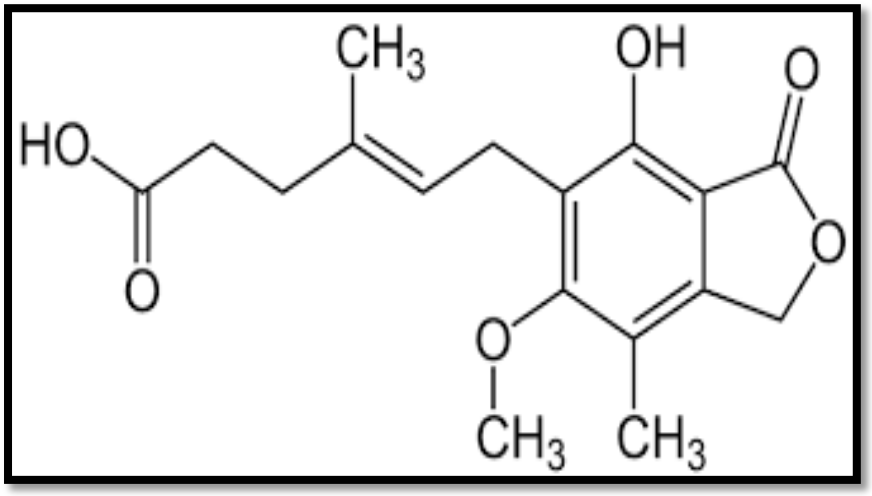

Figure 2.1A. Structure of mycophenolic acid

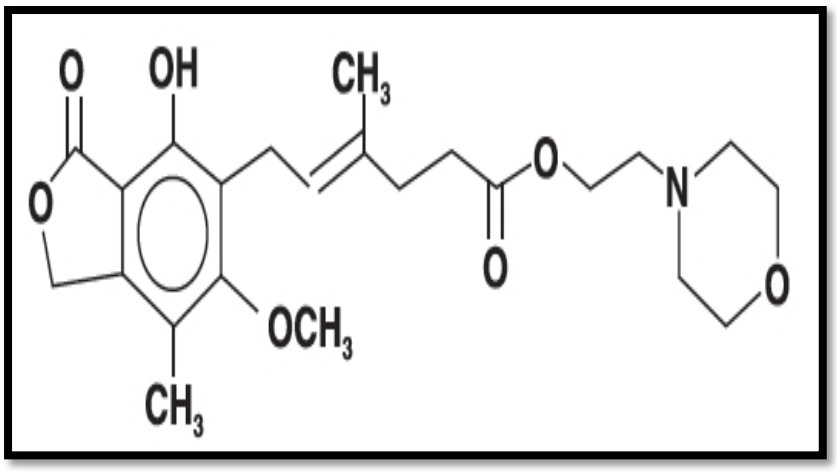

Figure 2.1B. Structure of mycophenolate mofetil

2.4 Biological Properties of Mycophenolic Acid

Mycophenolic acid has been shown to have several biological effects and has proven itself useful as a drug against psoriasis and as an immunosuppressant [Kitchin, Pomeranz, 1997]. The antibacterial property of mycophenolic acid was discovered by Gosio in the 19th century, but it would take until the nineteen forties before this and other biological properties were described further. In a publication from 1945 Abraham studies the effects of mycophenolic acid on a strain of *Staphylococcus aureus*. He finds that even though mycophenolic acid initially inhibits the growth of *Staphylococcus aureus*, the bacteria will become resistant [Abraham, 1945]. Mycophenolic acid does also have antiviral [Cline, et al., 1969; Kato, et al., 2020], antibacterial [Florey, et al., 1946; Quinn, et al., 1990; Siebert, et al., 2018; Williams Robert H, et al., 1968], antitumor [Ando, et al., 1970; Suzuki, et al., 1969], antifungal properties [Noto, et al., 1969; Williams RH, et al., 1968], and immunosuppressive agent [Chen, et al., 2022; Mitsui and Suzuki, 1969; Ohsugi, et al., 1976; Zhang, et al., 2018].

Because of various problems mycophenolic acid has never been used as a drug with any of these indications, even though clinical trials have been done an empting to use mycophenolic acid against different type of tumors [Carter, et al., 1969], but without success [Lee, et al., 1990].

2.5 Mycophenolic Acid: Mechanism of Interaction

Mycophenolic acid is a potent, selective, noncompetitive and reversible inhibitor of the human inosine monophosphate dehydrogenase (IMPDH) and of the guanosine monophosphate synthetase [Allison, et al., 1993; Franklin and Cook, 1969]. Inosine monophosphate dehydrogenase plays important role in the purine synthesis [Sweeney, et al., 1972]. It involved in the conversion of inosine monophosphate into xanthine monophosphate (XMP), an intermediate to the purine nucleotide, guanosine monophosphate (GMP). Due to the lack of guanosine triphosphate (GTP) the cells production of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and proteins will be compromised [Orvis, et al., 2009]. There are two major routes in which the cells can produce purine, namely the *de novo* and the salvage pathway (Figure 2.2). Mycophenolic acid only inhibits the *de novo* pathway, but since some cell types depend almost solely on this pathway, they will be more heavily affected than other cell types using both the *de novo* and the salvage pathway. The human T and B-lymphocytes are such cells that only use the *de novo* pathway, which explain the immunosuppressive effect that mycophenolic acid possesses [Allison and Eugui, 2000; Orvis, Wesson, 2009]. Figure 2.2 represents the two major pathways of purine biosynthesis.

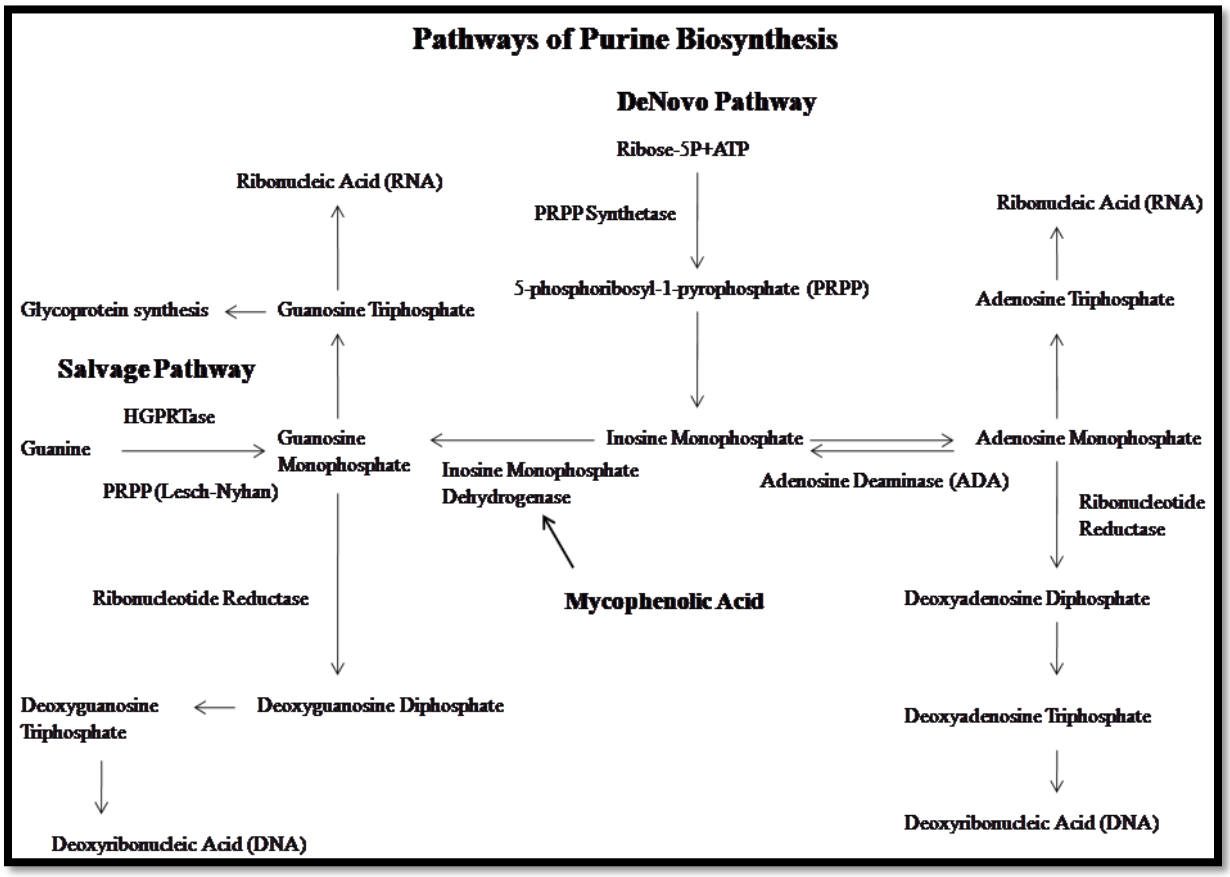

Figure 2.2. DeNovo and salvage pathways of purine biosynthesis, showing mechanism of action of mycophenolic acid.

2.6 Mycophenolic Acid Producing Microorganism

Mycophenolic acid is naturally produced by many strains of *Penicillium sp.* including *Penicillium brevicompactum, Penicillium stoloniferum, Penicillium roqueforti, Penicillium pinophilum, Penicillium bialowiezense, Penicillium corneum, Penicillium rugulosum* [Refai, et al.]. Some of the mutant strains of *Penicillium sp.* have also been developed with the help of chemical mutagens and irradiation to produce mycophenolic acid. Some of the mutant strains of *Penicillium sp.* have also been developed through chemical mutagens and irradiation [Queener and Nash III, 1978]. Schneweis et.al examined 233 samples of

contaminated and spoiled silage. They found that *Penicillium roqueforti*, a producer microorganism of mycophenolic acid were present in 206 samples [Schneweis, et al., 2000].

Puel *et.al* identified that *Byssochlamys nivea* produces mycotoxin patulin. They examined that degradation and spoilage of fruits occurs by this mycotoxin. They analyzed that *Byssochlamys nivea* also produces secondary metabolites like mycophenolic acid and its precursors such as 5-methylorsellinic acid and 5, 7- dihydroxy-4-methylphthalide [Puel, Tadrist, 2005]. Séguin *et.al* reported that mycophenolic acid also produced by *Eurotium repens* [Séguin, et al., 2014].

Mouhamadou *et.al* worked on xerophilic *Aspergillus* strains to produce mycophenolic acid. They were examined thirty xerophilic *Aspergillus* strains by molecular methods and found that some *Aspergillus* species produces mycophenolic acid. They identified that *Aspergillus pseudoglaucus* and the other strains of this species produced mycophenolic acid [Mouhamadou, et al., 2017].

2.7 Biosynthetic Pathway of Mycophenolic Acid

Biosynthesis of MPA mainly involves two major pathways. First one is polyketide pathway and second one is isoprenoid pathway [Bentley, 2000; Chain, 1940]. Biosynthesis of mycophenolic acid has involved two major routes, first one is polyketide pathway and second one is isoprenoid pathway [Chen, et al., 2019]. In 1958 Birch *et al* shown that methyl and methoxyl groups are attached in mycophenolic acid are derived from methionine, when they fed methionine in *Penicillium brevicompactum* culture [Birch, et al., 1958]. Later they found that the acetate pathway used for production of polyketide derivative mycophenolic acid and the aliphatic side chain is produced by incorporation of farnesyl diphosphate, leading to the

formation of side chain which is terpenoid in nature [Birch, et al., 1958; Dewick, 2002]. In 1973 the mycophenolic acid biosynthetic pathways order and nature of sequence was described by Bedford *et al* [Bedford, et al., 1971; Bedford, et al., 1973].

Canonica *et.al* performed incorporation experiments using different precursors and investigated that point where the methyl group and isoprenoid side chains are introduced, other than this, their experiments specifies that when formation of lactone ring and methylation of phenolic (–OH) occurred [Canonica, et al., 1970]. Canonica et al. found the critical aromatic intermediate onto which the terpenoid side chain is inserted in their prior experiment on the biosynthesis of mycophenolic acid. In *Penicillium brevicompactum*, the introduction and subsequent partial removal of a farnesyl side-chain occurs during the synthesis of mycophenolic acid. The methyl group is inserted before the formation of the group, resulting in 5, 7-dihydroxy-4-methylphthalide [Canonica, et al., 1971]. Mycophenolic acid has been shown to include an acetate-derived aromatic nucleus, a terpenoid side-chain, and two methionine derived methyl groups in its molecule [Canonica, et al., 1972].

The alkylation of 5, 7-dihydroxy-4-methylphthalide with a C_{15}-prenylated intermediate yields 6-farnesyl-5, 7-dihydroxy-4-methylphthalide. Mevalonic acid is the source of this compound. Farnesyl pyrophosphate in a cell-free environment is a substrate for this reaction [Bowen, et al., 1977].

As successive steps in the biosynthesis of mycophenolic acid, 5,7-dihydroxy-4-methylphthalide, 6-farnesyl-5,7-dihydroxy-4-methylphthalide, and normethylmycophenolic acid are involved [Colombo, et al., 1978].

Colombo *et.al* was used incorporation experiments and isotopic-trap experiments with advanced precursors to investigate the farnesyl-chain oxidation mechanism in mycophenolic acid production. The precursors were synthesized using two methods: a semi synthetic technique starting with mycophenolic acid and a total-synthesis process. They reveal that 6-farnesyl-5, 7-dihydroxy-4-methylphthalide may be transformed to mycophenolic acid by at least two pathways: direct oxidation of the central double bond and two-stage removal of the terminal and central groups [Colombo, et al., 1982; Colombo, et al., 1979].

A number of researchers have investigated the integration of various radioactive substances into the production of mycophenolic acid in order to better understand the process. Several phenolic intermediates originating from a tetraketide chain have been identified in the production of mycophenolic acid in these labelling experiments [Klangjorhor, et al., 2020]. A proposed biosynthetic pathway of mycophenolic acid was studied by Muth *et.al.* They studied the last step of the reaction where transfer of a methyl group from S-adenosylmethionine to demethylmycophenolic acid occurs [Muth, et al., 1975].

John W, Patterson examined the mechanism of methylation reaction. They were introduced the three necessary carbon substituents regioselectively into 2, 4-dihydroxybenzoic acid to produce mycophenolic acid. The insertion of the methyl substituent at position 5 by a fast, uncatalyzed replacement of the bromide in position 8 with methyllithium at low temperature is a critical alteration in this sequence [Patterson, 1995].

Regueira *et.al* reported the biosynthesis of mycophenolic acid in *Penicillium brevicompactum* on molecular basis. They describe the identification of MpaC, a polyketide

synthase (PKS) in Penicillium brevicompactum that we successfully characterized and identified as responsible for MPA synthesis [Regueira, et al., 2011]. Figure 2.3 represents the biosynthetic pathway of mycophenolic acid.

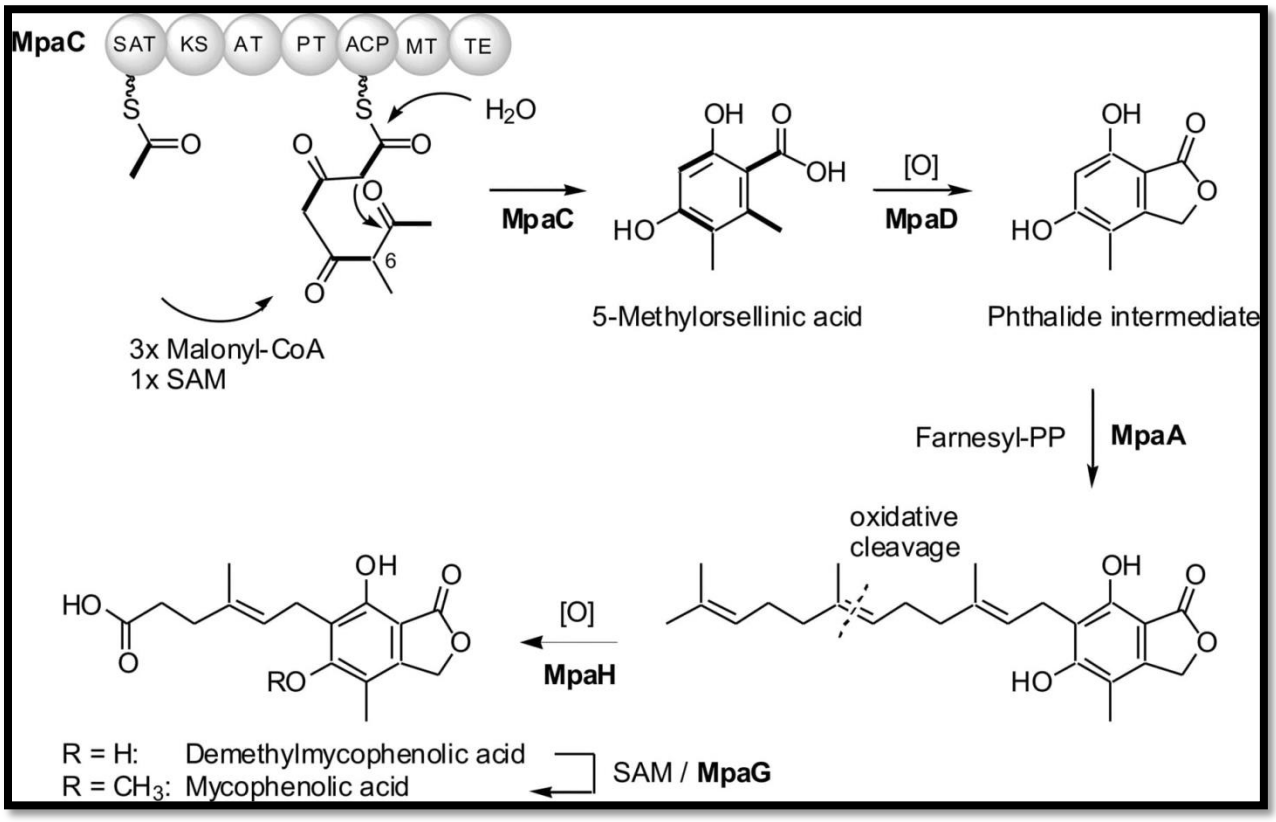

Figure 2.3. Proposed biosynthetic pathway of mycophenolic acid from acetyl-CoA and malonyl-CoA

Hansen *et.al* investigated the role of a natural fusion of a cytochrome P450 and a hydrolase in the biosynthesis of mycophenolic acid. The MPA biosynthesis pathway gene cluster was recently found in *Penicillium brevicompactum*, indicating that MpaC, a polyketide synthase that produces 5-methylorsellinic acid, catalyses the first step (5-MOA). The biological function of the enzymes encoded by the other genes in the MPA gene cluster, on the other hand, remains unclear.

In this study they postulated that the step after 5-MOA formation in the pathway is carried out by a natural fusion enzyme MpaDE, which consists of a cytochrome P450 (MpaD) in the N-terminal region and a hydrolase (MpaE) in the C-terminal area, based on bioinformatic study of the MPA gene cluster. They obtained the full-length sequence of the MpaDE cDNA generated from the isolated RNA to confirm that the fusion gene is expressed in *P. brevicompactum*. In the MPA-nonproducer *Aspergillus nidulans*, heterologous coexpression of MpaC and the fusion gene MpaDE resulted in the creation of 5, 7-dihydroxy-4-methylphthalide (DHMP), the second stage in MPA biosynthesis. According to a strain coexpressing MpaC and the MpaD component of MpaDE (DHMB), the P450 catalyses the hydroxylation of 5-MOA to 4, 6-dihydroxy-2-(hydroxymethyl)-3-methylbenzoic acid. According to their findings, the hydrolase domain appears to facilitate this second phase by serving as a lactone synthase those catalyses the ring closure. Overall, the chimeric enzyme MpaDE sheds light on the MPA biosynthesis pathway's genetic architecture [Hansen, et al., 2012; Zhang, et al., 2019]. Figure 2.4 showed the enzymes involved in mycophenolic acid biosynthesis.

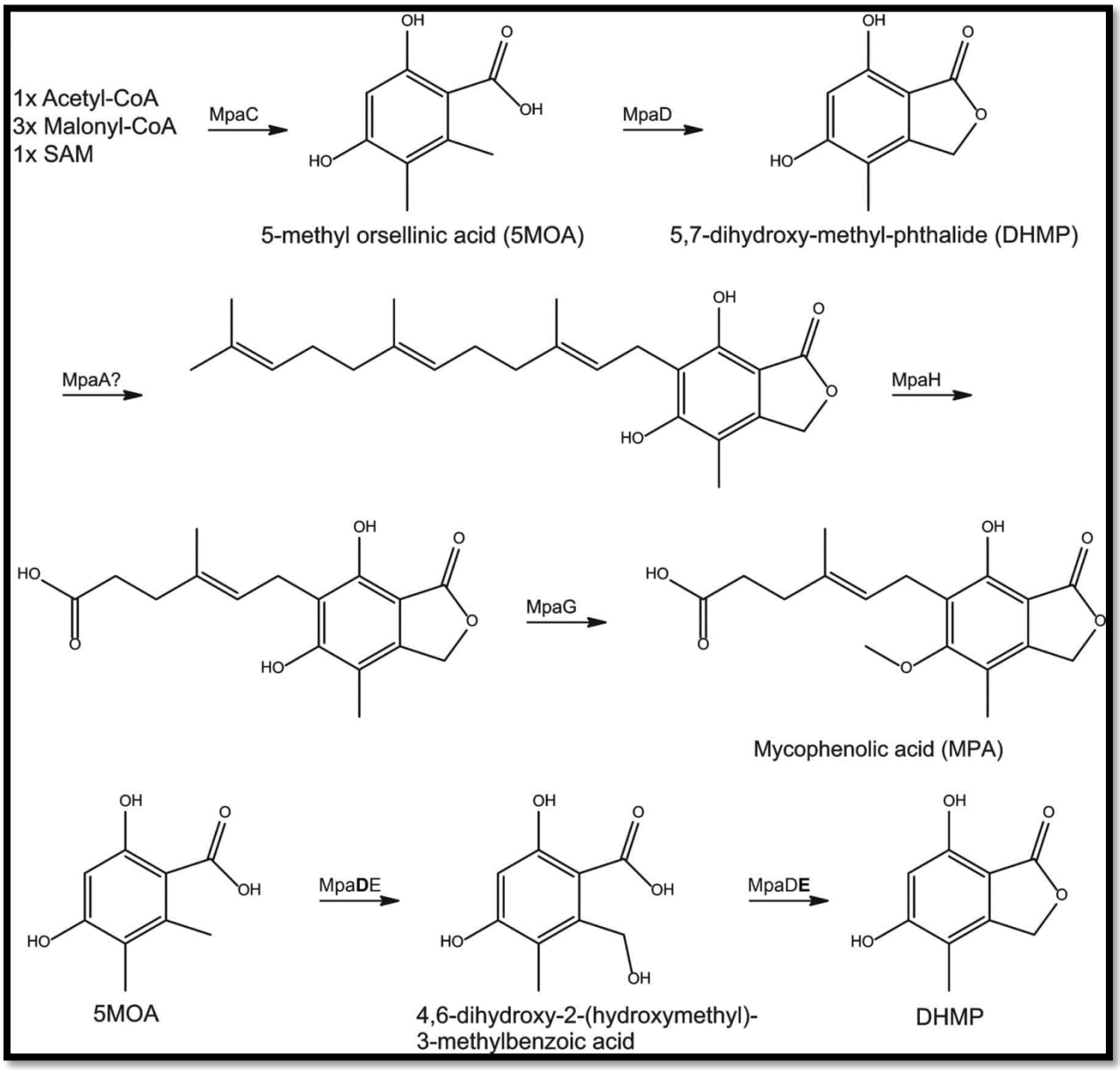

Figure 2.4. Mycophenolic acid biosynthetic pathway, including the enzymes proposed by Regueira *et.al*

Del cid *et.al* studied on *Penicillium roqueforti* and identifies the mycophenolic acid gene cluster. They were found a genomic area of approximately 24.4 kb containing a seven-gene cluster that may be involved in MPA production in P. roqueforti using a bioinformatic technique. Silencing each of these seven genes (called mpaA, mpaB, mpaC, mpaDE, mpaF,

mpaG, and mpaH) resulted in a significant decrease in MPA production, indicating that they are all involved in the compound's biosynthesis [Del-Cid, et al., 2016]. Figure 2.5 represents the gene cluster present in *Penicillium roqueforti* for biosynthesis of mycophenolic acid.

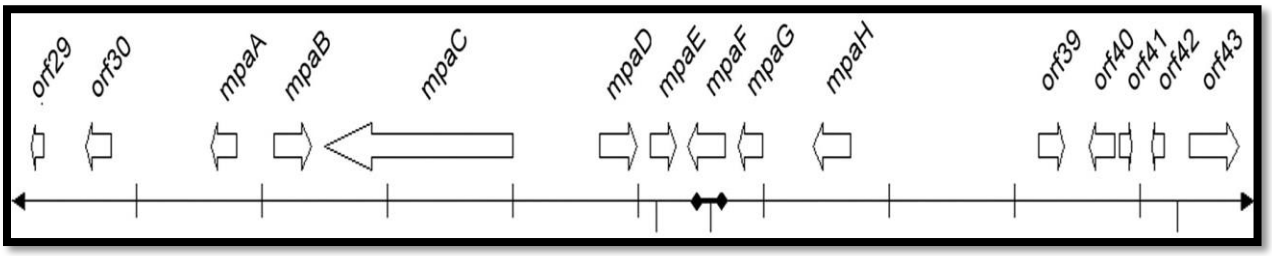

Figure 2.5. Schematic organization of the mycophenolic acid biosynthetic gene cluster

2.8 Microbial Production of Mycophenolic Acid

Microbial production of a compound can vary with the mode of fermentation used. Solid state fermentation and submerged state fermentation are used to produce mycophenolic acid by using *Penicillium* species.

In the year 1977, Nulton *et.al* found that *Penicillium brevicompactum* produces mycophenolic acid on Czapek Dox medium during steady growth [Nulton and Campbell, 1977]. After this study Doerfler *et.al* found that mycophenolic acid was produced by using *Penicillium brevicompactum* in simple and richer medium [Doerfler, et al., 1979].

In 1979, Lafont *et.al* isolated sixteen strains of *Penicillium roqueforti* from blue-molded cheese [Lafont P, et al., 1979]. They also studied the production of mycophenolic acid from these strains and found that mycophenolic acid produced by all strains. The maximum yield obtained after 10 days of incubation period at 15 ºC was 4 mg/g of dry culture. According to the result reported by the authors, the nature of the substrate on which

the fungus grows plays an important role in production of mycophenolic acid [Lafont PHILIPPE, et al., 1979]. Bartman *et.al* used solid media to produce mycophenolic acid. They used *Penicillium brevicompactum* Dierckx ATCC 9056 strain for mycophenolic acid production, was grown on Czapek Dox media. They were used wheat, rice, corn-meal, and oat-meal as a substrate. They extracted each sample after eight days with ethyl acetate and assayed for mycophenolic acid production. Mycophenolic acid obtained from wheat, rice, corn-meal and oat-meal was 17.9, 2.3, 3.6 and 7.6 mg/plate, respectively [Bartman, et al., 1981].

Ozaki *et.al* derived a mutant form of the strain *Penicillium brevicompactum* ATCC 16024, which is a drug resistant mutant No 4-23-11. It was cultured using submerged fermentation for the production of mycophenolic acid. Mycophenolic acid production was observed both in the presence and absence of adsorbents such as celite, alumina, zeolite, talc, silica, charcoal, carbon blacks, natural graphite and carbonaceous mesophase spheres. In the absence of adsorbents a production of 0.2-4.8 g/L of mycophenolic acid was observed. In the presence of one of these adsorbents, production of mycophenolic acid was found to increase up to 4.5-5.4 g/L. They also observed that in the absence of adsorbents the size of pellets were (0.5 -5) mm which in turn depended on the inoculated spore concentration, while in the presence of adsorbents, the size of pellets observed was approximately 1 mm. The pellets were microscopically observed and it was discovered that the adsorbent particles lay affixed on the surface of hyphae. Their study revealed that the sticking of adsorbent particles to the hyphae slowed down the formation of larger pellets. Hence, small pellet size lead to increased production due to less mass transfer limitations [Ozaki, et al., 1987].

Sadhukhan *et.al* studied solid state fermentation for production of mycophenolic acid by using *Penicillium brevicompactum* ATCC 16024. They used moist wheat bran for production of mycophenolic acid. The maximum production of mycophenolic acid was observed to be 3.29 g/kg of wheat bran [Sadhukhan, et al., 1999]. Schneweis *et.al* examined 233 silage samples and found that, molds were present in 206 samples. After detection of these samples by liquid chromatography-mass spectrometry (LC-MS), they found Mycophenolic acid in 74 samples [Schneweis, Meyer, 2000].

Puel *et.al* studied production of mycophenolic acid in submerged state fermentation by using *Byssochlamys nivea.* They examined and found that about 0.02 g/L of mycophenolic acid was produced by this strain [Puel, Tadrist, 2005]. Vinokurova *et.al* worked on various strains of *Penicillium* species that can produce mycophenolic acid. They took 36 strains of Penicillium fungus and found that 14 strains out of these 36 strains can produce mycophenolic acid [Vinokurova, et al., 2005].

Research for production of mycophenolic acid has also been extended to the immobilization of *Penicillium brevicompactum* for reduced fermentation time and increase in volumetric productivity. Xu *et.al* immobilized *Penicillium brevicompactum* in rotating fibrous bed bioreactor (RFB) for repeated use of culture and cell-free fermentation broth which can ease the purification process. The immobilized-cell fermentation in the rotating fibrous bed bioreactor reached a high mycophenolic acid concentration of 5.7 g/L in about 14 days of incubation [Xu and Yang, 2007].

Alani *et.al* studied the productivity of mycophenolic acid by solid state fermentation. Higher productivity was obtained by solid state fermentation then submerged state

fermentation using pearl barley as compared to other substrates. Other parameters such as moisture content, inoculum concentration and incubation time were optimized to obtain yield of 6.9 mg/g using a packed bed bioreactor [Alani, et al., 2009].

To determine the effect of continuous culture Ardestani *et.al,* studied the effect of continuous culture in 2 L stirred tank fermentor on *Penicillium brevicompactum* MUCL 19011, which resulted in steady rate of production during 120 h of fermentation. They studied a variety of structured and unstructured growth kinetics models for batch culture of *Penicillium brevicompactum* MUCL 19011 and found Contois model to be the best fit with the experimental data [Ardestani, Fatemi, 2010].

Ramos *et.al* studied the production of mycophenolic acid in submerged fermentation by using *Penicillium pinophilum*. After 10 days of incubation they reported the maximum production of mycophenolic acid about 923 mg/L [Ramos-Ponce, et al., 2012].

Ismaiel *et.al* studied mycophenolic acid production in submerged fermentation by using two isolated strains of *Penicillium roqueforti* (AG101 and LG109) from blue-molded cheese. They observed that under optimized medium conditions the maximum mycophenolic acid production by AG101 and LG109 was 2.70 and 2.54 mg/L, respectively [Ismaiel, Ahmed, 2014]. They further studied production of mycophenolic acid in different agricultural waste using these strains, and found that sugarcane bagasse produce higher mycophenolic acid [El-Sayed, et al., 2019].

Mycophenolic acid production in submerged fermentation by using *Penicillium brevicompactum* MTCC 8010 was reported by Patel *et.al.* They optimized different carbon source, nitrogen source and precursors by using one variable at a time (OVAT) and central

composite design (CCD) for mycophenolic acid production. By using optimized medium in OVAT approach the maximum mycophenolic acid yield was 1232 mg/L and by using CCD the maximum MPA yield was 1737 mg/L. They reported that the production of mycophenolic acid increases 9- folds in central composite design than single parameter optimization [Patel, et al., 2016].

Patel *et.al* reported mycophenolic acid production in solid state fermentation by using *Penicillium brevicompactum*. They were used whole wheat, cracked wheat, long grain Basmati rice, and short grain Parmal rice as substrate to produce mycophenolic acid. Under optimized condition they found that production of mycophenolic acid was higher in Parmal rice about 3.4 g/kg [Patel, et al., 2017].

Different fermentation processes were studied for microbial production of mycophenolic acid. Table 2.1 shows comparison of different fermentation processes for mycophenolic acid production.

Table 2.1: Comparison of MPA production in various fermentation processes

Fermentation process	**Scale of fermentation**	**Microbial strain**	**Fermentation time (h)**	**MPA titer (g/kg or g/L)**	**Reference**
Submerged	Shake flask	*P. brevicompactum* MTCC 8010	280	1.73	[Patel, Patil,

					2016]
Submerged	Shake flask	*P. brevicompactum* MUCL 19011	280	1.38	[Ardestani, Fatemi, 2010]
Submerged	Shake flask	*P. brevicompactum* MUCL 19011	280	3.63	[Ardestani, Fatemi, 2010]
Submerged	2 L fermentor	*P. brevicompactum* MUCL 19011	240	1.37	[Ardestani, Fatemi, 2010]
Submerged	2 L fermentor	*P. brevicompactum* MUCL 19011	120	1.46	[Ardestani, Fatemi, 2010]
Submerged	Shake flask	*P. brevicompactum* ATCC 16024	144	0.06	[Alani, Grove, 2009]
Solid state	Shake flask	*P. brevicompactum* ATCC 16024	144	1.219	[Alani, Grove, 2009]
Submerged	5 L fermentor	*P. brevicompactum*	264	2.31	[Xu and

		ATCC 16024			Yang, 2007]
Submerged	5 L (RFB) fermentor	*P. brevicompactum* ATCC 16024	338	5.70	[Xu and Yang, 2007]
Submerged	Shake flask	*Byssochlamys nivea*	670	0.02	[Puel, Tadrist, 2005]
Solid-state	Shake flask	*P. brevicompactum* ATCC 16024	144	3.29	[Sadhukhan, Murthy, 1999]
Submerged	Shake flask	*P. brevicompactum* mutated	336	4.5-5.4	[Ozaki, Ishihara, 1987]
Submerged	Shake flask	*P. brevicompactum* ATCC 16024	336	0.2-4.8	[Ozaki, Ishihara, 1987]
Submerged	3.7 L Bioreactor	*P. brevicompactum* MTCC 549	240	1.84	[Anand, et al., 2020]
Submerged	3.7 L	*P. brevicompactum*	288	1.91	[Anand

	Bioreactor	MTCC 549			and Srivastava, 2021]

2.9 Strain improvement

In all fermentation processes, strain enhancement tactics and better production conditions are critical for increasing secondary metabolite yield. Because the microbial strain is at the heart of the fermentation sector, improving the strain provides the most cost-cutting prospects with the least amount of capital outlay.

Ismaiel *et.al* used immobilization of two strains of *Penicillium roqueforti* (AG 101 and LG 109) for more efficient production of mycophenolic acid. They used UV and gamma radiation to improve the formation of mycophenolic acid in immobilized spores and mycelia. For immobilization, they used several entrapping carriers. They used a 3 % (w/v) alginate concentration and a 10 % (w/v) mycelia fresh weight to obtain maximum mycophenolic acid synthesis. They discovered that alginate immobilized spores efficiently generate mycophenolic acid. They also found that exposing immobilized strain to UV (250 nm) for 120 and 90 minutes significantly increased MPA synthesis [Ismaiel, et al., 2015].

The influence of different light sources and light intensity on mycophenolic acid by *Penicillium brevicompactum* in batch culture was significantly demonstrated by Chin-Hang Shu *et.al*. In a light-controlled photo-bioreactor, MPA batch cultures were examined under

various light sources, including fluorescent light, red light emitting diode (LED) light (630 nm), and blue LED light (470 nm) [Shu, et al., 2010].

Ultra violet radiation (UV), ethyl methane sulphonate, and methyl viologen were used to enhance the strain of *Penicillium brevicompactum* for production of mycophenolic acid. For various periods of mutagen exposure, the proportion of spores that survived was calculated. The mutagenesis experiments yielded two mutant colonies. Ethyl methane sulphonate and methyl viologen treatment resulted in the colonies P120EMS1 and P90MV2, respectively. The yield varied from mutant to mutant; with the methyl viologen mutant P90MV2 producing the highest yield of 10.942 mg/g and the ethyl methane sulphate mutant P120EMS1 is producing the lowest yield of 10.216 mg/g. According to a comparison, MV increased MPA yield by 28%, while ethyl methane sulphonate and UV light increased MPA yield by 20% and 5%, respectively.

The first report of MPA production by a local strain of the *Penicillium glabrum* species was reported by mahmoudian *et.al*. As secondary metabolites, soil-dwelling fungus create a range of mycotoxins, one of which is mycophenolic acid, an antibiotic and immunosuppressive agent. They were isolated the Ascomycete cultures from mouldy food and fruits, as well as soils collected from various locations of Iran, and screened. HPLC was used to examine the MPA production of a total of 140 *Penicillium* isolates. Three MPA producing isolates were found, with the most producers being further characterised using morphological and microscopic research, as well as a molecular method (ITS, rDNA and beta-tubulin gene sequences). After the experiment they found that the best MPA producer was *P. glabrum* IBRC-M 30518, which can produce 1079 mg/L MPA in Czapek-Dox medium [Mahmoudian, et al., 2021].

2.10 Optimization of production parameters

The cultivation of filamentous microorganisms in industries is primarily done in a liquid medium that contains all of the essential nutrients to ensure the microorganism's development and the generation of the desired secondary metabolite. The culture media primarily contain carbon and nitrogen sources, as well as a different variety of organic and inorganic nutrients, as well as phosphates, sulphates, and other minor nutrients. It also contains trace elements. The availability and cost of the components used in cultivation media influence the selection. In the fermentations of *Penicillium brevicompactum* for the generation of mycophenolic acid, the cultivation media have been widely explored [Ardestani, Fatemi, 2010; El-Sayed, Ahmed, 2019; Patel, Patil, 2017; Patel, Patil, 2016; Zhou, Han, 2018].

Sadhukhan *et.al* used solid state fermentation process for production of mycophenolic acid using *Penicillium brevicompactum*. They were used wheat bran for the production and found that 425 mg of mycophenolic acid produced per kg of wheat bran. They were also used statistical approach for optimization of different nutrients [Sadhukhan, Murthy, 1999].

Ardestani *et.al* studied the different nutrients for production of mycophenolic acid. They were optimized nutrients using statistical approach. They were optimized different media components for enhancing the production of MPA. After addition of enzymatically hydrolyzed casein in base medium they found an increase in production and productivity of mycophenolic acid [Ardestani and Najafpour, 2012].

Patel *et.al* reported the production of MPA by submerged fermentation using *Penicillium brevicompactum*. They were optimized pH, temperature, carbon sources,

nitrogen sources, and precursors for maximizing the MPA production. They were used different carbon sources and found that glucose gives better results. Similarly they were found peptone as a potential nitrogen source for maximizing the production of MPA [Patel, Patil, 2016].

2.11 Influence of pH and Temperature

The pH of the broth influences mycophenolic acid synthesis as well as the growth of *Penicillium brevicompactum*. The generation of mycophenolic acid and biomass could be increased by changing the pH of the fermentation broth at regular intervals. The optimum temperature for development and synthesis of mycophenolic acid was found to be 28°C, and optimum pH was 5.5 [Ardestani, Fatemi, 2010; El-Sayed, Ahmed, 2019; Ismaiel, Ahmed, 2014; Patel, Patil, 2016].

2.12 Use of Different Bioreactor for Production of MPA

Different bioreactor studies were performed for maximizing the production of mycophenolic acid. Alani used packed bed bioreactor for production of mycophenolic acid using solid state fermentation. They were use pearl barley, wheat bran, oats and rice for production in solid state fermentation. They were found an increase in production of mycophenolic acid in solid state fermentation then submerged fermentation [Alani, Grove, 2009].

Xu *et.al* used immobilized cell of *Penicillium brevicompactum* in rotating fibrous bed bioreactor for production of mycophenolic acid. They were optimized a complex media for production of mycophenolic acid. In 5 L fermentor, the optimized medium was evaluated for

mycophenolic acid synthesis. The typical stirred-tank fermentor was found to be difficult to run, with fungal mycelia growing uncontrollably throughout the fermentor, resulting in low MPA yield. P.brevicompactum spores were trapped in a rotating fibrous-bed (RFB) in the bioreactor to solve this problem. Almost all mycelia were adhered to the RFB after spore germination, and the fermentation broth remained clear throughout the batch fermentation [Xu and Yang, 2007].

Patel *et.al* used stirred tank bioreactor for the production of mycophenolic acid. They were performed different fermentation modes for enhancing the production [Patel, et al., 2018].

2.13 Fed Batch Strategy

Fed-batch culture is defined in the broadest sense as a biotechnological process operation in which one or more nutrients are fed to the bioreactor during cultivation and the products remain in the bioreactor until the conclusion of the run. When altering the amounts of a nutrient impacts the production or productivity of the target metabolite, fed-batch culture is preferable than standard batch culture.

A combination feeding strategy for boosting mycophenolic acid synthesis via fed-batch fermentation was explored using *Penicillium brevicompactum*. They were investigated carbon and nitrogen feeding strategy and methionine feeding strategy and pH shift strategy for production. By comparing these three strategy in fed batch fermentation they found an improved production of mycophenolic acid [Dong, et al., 2015].

The synthesis of mycophenolic acid (MPA) was adjusted and compared using batch and fed-batch fermentation techniques by Patel *et.al*. In a 14 L stirred tank bioreactor, different feeding strategies were examined in fed-batch fermentations [Patel, Biswas, 2018].

2.14 Extraction and Purification of Mycophenolic acid

Mycophenolic acid produced by fermentation of *Penicillium brevicompactum* was further extracted by solvent extraction process using different solvent [Živanović, et al., 2008]. Purification of mycophenolic acid was done by using high performance liquid chromatography, and column chromatography [Muth, Nash III, 1975; Sircar, et al., 2005].

Willis *et.al* reported a high-performance liquid chromatography tandem mass spectrometry approach with indomethacin as an internal standard to make investigating free mycophenolic acid concentrations easier. They were used ultra filtration method for isolation of free drug from plasma samples [Willis, et al., 2000].

Klepacki *et.al* used liquid-handling robotic extraction to develop and test an automated high-throughput ultra-high performance chromatography–tandem mass spectrometry (U-HPLC–MS/MS) assay for the quantification of MPA [Klepacki, et al., 2012].

The development and full validation of a rapid, high throughput sensible and accurate UPLC method for mycophenolate acid and its metabolites, MPA glucuronide (MPAG), and acyl MPA glucuronide (AcMPAG) concentration determination in human plasma using tandem mass spectrometry detection with MPA-D3 as internal standard were described by delavenne *et.al* [Delavenne, et al., 2011].

For the determination of mycophenolic acid and its phenol glucuronide (MPAG) and acyl glucuronide metabolites, annesley *et.al* developed and validated a quick and reliable liquid chromatography–tandem mass spectrometry (LC-MS/MS) technique [Annesley and Clayton, 2005].