Chapter

The Application of Solid State Fermentation for Obtaining Substances Useful in Healthcare

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Abstract

In the current review we summarised the research involving solid state fermentation (SSF) for the production of compounds that could be used in healthcare (terpenoids, polyphenols, fibrinolytic enzymes, mycophenolic acid and others). We described several groups of obtained agents which hold various activity: antimicrobial, anti-inflammatory, immunosuppressive, anticoagulant and others (e.g. anticancer or anti-diabetic). It seems that especially terpenoids and polyphenols could be useful in that field, however, other substances such as enzymes and fatty acids play important role as well. We described main groups of microorganisms that are applied in SSF of those compounds, particularly *Bacillus* genus and fungi, and where possible provided information regarding genes involved in those processes. We also compared various approaches toward optimisation of SSF.

Keywords: solid state fermentation, healthcare, agricultural waste, *Bacillus*, fungi

1. Introduction

Solid state fermentation (SSF) is a process during which microorganisms (in the presence of small amounts of water) transform agro-industrial waste into valuable compounds [1]. Based on the literature research, wheat bran was commonly used for those processes (**Table 1**). It is composed of about 53% of dietary fibre (xylans, lignin, cellulose, and galactan, fructans) and contains variety of phenolic acids e.g. ferulic acid, vanillic acid, coumaric acid, caffeic acid, and chlorogenic acid [71]. Researchers also applied other materials rich in polysaccharides (e.g. rice, whole grain wheat, millet, barley) or simple sugars (e.g. fruit pomace) (**Table 1**). Other substrates which were utilised for SSF are not only sources of carbohydrates, but also protein e.g. soybeans, lentil flour, silkworm larvae, fish meal, cuttle fish waste and king oyster mushroom (**Table 1**). The selection of waste products used in SSF should ensure the proper balance of nutrients to allow microbial growth and production of terpenoids, polyphenols, enzymes, biosurfactants, short chain fatty acids or others. Therefore, industrial waste with a high content of carbohydrates, protein, pectin or lipids is a suitable substrate.

There were various review papers regarding SSF but in the current chapter we focused only on selected substances which could be used in healthcare and demonstrate antimicrobial, anti-inflammatory properties or/and are immunosuppressants, anticoagulants and anticancer agents, e.g. enzymes, surfactants, terpenoids,

Name of the substance	Microorganism	Agricultural waste	Reference
	Antimicrobial properties		
Nonactin, monactin, dynactin, trinactin	*Streptomyces cavourensis* TN638	Immobilised bacterial spores (XAD-16) on potato dextrose agar	[2]
Surfactin homologues	*Bacillus natto* NT-6	Potato dextrose medium	[3]
Biosurfactant	*Bacillus subtilis* SPB1	Millet	[4]
Surfactin	*Bacillus pumilus* UFPEDA 448	Okara and sugarcane bagasse	[5]
Biosurfactant	*Tremetes versicolor* TV-6	Two-phase olive mill waste, wheat bran and olive stone	[6]
Biosurfactant	*Aspergillus niger*	Wheat bran and corncob	[7]
Not specified	*Pediococcus acidilactici* KTU-05-7	Milk thistle seeds	[8]
Not specified	*Bacillus licheniformis*	Wheat bran, soybean meal, yeast, fish meal	[9]
Sambacide	*Fusarium sambucinum* B10.2	Potato	[10]
γ-Decalactone	*Yarrowia lipolytica* W29 (ATCC 20460)	Luffa sponge, cellulose sponge, corncob, castor seed	[11]
Phenolic acids	*Pleurotus sapidus*	Sunflower seed hulls, golden rice straw and husks	[12]
Curcumin	*Trichoderma* strains	Turmeric	[13]
Coumarins and oxylipins	*Aspergillus oryzae* KCCM 12698	Malt extract agar	[14]
Phenolic compounds	*Lentinus edodes*	Cranberry pomace	[15]
Phenolic compounds	*Trichoderma* strains	Commercial turmeric	
Phenolic compounds	*Trichoderma* strains	Ginger powder	[16]
Phenolic compounds	*Trichoderma reesei*	Garden cress seeds	[17]
Phenolic compounds	*Aspergillus oryzae* NCH 42	Chinese cucumber, Chinese sage, houpu magnolia, liquorice root	[18]
Phenolic compounds	*Bacillus clausii*	Spent coffee grounds (Arabica)	[19]
	Anti-inflammatory agents		
Phenolic compounds	*Trametes versicolor* TV-6	Grape pomace	[20]
Not specified	*Taiwanofungus camphoratus* obtained by SSF		[21]
Rutin	*Rhizopus oligosporus* NRRL 2710	Buckwheat groats	[22]
Betulinic acid	*Inonotus obliquus*	The spent substrate of king oyster mushroom, grain including corn, rice grain, white birch and mulberry powder	[23]

Name of the substance	Microorganism	Agricultural waste	Reference
Phenolic compounds	*Xylaria nigripes*	Wheat bran	[24]
limonene-1,2-diol, α-terpineol, (−)-carvone, α-tocopherol, dihydrocarveol and valencene	*Diaporthe sp. (Phomopsis sp.)*	Orange peel and bagasse	[25]
Phenolic compounds, lignans	*Pediococcus acidilactici* LUHS29	Grounded barley by-products	[26]
α-Pinene	*Saccharomyces cerevisiae* AXAZ-1 and *Kluyveromyces marxianus* IMB3	Mixed solid and liquid food industry wastes	[27]
δ-Octalactone γ-Undecalactone γ-Dodecalactone δ-Dodecalactone	*Trichoderma viride* EMCC-107	Dried and grounded sugarcane bagasse	[28]
cis-Linaloloxide, Phenanthrene	*Trichoderma viride Pers. ex Fr.; Aspergillus niger van Tieghem*	Pu-erh tea	[29]
Various terpenes	*Antrodia camphorata*	Millet	[30]
Neochlorogenic acid), chlorogenic acid, rutin, 6″acetyl-glucoside	*Aspergillus niger* ATCC-6275 *Rhizopus oligosporus* ATCC-22959	Stones and pomace from fully ripened apricot	[31]
Quercetin and phenolic acids: gallic, vanillic, p-hydroxybenzoic, ferulic	*Lactobacillus plantarum* CECT 748 ATCC 14917	Cowpeas	[32]
Phenolic compounds	*B. subtilis* BCRC 14715	Black soybeans	[33]
Daidzin, daidzein, genistin and genistein	*Eurotium cristatum* YL-1	Soybeans seeds	[34]
Gallic acid	*Rhizopus oryzae* (RO IIT RB-13, NRRL 21498) *Aspergillus foetidus* (GMRB013 MTCC 3557)	Powdered fruits of Myrobalan and Teri pod	[35]
4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid and unidentified compounds	*Rhizopus oryzae* RCK2012	Whole grain wheat	[36]
3,4-di-hydroxybenzoic acid, ferulic acid, vanillic acid, quercetin	*Aspergillus oryzae* LBA01, *A. niger* LBA02	Lentil flour	[37]
	Immunosupresants		
Mycophenolic acid	*Penicillium brevicompactum* DSM 2215	Rice bran-potato peel mixture	[38]
Mycophenolic acid	*Penicillium brevicompactum* ATCC 16024 (AFI 668)	Pearl barley	[39]
Mycophenolic acid	*Penicillium brevicompactum* ATCC 16024	Wheat bran	[40]
Mycophenolic acid	*Penicillium brevicompactum* MTCC 8010	Rice bran	[41]
Mycophenolic acid	*Penicillium brevicompactum* (various strains)	Various agricultural waste	[42]
Mycophenolic acid	*Penicillium roqueforti* (AG101 and LG109)	Sugarcane bagasse	[43]

Name of the substance	Microorganism	Agricultural waste	Reference
Cyclosporin A	*Tolypocladium inflatum* MTCC 557	Hydrolysed wheat bran flour and coconut oil cake	[44]
Cyclosporin A	*Tolypocladium inflatum* MTCC 557	Wheat bran flour and coconut oil cake	[45]
Cyclosporin A	*Tolypocladium inflatum* ATCC 34921	Wheat bran	[46]
Cyclosporin A	*Tolypocladium inflatum* DRCC 106 (mutated srain)	Wheat bran	[47]
Cyclosporin A	*Tolypocladium* sp.	Wheat bran	[48]
Tacrolimus	*Streptomyces hygroscopicus*	Various agricultural waste	[49]
	Anticoagulant agents		
Halotolerant chitinase	*Citrobacter freundii* str. nov. *haritD11*	Wheat bran with fish scale	[50]
Halotolerant Chitinase	*Citrobacter freundii* str. nov. *haritD11*	Wheat bran with shrimp shellfish	[51]
Fibrynolytic enzyme	*Bacillus amyloliquefaciens* LSSE-62	Chickpeas	[52]
Fibrinolytic enzyme	*Bacillus* sp. IND6	Wheat bran	[53]
Fibrinolytic enzyme	*Bacillus sp.* IND12	Cow dung	[54]
Nattokinase	*Bacillus subtilis* natto	Soybean	[55]
Fibrinolytic enzyme	*Bacillus subtilis* XZI125	Soybean meal	[56]
Fibrinolytic enzyme	*Bacillus subtilis* WR350	Corn steep	[57]
Fibrinolytic enzyme	*Bacillus halodurans* IND18	Wheat bran	[58]
Fibrinolytic enzyme	*Bacillus cereus* GD55	Apple pomace	[59]
Fibrinolytic enzyme	*Bacillus cereus* IND5	Cuttle fish waste and cow dung	[60]
Fibrinolytic enzyme	*Paenibacillus* sp. IND8	Wheat bran	[61]
Fibrinolytic enzyme	*Pseudoalteromonas* sp. IND11	Sun-dried cow dung	[62]
Fibrinolytic enzyme	*Xanthomonas oryzae* IND3	Cow dung	[63]
Fibrinolytic enzyme	*Bacillus firmus* NA-1	Soybean grits	[64]
Fibrinolytic enzyme	*Mucor subtillissimus* UCP 1262	Wheat bran	[65]
Fibrinolytic enzyme	*Fusarium oxysporum*	Rice chaff	[66]
Fibrinolytic enzyme	*Fusarium oxysporum*	Rice chaff	[67]
	Anticancer agents		
Short chain fatty acids	*Aspergillus kawachii* KCCM 32819	Silkworm larvae powder	[68]
Putative phytoestrogen	*Aspergillus fumigatus* F-993 or *A. awamori* FB-133	Defatted soybean	[69]
Andrastin A and C	*Penicillium expansum* KACC 40815	Malt extract agar	[70]

Table 1.
Examples of substances produced by solid state fermentation that could be used in healthcare.

polyphenols and short chain fatty acids. We also described main groups of microorganisms that were involved in cited studies and compared various approaches for optimising SSF.

2. Main properties of substances obtained from SSF

2.1 Antimicrobial properties

In the majority of cited studies (**Table 1**) authors did not verify which particular compound contributed to antimicrobial properties. In most of cases they concluded that polyphenols contributed to that phenomenon [13, 15–19] because in comparison to control groups, extracts obtained after SSF demonstrated stronger antimicrobial effects containing more phenolic compounds (PC) at the same time. In the paper written by Mohamed et al. [13, 72] authors did not carry out detailed qualitative and quantitative analysis of fungal metabolites – they assumed that only curcumin would be the substance demonstrating antibacterial properties.

Some studies involved detailed analysis of polyphenol profiles and authors assigned antibacterial and antifungal properties to phenolic acids which concentration was increased by *Pleurotus sapidus* [12]. Others indicated that antimicrobial activity was achieved due to the occurrence of coumarins and oxylipins detected in post-fermentation extracts when *Aspergillus oryzae* KCCM 12698 was used for SSF [14]. Kaaniche et al. [2] additionally analysed structures of obtained bioactive compounds and they proved that four most potent antimicrobials produced by *Streptomyces cavourensis* TN638 were macrotetrolides. Similar approach was applied to identify antimicrobial compounds produced by *Fusarium sambucinum* B10.2 and proved it was sambacide [10]. When surfactants produced by various *Bacillus* strains were tested for antimicrobial properties, researchers additionally tested their properties like emulsification activities [4] or tensioactive activity [5]. Except for latest reports regarding surfactin, we did not include antibiotics in our chapter because currently there are various resistant strains so some alternatives are required.

The majority of identified antimicrobial compounds demonstrated activity equal to [2, 13, 17] or greater [10, 13, 15, 16] than well-known antibiotics. In some cases authors did not provide results for control samples so it was not possible to assess how those substances were effective, however, inhibition zones in diffusion disk method were very prominent [4, 9, 14, 15]. In other studies MIC (Minimum Inhibitory Concentration) of extracted substances were not higher than for antibiotics, however, since those substances were obtained from agricultural waste which is a cost effective substrate, they still could be considered as potential antimicrobials [2, 12, 18, 19]. Only metabolites produced by *Pediococcus acidilactici* KT-05-7 demonstrated very weak antimicrobial properties [8].

2.2 Anti-inflammatory agents

Anti-inflammatory properties of terpenoids were already described in various reviews [73, 74] but they were not investigated in cited papers so we did not discuss obtained results. It is worth mentioning that each extract obtained after SSF contained at least one compound that could demonstrate such activity: lactones which were produced by *Trichoderma viride* EMCC-107 [28]; limonene-1,2-diol, α-terpineol, (−)-carvone, α-tocopherol produced by *Diaporthe* sp. KY113119 [25]; 1-terpineol, L-linalool produced by *Antrodia camphorata* [30]; betulinic acid – *Inonotus obliquus* and [23]; α-pinene produced by *Saccharomyces cerevisiae* AXAZ-1 and *Kluyveromyces marxianus* IMB3 [27].

In the case of polyphenols we took the same approach – we only summarised the research that investigated anti-inflammatory properties of extracts obtained after SSF. When grape pomace was treated with *Trametes versicolor* TV-6 the concentration of phenolic acids, flavan-3-ols and rutin increased while the concentration of anthocyanins decreased. Those changes resulted in enhanced anti-inflammatory activity of obtained extracts which was measured by the inhibition of 5-lipoxygenase and hyaluronidase [20]. Also polyphenols produced by *Xylaria nigripes* demonstrated enhanced anti-inflammatory properties which were verified by the inhibition of cyclooxygenase-2 [24]. Additionally, in both cases, obtained extracts demonstrated neuroprotective properties. Studies carried out by Yin et al. [75] demonstrated that *A. niger* was able to release ferulic acid bound to various polysaccharides in the wheat bran. Obtained substances exhibited stronger anti-inflammatory activity than that of free ferulic acid which probably took place due to the presence of accompanying compounds in obtained extracts. Moreover, those released compounds could significantly inhibit intracellular malondialdehyde formation and the LPS-induced inflammation. It is difficult to assess which extracts demonstrated greater activity because authors of all abovementioned studies used different way to express results – the provided IC_{50} values or % of inhibition.

In the majority of cited papers anti-inflammatory properties of extracts containing polyphenols were not verified despite the fact that there were some compounds among them which demonstrate such activity [76, 77]. Research mainly focused on antioxidant properties [20, 22, 23, 31]. This applies to various studies involving organisms providing increase of particular phenolic compounds: *Rhizopus oligosporus* NRRL 2710 – rutin [22], *Prunus armeniaca* L – cinnamic acids and selected flavonols [31]; *Lactobacillus plantarum* CECT 748 – hydroxybenzoic acids and flavonols [78]; *Eurotium cristatum* YL-1 – isoflavones (daidzin, daidzein, genistin and genistein) [34]; *Rhizopus oryzae* and *Aspergillus foetidus* – tannins [35]; *Rhizopus oryzae* RCK2012 – phenolic acids [36]; *Aspergillus oryzae* LBA01 – 3,4-di- hydroxybenzoic acid, ferulic acid, vanillic acid and quercetin [37].

2.3 Immunosuppressants

Immunosuppressants could be obtained by SSF as well. It seems that one of the most common substance which was detected in studies involving SSF is mycophenolic acid (MA, **Table 1**). It works as a blocker in producing precursors for the synthesis of RNA and DNA, so as the result it blocks proliferative response of T and B lymphocytes [79]. In the research that involved SSF, authors did not verify properties of the obtained MA.

Another substance belonging to that group, classified as calcineurin inhibitor [80], is cyclosporin A (CA). This substance is used not only in transplant patients but also in treatment of glomerular disease. CA prevents calcineurin-dependent transcription in activated T cells. Based on studies that were aiming to produce CA we concluded that authors did not verify properties or safety of obtained substances but they focused on various methods for its extraction and purification. They mostly used butyl acetate [44, 48] or ethyl acetate [47]. Tacrolimus could be also produced by SSF by *Streptococcus hygroscopicus*. The whole process of bacterial cultivation and the extraction of that compounds was covered by the patent [49].

2.4 Anticoagulants

Another group of substances that could be produced by SSF is anticoagulants. Blood coagulation is a physiological process, which consists of a series of coagulation factors and proteolytic activation steps, which lead to the production of

thrombin – the main coagulation enzyme. The majority of research was focused on fibrinolytic enzymes (FE, **Table 1**) – subtilisin, however, in one case it was nattokinase [55]. In the majority of cited papers authors verified anticoagulant properties of obtained enzymes and they used different methods – some of them applied spectrophotometric method which measured the increase of turbidity at 275 nm caused by added enzyme [53, 54, 58, 60, 62, 65] and others measured zones of clearance in solid media containing fibrinogen [64, 66, 67].

Another compound which could be considered as a putative anticoagulant is halotolerant chitinase. Its properties were confirmed [50, 51] by testing how this enzyme could dissolve fibrin in time. Moreover, Meruvu et al. [51] showed that it held antifungal activity.

2.5 Anticancer agents

Terpenoids are well-known for their cytotoxic activity and there were various studies investigating such activity against cancer cells. Anticancer mechanism of terpene or essential oils that contain them was described in various review papers [81–83]. Several compounds belonging to that class could be obtained by SSF (**Table 1**): limonene from orange waste [25]; linalool, geraniol and β-caryophyllene from millet [28, 30]; andrastin A and C on malt extract agar [70]. Properties of extracts that contained those and other terpenoids were not investigated in cited papers so we decided not to discuss that aspect in the current chapter.

Another group of bioactive compounds which was shown to hold anticancer activity is polyphenols [84, 85]. The following substances occurred in cited reviews and studies that we summarised in **Table 1**: cinnamic acids [22], daidzein and genistein [86], quercetin [35, 87], and tannins [34]. Since authors of cited research papers (**Table 1**) did not test obtained extracts against those properties, we did not discuss that phenomenon.

In the study of Cho et al. (2019) authors demonstrated that fatty acids detected in extracts obtained from silkworm larvae powder fermented by *Aspergillus kawachii* demonstrated such activity against human hepatocellular carcinoma [68]. It was shown that fermentation increased concentration of those compounds, especially oleic and linoleic acids. This phenomenon took place due to the enhancement of cell apoptosis and suppression of protein responsible for preventing the apoptosis. The value of that research is particularly significant because so far, polyunsaturated fatty acids (PUFAs) attracted most attention in the context of colorectal cancer [87].

3. Groups of microorganisms demonstrating greatest potential for the production of health-promoting properties in SSF

3.1 *Bacillus* genus

It was demonstrated that the representatives of the *Bacillus* genus were able to produce fibrinolytic enzymes by SSF [52–58, 60]. That ability is mostly assigned to the expression of *fibE* gene which encodes enzyme called subtilisin [88] which solubilises blood clots. Gene expression was not investigated in cited papers so the ability to produce those enzymes by tested strains could be the result of other genes expression.

Bacillus genus is successfully used in solid-state fermentation to improve antimicrobial activity of fermented food. Rochín-Medina et al. [19] who tried to determine optimal bioprocessing conditions for SSF of spent coffee grounds by *Bacillus clausii* achieved increase of flavonoid and total phenolic contents by 13 and 36%,

respectively. SSF also enhanced antibacterial activity of obtained extracts. That phenomenon could be explained by the fact that *Bacillus sp*. strains could metabolise fibre which releases phenolic compounds as a result of lignocellulolytic activity, and demonstrate strong correlation between the increase of phenolic compounds and the synthesis of cellulases and pectinases [19]. Those enzymes can break down plant cell wall components which leads to the hydrolysis of ester bonds that bind phenolic compounds [19].

The representatives of the *Bacillus* genus are able to produce surfactants as well (**Table 1**). There are various enzymes involved in the production of surfactin which form multienzyme peptide synthetase complex. One of those proteins is Srf (which consists of three units A, B and C). There are also two other genes involved in that process – *sfp* and *com*X. However, the role and interactions of protein and genes was described in more details in other review papers [89], therefore, we did not discuss that phenomenon in details.

3.2 Other bacteria

There are also other bacteria that were tested against the production of substances holding the potential for application in healthcare. For instance, there are several species that are able to produce fibrinolytic enzymes which could serve as potential anticoagulants: *Paenibacillus* sp. IND8 [61], *Pseudoalteromonas* sp. IND11 [62], *Xanthomonas oryzae* IND3 [63] or *Citrobacter freundii nov. haritD11* [50, 51]. In the case of *Paenibacillus* its ability for producing such enzymes could be assigned to the expression of *PPFE-I* gene [90]. It seems that the synthesis of protein encoded by that gene could be stimulated by Zn^{2+}, Mg^{2+} and Fe^{+} so the concentration of these ions could be considered in future studies focusing on optimisation of enzyme production. In the case of *Citrobacter freundii*, molecular mechanism seems much simpler because so far, only *chiX* gene was assigned to its ability for chitinase production [91]. It is still unclear which genes are involved in the production of anticoagulant agent by *Pseudoalteromonas* or *Xanthomonas oryzae* so it is an aspect that could be investigated in the future, since the quantity of the enzyme was very prominent – up to 1,388 U/ml.

It was also demonstrated that *Streptococcus hygroscopicus* is able to produce immunosuppressant in SSF based on agricultural waste with added supplements [49] and it seems that a*roA*, *fkbN,* and *luxR* genes are mostly responsible for that phenomenon [92]. On the other hand, molecular mechanisms standing behind the ability of *Yarrowia lipolytica* W29 (ATCC 20460) to produce γ-decalactone [11] which analogues demonstrate antiviral and antifungal properties [93] is simpler because it involves only *POX2* overexpression [94]. Similarly, in the case of *Lactobacillus plantarum* CECT 748 [95] which increased the concentration of particular phenolic compounds, probably only *est_1092* gene was involved – it encodes phenolic esterase [96].

None of the cited paper evaluated molecular mechanisms involved in processes carried out by tested strains.

3.3 Fungi

The majority of research involving SSF is carried out with various fungi (**Table 1**). Many of analysed examples focused on the increase of phenolic compounds. One of the genera which were involved in that process was *Trichoderma* [13, 16, 17]. Those fungi are known to produce cellulolytic, ligninolytic and xylanolytic enzymes [97] and it has been demonstrated in other studies that cellulase could significantly increase concentrations of various polyphenols e.g. caffeic acid, vanillin, p-coumaric acid, and ferulic acid [98]. In fact, similar strategies

might apply to *Aspergillus* spp. [22, 37, 99] because representatives of that genus could produce enzymes demonstrating such activities [98] as well. *Rhizopus oryzae* [36, 100] synthesises cellulase, xylanase and pectinase [101]. Other fungi applied in other cited studies could produce the following enzymes that participate in polyphenol increase: *Lentinus edodes* [15] – xylanase and cellulase [102], *Pleurotus sapidus* – ligninolytic enzymes [12], *Trametes versicolor* TV-6 – β-glucosidase [20].

Genetic background of the synthesis of abovementioned enzymes by *Trichoderma* spp. and *Aspergillus* spp. was revised by Amore et al. [103] while in the case of *R. oryzae* and *P. chrysopsorium* it was described by Battaglia et al. [104] so we did not provide details of those processes, especially that many genes are involved.

There are also some fungi which could be natural sources of polyphenols: *Taiwanofungus camphoratus* [21], *Inonotus obliquus* [23], and *Xylaria nigripes* [24]. In all cases SSF increased their anti-inflammatory properties by increasing concentration of particular phenolic compounds which could also originate from substrates that were used for their cultivation and those were: spent substrate of *Pleurotus eryngii*, sunflower seed hulls, corn and rice grain, white birch and mulberry in the case of *I. obliqus*; wheat bran in the case of *X. nigripes*.

Another significant group of bioactive compounds which concentration was increased by SSF was terpenes (**Table 1**) and those processes were carried out by various fungi: *Fusarium sambucinum* B10 [10], *Penicillium expansum* KACC 40815 [70], *Diaporthe* sp. KY113119 [25], *Antrodia camphorata* [30], *Saccharomyces cerevisiae* AXAZ-1 and *Kluveromyces marxianus* IMB3 [27], *Trichoderma viride* EMCC-107 [28], and *Aspergillus niger* van Tieghem [29]. *A. camphorate* is the natural source of terpenes and their concentration was increased by selecting millet as the main substrate for SSF. *K. marxianus* and *S. cerevisiae* could probably increase the concentration of tested compounds by releasing terpenes from their glycosidic forms by β-glucosidases. In the case of *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. it was demonstrated that those genera could produce several sesquiterpene synthases [105]. As in the case of polyphenols, molecular background of terpene transformations are very complex so we decided not to describe it in the current chapter, but refer to the review of Quin et al. [105] instead. It must be highlighted that except for the study regarding *P. expansum* KACC 40815 demonstrating that terpenoid cyclase was mostly involved in described processes [70], none of the authors investigated enzymatic activity during terpene transformation nor determined gene expression.

Based on cited papers it might be stated that *Penicillium brevicopactum* is most common in the research regarding production of mycophenolic acid in SSF [38–43]. Those fungi produce polyketide synthase encoded by *mpaC* gene along with other enzymes such us: protein transacylase, β- ketoacylsynthase, acyltransferase, acyl carrier protein, and methyltransferase (MT) domains [106]. There is another taxon which is able to produce immunosuppressants, namely cyclosporin A, and it is *Tolypocladium inflatum* [44–48] which has got nonribosomal peptide synthetase that encodes for cyclosporin synthetase (*simA* gene) [107].

Mucor subtilissimus UCP 1262 [65] and *Fusarium oxysporum* [66, 67] were shown to produce fibrinolytic enzymes. It has been already demonstrated that *FP* gene is responsible for encoding fibrinolytic protease in *Fusarium* sp. [108] but in the case of *Mucor* sp. it remains unknown. Molecular background of *A. kawachii* KCCM 32819 production of short chain fatty acids could be the same as for *A. nidulans* and other filamentous ascomycetes – *farB* gene is mostly responsible for that ability, however, *farA* participates as well [109]. Genetic background of biosurfactant production in moulds is still unknown.

3.4 Modified strains

It has been demonstrated that in some cases the yield of microbial metabolites significantly increased when the strain was subjected to gamma ray – it increased the quantity of obtained mycophenolic acid produced by two strains of *Penicillium requeforti* [43] in comparison of other cited studies which involved unmodified *Penicillium brevicompactum* [39, 41]. On the other hand, UV radiation was used for the modification of *Tolypocladium inflatum* which was used for the production of Cyclosporin A [47], however, in the case of that substance there were other studies which resulted in much higher yields [44, 45].

4. Optimisation of SSF conditions

One of the approaches that were applied for the optimisation of the concentration of bioactive substances by SSF is to provide pre-treatment to the main substrate. Heat-treatment was one of the main methods e.g. autoclaving, moistening in boiling water, cooking with deionised water, drying, freezing, freeze-drying, vacuum-drying and roasting. It inactivates native microorganisms and enzymes.

Once the main substrate is prepared, there are other experimental conditions that need to be optimised. In cited papers the major factor contributing to obtained results was moisture which was at least 50% [69, 110]. The next crucial factor was medium composition. In few cases authors used solid media commonly used for the cultivation of microorganisms [2, 70]. However, in the majority of cases authors added some nutrients to agricultural waste to provide better results of releasing bioactive compounds. In the case of mycophenolic acid produced *P. brevicompactum* MTCC 8010 from rice bran those supplements were: peptone, KH_2PO_4, glycine and methionine [41]. On the other hand, in the case of *P. brevicompactum* ATCC 16024, the addition of mannitol or $(NH_4)_2HPO_4$ to pearl barley did not enhance the MA synthesis [39]. Surprisingly, the quantity of MA was higher in the latter case – 5.47 g/kg of the substrate in comparison to 4.5 g/kg under optimised conditions. It seems that pearl barley has got chemical composition which is more preferable for obtaining MA.

On the other hand, Plackett-Burman design was applied for the optimisation of cyclosporin A production by *Tolypocladium inflatum* MTCC 557 which resulted in 8,166 mg/kg [45] which is 1.26-fold higher than in studies carried out by Survase et al. [44, 45] who applied the same fungal strain or even 45.62-fold higher than reported by Nisha and Ramasamy [46] who used *T. inflatum* ATTC 34921 strain. In the last study authors firstly used Plackett-Burman design for the selection of nutrients and later on, they used half-factorial central composite rotatable design (CCRD) of response surface methodology (RSM) to select optimum concentrations of those substances which resulted in more than 10-fold increase of tested compound [47].

When the production of fibrinolytic enzyme was optimised with RSM, its activity increased 3 times in the case of *Pseudoalteromonas* sp. IND11 [62]; 4 times in the case of *Xanthomonas oryzae* sp. IND3 [63]; while when central composite design (CCD) was used, 4.5-fold increase was noted when *Paenibacillus* sp. IND8 was used [61]. In the case of *Xanthomonas oryzae* sp. IND3, CCD was additionally used for estimating optimal values of the following variables: sucrose, yeast extract, and pH of the medium [63]. Among all mentioned microorganisms *Paenibacillus* sp. IND8 produced greatest quantities of the enzyme – 4,418 U/ml.

Various *Bacillus* strains were used for the production of fibrinolytic enzyme (**Table 1**) and those proved to be more efficient enzyme producer

that abovementioned bacteria. When authors used two-level factorial design in the case of *Bacillus* sp. IND12 examining the impact of moisture, sucrose, and $MgSO_4$ levels added to the cow dung, maximum enzyme activity reached 4,143 U/g [54]. Further, orthogonal design (corn steep powder, sucrose and $MgSO_4 \cdot 7H_2O$) provided even greater enzyme activity (5,865 U/ml) in 100 l fermenter when applying *Bacillus subtilis* WR350 [57]. However, the greatest activity was achieved when *Bacillus halodurans* IND18 was used. It produced 6,851 U/g when two-level full factorial design was applied and the optimum conditions were as follows: 1% peptone, 80% moisture and pH 8.32, using wheat bran as the main substrate [58].

Ghribi et al. [4] took a different approach for the optimisation of surfactin production – firstly, authors applied Plackett-Burman design to assess which of the five variables were the most important and then they optimised the process with CCD involving three selected variables. They found parameters (temperature – 37°C, inoculum age – 14 h, and moisture – 88%) that were the most favourable for the production of surfactin and increased its yield by 2-fold (up to 2 g/l). Sun et al. [3, 111] carried out step-by-step optimisation and found out that the addition of attapulgite by 1.96-fold (4.3782 g/kg). This would suggest that *B. natto* NT-6 was the most suitable for that application among all tested strains.

Optimization of halotolerant chitinase was carried out using RSM –Box Behnken method which involved *Citrobacter freundii* and that process slightly improved enzyme production in comparison to initial optimisation experiments from 112.43 U/g dry substance to 124.73 U/g dry substance [51]. That optimisation was mainly focused on the ratio of main substrates (wheat bran and shrimp shellfish), temperature and moisture content. Similarly, minor changes were observed when wheat bran and powdered fish scales were used for the statistical optimisation of chitinase production [50].

5. Conclusions

Solid-state fermentation could provide various substances useful in healthcare: antimicrobials, immunosuppressants, anticoagulants, substances holding anti-inflammatory properties and anticancer agents. It seems that polyphenols and terpenes are especially versatile in their applications. The majority of studies involved various fungi mainly due to their enzymatic activity which supports the release of bioactive compounds from agricultural waste. Molecular mechanisms of those processes are usually very complex; however, they remain unknown for some fungi. Further studies are necessary to assess which genes could be expressed during those processes because those could be used for modification of microorganisms to increase their yield. It is also important to bear in mind that SSF requires the presence of various supplements and fermentation could be optimised by statistical tools, especially Response Surface Methodology and Central Composite Design. In some cases step-by-step optimisation could be sufficient.

Conflict of interest

The authors declare no conflict of interest.

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