Extracellular polysaccharides from Ascomycota and Basidiomycota: production conditions, biochemical characteristics, and biological properties

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Abstract  Fungal polysaccharides (PSs) are the subject of research in many fields of science and industry. Many properties of PSs have already been confirmed and the list of postulated functions continues to grow. Fungal PSs are classified into different groups according to systematic affinity, structure (linear and branched), sugar composition (homo- and heteropolysaccharides), type of bonds between the monomers (β-(1 → 3), β-(1 → 6), and α-(1 → 3)) and their location in the cell (cell wall PSs, exoPSs, and endoPSs). Exopolysaccharides (EPSs) are most frequently studied fungal PSs but their definition, classification, and origin are still not clear and should be explained. Ascomycota and Basidiomycota fungi producing EPS have different ecological positions (saprotrophic and endophytic, pathogenic or symbiotic-mycorrhizae fungi); therefore, EPSs play different biological functions, for example in the protection against environmental stress factors and in interactions with other organisms. EPSs obtained from Ascomycota and Basidiomycota fungal cultures are known for their antioxidant, immunostimulating, antitumor, and antimicrobial properties. The major objective of the presented review article was to provide a detailed description of the state-of-the-art knowledge of the effectiveness of EPS production by filamentous and yeast Ascomycota and Basidiomycota fungi and techniques of derivation of EPSs, their biochemical characteristics, and biological properties allowing comprehensive analysis as well as indication of similarities and differences between these fungal groups. Understanding the role of EPSs in a variety of processes and their application in food or pharmaceutical industries requires improvement of the techniques of their derivation, purification, and characterization. The detailed analyses of data concerning the derivation and application of Ascomycota and Basidiomycota EPSs can facilitate development and trace the direction of application of these EPSs in different branches of industry, agriculture, and medicine.

Keywords  Exopolysaccharides · Fungi · Ascomycota · Basidiomycota · Biological properties

Abbreviations
Arab  Arabinose
CTAB-PS  Isolation of EPS by cetyltrimethylammonium bromide precipitation
EPS  Exopolysaccharide
ET  Ethylene
FBBs  Fungal–bacterial biofilmed biofertilizers
FRBs  Fungal–rhizobial biofilmed biofertilizers
Fuc  Fucose
Gal  Galactose
Glc  Glucose
GlcA  Glucuronic acid
GPC  Gel permeation chromatography
HPAEC  High performance anion exchange chromatography
IEC  Ion exchange chromatography
IFN  Interferon
IL  Interleukin
ISR  Induced systemic resistance
Introduction

Microbiological polysaccharides (PSs) are classified into different groups according to their location in the cell, structure, sugar composition, type of bonds between monomers, and systematic affinity. Exopolysaccharides (EPSs) are the most frequently studied microbial PSs, besides cell wall PSs and intracellular cytosolic PSs. Several definitions of EPS are reported in the literature (Mahapatra and Banerjee 2013b). In many cases, EPSs are rather defined by the separation/extraction method used than by theoretical consideration of the composition of the cell wall and macromolecules outside the cell wall. Bound or soluble EPSs contain not only high-molecular-weight polymeric compound exudates from microorganisms, but also products of cellular lysis and hydrolysis of macromolecules.

EPSs are particularly intensively studied in bacterial cultures, where they are synthesized in high concentrations in young cultures growing on various carbon (C) and nitrogen (N) sources, but at a relatively high temperature (Donot et al. 2012). Filamentous and yeast Ascomycota and Basidiomycota fungi are used for biotechnological derivation of EPSs in laboratory conditions using similar techniques, culture conditions, sources of C, N and other elements, culture periods, and mostly acidic pH of the medium (Shu and Lung 2004; Mahapatra and Banerjee 2013b). Fungal strains belonging to both these groups are an important component, characterized by the highest biomass, of the microbiome of various environments (Olsson et al. 1999). It worth mentioning that the optimal period of EPS synthesis by these fungi does not correspond to the period of biomass formation (Madla et al. 2005; Shih et al. 2008). Ascomycota and Basidiomycota fungi exhibit a very high variation of the weight of synthesized EPSs and their biochemical and biological properties (Donot et al. 2012; Mahapatra and Banerjee 2013b).

Microbial PSs are the subject of research in many fields of science. Many properties of PSs have already been confirmed and the list of postulated functions continues to grow. The directions of research on PSs are mainly focused on identification of factors responsible for their synthesis/release, optimization of production (connected with the cost and productivity), and elucidation of the role and application of PSs in interactions between various organisms. The knowledge of the diversity of fungal PSs and the mode of interaction between PSs with microorganisms and higher organisms is still limited, since identification of PSs and investigation of the mechanisms of this interaction depends on the availability of isolation and diagnostics methods.

PS isolation methods are based on fractionation and use of various solvents and PS procedures. Lack of uniformity in these methods often makes the results of different studies incomparable, or even contradictory. Understanding the role of PSs in a variety of processes and the application of PSs in food or pharmaceutical industries or as plant resistance inducers (elicitors) requires improvement of the techniques of derivation, purification, and exploration of properties. The process of PS derivation involves selection of suitable microorganisms, the type of culture, and the method for PS preparation/extraction. Fungal EPSs are derived through ethanol precipitation with different proportions of the culture/water suspension and alcohol (Shu and Lung 2004; Chen et al. 2011; Ma et al. 2015).

Exploration of the similarities and differences in the conditions as well as the efficiency of EPS production and derivation is of great importance as it could contribute to standardization of technologies and selection of optimum production conditions and techniques of purification of fungal EPSs. Very important is also identification of the similarities and differences in the structure and properties of EPSs produced by Ascomycota and Basidiomycota strains, which could facilitate development of mixed formulas containing both Ascomycota and Basidiomycota mycelia or the EPSs of these strains. Given the complementarity of their components, such preparations should
have a broader spectrum of activity in various fields of biotechnology, medicine, agriculture, and environmental protection.

Production of EPS by Ascomycota and Basidiomycota fungal strains

Parameters of the growth medium and culture conditions

Recently, many fungal filamentous and yeast Ascomycota and Basidiomycota strains, are known for their ability to produce EPSs in various culture conditions. The most commonly encountered EPS producers from these both group of microorganisms and parameters of culture growth facilitating EPS production are collected, selected, and presented in Table 1.

The growth conditions of the fungus e.g. the type of culture, temperature, pH value, C and N source, appropriate salt content, and time of culture are essential for the type and amount of EPS obtained. The maximal yield of EPS produced by Ascomycota and Basidiomycota fungi was in the range from 0.12 to 42.24 g l\(^{-1}\) and depended mainly on the tested strain and culture conditions used (Table 1). The production of fungal EPS was reached in shaking cultures with a constant supply of oxygen. Bolla et al. (2010) examined production of EPS by *Trametes versicolor* and reported that the yield of EPS in shaking cultures was 8.0 g l\(^{-1}\). In turn, also the batch fermentation technique for EPS production (10.92 g l\(^{-1}\)) by *Phellinus* sp. PO988 was very effective among Basidiomycota strains (Ma et al. 2014). The greatest efficiency in EPS production, i.e. up 42.24 g l\(^{-1}\), was reported by Yadav et al. (2014) in cultures of the Ascomycota fungus *Aureobasidium pullulans* RYLF-10. The cultivation time is also an important factor affecting EPS production and depends mainly on the type of culture and the type of the fungal strain. The optimal time for EPS synthesis in cultures of Ascomycota and Basidiomycota strains usually ranges from 3 to 40 days (Table 1). It is known that the most effective EPS synthesis occurs at the end of the logarithmic growth phase or early stationary phase. In contrast to bacteria, Ascomycota and Basidiomycota fungi usually require a long incubation time to produce more EPS although Wu et al. (2006) showed that *Auricularia auricula* produced relatively large amounts of EPS (7.5 g l\(^{-1}\)) after 4 days of growth and Ascomycota *Botryosphaeria rhodina* strain DABAC-P82 synthesized up to 17.7 g l\(^{-1}\) of EPS after only 1 day of culture (Selbmann et al. 2003).

An excessively long cultivation time contributed to reduction of EPS amounts in the liquid medium and was related to formation of low molecular EPS (Shu and Lung

| EPS producer | Main parameters of growth medium, type of medium and concentration of components (g l\(^{-1}\)) | Culture conditions | EPS yield (g l\(^{-1}\)) | Optimal time (day) |References |
|--------------|-------------------------------------------------------------|--------------------|------------------------|-------------------|-----------|
| *Alternaria alternate* | Carbon source: Glucose (40) | YE (20) | KH\(_2\)PO\(_4\) (0.5), MgSO\(_4\)/C\(_{17}\)H\(_2\)O (0.5) | Shaking (150) | 6.58 | 9 | Nehad and El-Shamy (2010) |
| *Aspergillus sp.* Y16 | Carbon source: Glucose (20), YE (3), Peptone (5), ME (3) | KH\(_2\)PO\(_4\) (0.5), NH\(_4\)Cl (0.5), Sea salt (24.4) | Shaking (–) | 6.0–6.5 | 7 | Chen et al. (2011) |
| *Aspergillus sp.* RYLF17 | Carbon source: Glucose (–) | PE in PDB | – | Fermentation | 6.0 | 14 | Yadav et al. (2012) |
| *Aspergillus versicolor* | Carbon source: Sorbitol (20), maltose (20) | YE (1) | KH\(_2\)PO\(_4\) (0.5), NH\(_4\)Cl (0.5), Sea salt (33.3) | Shaking (–) | 6.5 | 30 | Chen et al. (2013a) |
| *Aureobasidium pullulans* RYLF-10 | Carbon source: Sucrose (50) | YE (1) | KH\(_2\)PO\(_4\) (0.1), MgSO\(_4\) (0.1) | Shaking (–) | 42.24 | 7 | Yadav et al. (2014) |
| EPS producer                        | Main parameters of growth medium, type of medium and concentration of components (g l⁻¹) | Culture conditions | EPS yield (g l⁻¹) | References                                      |
|------------------------------------|------------------------------------------------------------------------------------------|--------------------|------------------|------------------------------------------------|
|                                    | Carbon source | Nitrogen source | Salts | Type of culture (rev. min⁻¹) | Temperature (°C) | Initial pH | Optimal time (day) |                                                      |
| **Botryosphaeria rhodina**          | Sucrose (50)  | NH₄NO₃ (2)      | VMSM* | Shaking (180) | 28                  | –        | 3               | 1.80 Barbosa et al. (2003) and Vasconcelos et al. (2008) |
| **Botryosphaeria rhodina**          | Sucrose (50)  | NH₄NO₃ (2)      | VMSM* | Shaking (180) | 28                  | –        | 3               | 1.50                                                      |
| **Botryosphaeria rhodina**          | Sucrose (50)  | NH₄NO₃ (2)      | VMSM* | Shaking (180) | 28                  | –        | 3               | 0.40                                                      |
| **Botryosphaeria rhodina**          | Sucrose (50)  | NH₄NO₃ (2)      | VMSM* | Shaking (180) | 28                  | –        | 3               | 1.30                                                      |
| **Botryosphaeria rhodina**          | Glucose (30)  | NaNO₃ (2), YE (1)| KH₂PO₄ (1), MgSO₄·7H₂O (0.5), KCl (0.5)| Shaking (150) | 28                  | 3.7      | 1               | 17.70 Selbmann et al. (2003) |
| **Cordyceps sinensis**             | Sucrose (40)  | YE (10), peptone (5) | KH₂PO₄ (1), MgSO₄·7H₂O (0.5) | Shaking (150) | 20                  | 6.8      | 7               | 1.02 Leung et al. (2009) and Huang et al. (2013) |
| **Fusarium coccophilum**           | Glucose (–)   | PE in PDB       | –      | Shaking (150) | 25                  | –        | 21              | 2.83 Madla et al. (2005) |
| **Fusarium oxysporum**             | Glucose (20)  | YE (3), peptone (5), ME (3) | KH₂PO₄ (0.5), NH₄Cl (0.5), sea salt (24.4) | Fermentation | 25                  | 6.0–6.5  | 40              | 0.59 Chen et al. (2015) |
| **Fusarium oxysporum**             | Glucose (50)  | Peptone (13)    | KH₂PO₄ (0.6), MgSO₄·7H₂O (0.2), NaCl (0.6) | Shaking (150) | 25                  | –        | 14              | 0.21 Li et al. (2014) |
| **Fusarium oxysporum**             | Glucose (20)  | YE (3), peptone (5), NH₄Cl (0.5) | KH₂PO₄ (0.5), NH₄Cl (0.5) | Shaking (–) | 25                  | 6.0–6.5  | 7               | 0.12 Guo et al. (2010, 2013) |
| **Fusarium solani**                 | Glucose (9.8), PE in PDB | YE (0.69) | KH₂PO₄ (0.5), KCl (0.5) | Shaking (120) | 28                  | 6.46     | 13.7            | 2.28 Mahapatra and Banerjee (2012) |
| **Hypocreales sp.**                | Sucrose (10.6) | YE (10.92) | KH₂PO₄ (1), MgSO₄ (1) | Shaking (100) | 25                  | 6.5      | 5               | 1.33 Yeh et al. (2014) |
| **Morchella crassipes**            | Maltose (44.8)| Tryptone (4.21) | –      | Shaking (150) | 28                  | 6.0      | 7               | 9.67 He et al. (2012) |
Table 1 continued

| EPS producer       | Main parameters of growth medium, type of medium and concentration of components (g l⁻¹) | Culture conditions                                                                 | EPS yield (g l⁻¹) | References                      |
|--------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------|----------------------------------|
| **Carbon source**  | **Nitrogen source** | **Salts** | **Type of culture** (rev. min⁻¹) | **Temperature (°C)** | **Initial pH** | **Optimal time (day)** |                      |
| Penicillium        | Mannitol (20), Maltose (20), Glucose (10)                                                   | MG (10), corn syrup (1), YE (3) | Fermentation       | 28                   | 6.5                 | 10                   | 0.43                | Chen et al. (2013c)  |
| commune            | Penicillium griseofulvum                                                                   | MG (10), YE (3), maize paste (1)                                                 | Static            | 20                   | 6.5                 | 30                   | 0.25                | Chen et al. (2013b)  |
| Penicillium        | Glucose (90)                                                                               | NaNO₃ (2)                                                                         | Shaking (--)      | 28                   | 6.3                 | 7                    | 4.8                 | Kogan et al. (2002)  |
| vermilionum CCM    | F-276                                                                                      | KCl (0.5), KH₂PO₄ (1), MgSO₄ (0.5), MgSO₄.7H₂O (0.5) | Shaking (150)     | 28                   | 4.7                 | 4                    | 13.6                | Selbmann et al. (2002) |
| Phoma herbarum     | Sorbitol (60)                                                                              | NaNO₃ (3)                                                                         | Shaking (150)     | 28                   | 4.7                 | 4                    | 13.6                | Selbmann et al. (2002) |
| Trichoderma        | Glucose (--)                                                                               | PE (--)                                                                          | Shaking (180)     | 28                   | --                  | 10                   | --                  | Huanga et al. (2012) |
| pseudokoningii     |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Basidiomycota      |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Agaricus nevoi     | Mannitol (20)                                                                               | Corn steep liquor (20 mM), YE (3)                                                 | Shaking (150)     | 25                   | 6.0                 | 5                    | 3.00                | Elisashvili et al. (2009) |
| HAI 610            |                                                                                           |                                                                                   | Batch fermentation (300) | 28                   | 5.0                 | 14                   | 0.12                | Shu and Lung (2004) |
| Antrodia camphorate| Glucose (25)                                                                                | Peptone (5), ME (3), YE (3)                                                      | Shaking (150)     | 28                   | 5.5                 | 14                   | 0.38                | Lin and Sung (2006)  |
| Antrodia            |                                                                                           |                                                                                   | Shaking (150)     | 28                   | 5.4                 | 4                    | 4.3                 | Wu et al. (2006)     |
| cinnamomea BCRC    |                                                                                           |                                                                                   | Batch fermentation (200) | 28                   | 5.4                 | 4                    | 7.50                | Wu et al. (2006)     |
| 35396              |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Auricularia        |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| auricula AA-2      |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Clavariadelphus     |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| truncatens PDB     |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Cerrena unicolor   |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| IBB 681            |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Cerrena maxima     |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| Cryptococcus        |                                                                                           |                                                                                   |                   |                     |                     |                      |                     |                      |
| laurentii DSMZ 70766|                                                                                          |                                                                                   |                   |                     |                     |                      |                     |                      |
Table 1 continued

| EPS producer                        | Main parameters of growth medium, type of medium and concentration of components (g l\(^{-1}\)) | Culture conditions              | EPS yield (g l\(^{-1}\)) | References                |
|-------------------------------------|------------------------------------------------------------------------------------------------|--------------------------------|-------------------------|----------------------------|
|                                     | Carbon source | Nitrogen source | Salts | Type of culture (rev. min\(^{-1}\)) | Temperature (°C) | Initial pH | Optimal time (day) | getopt..                                     |
| **Cryptococcus neoformans**         | Glucose (2.7) | Glycine (1), thiamine (0.001) | MgSO\(_4\) (1.2), KH\(_2\)PO\(_4\) (4) | – | 30 | 5.5 | – | Frases et al. (2008) |
| **ATCC 24067**                      |                                                          |                                |                          |                                          |
| **Flammulina velutipes SF-06**       | Glucose (10)  | Potato (cut into pieces) (200) | KH\(_3\)PO\(_4\) (1.5), MgSO\(_4\)\(_7\) H\(_2\)O (1) | Batch fermentation | 25 | – | 7 | – | Ma et al. (2015) |
| **Fomes fomentarius**               | Glucose (60)  | Silkworm chrysalis (15), YE (3) | KH\(_3\)PO\(_4\) (1), MgSO\(_4\)\(_7\) H\(_2\)O (0.8), CaCl\(_2\) (0.5) | Shaking (150) | 25 | 6.0 | 8 | 3.64 | Chen et al. (2008a, b) |
| **Ganoderma applanatum**            | Glucose or maltose (60) | YE (2), glutamic acid (1) | KH\(_3\)PO\(_4\) (2), MgSO\(_4\) (0.5) | Shaking (100) | 25 | 4.5 | 12 | 1.35 | Lee et al. (2007) |
| **KFRI 646**                        |                                                          |                                |                          |                                          |
| **Ganoderma carnosum**              | PDB (24) | ME (10), peptone (1) | – | Shaking (150) | 25 | – | 7 | – | Demir and Yamac (2008) |
| **Ganoderma lucidium**              | Glucose (17.5) | Peptone (2.5), wheat grains, extract (–) | KH\(_3\)PO\(_4\) (1.5), MgSO\(_4\)\(_7\) H\(_2\)O (1) | Shaking (150) | 25 | 4.0 | 14 | 0.53 | Fraga et al. (2014) |
| **UF20706**                         |                                                          |                                |                          |                                          |
| **Ganoderma lucidium**              | Lactose (35) | YE (5), Peptone (5) | KH\(_3\)PO\(_4\) (1), MgSO\(_4\)\(_7\) H\(_2\)O (0.5) | Fed-batch fermentation (–) | 30 | – | 14 | 1.25 | Tang and Zhang (2002) |
| **CCGMC 5.616**                     |                                                          |                                |                          |                                          |
| **Ganoderma lucidium**              | Glucose (20) | YE (3), (NH\(_4\))\(_2\)SO\(_4\) (2) | KH\(_3\)PO\(_4\) (0.8), Na\(_2\)HPO\(_4\) (0.4), MgSO\(_4\)\(_7\) H\(_2\)O (0.5) | Shaking culture (150) | 25 | 6.0 | 5 | 1.6 | Elisashvili et al. (2009) |
| **HAI 447**                         |                                                          |                                |                          |                                          |
| **Grifola frondosa**                | Glucose (40) | YE (8) Corn steep powder (12) (NH\(_4\))\(_2\)SO\(_4\) (1) | MSS** | Fed-batch fermentation (250) | 25 | 5.0 | 13 | 3.88 | Shih et al. (2008) |
| **Inonotus levis**                  | Glucose (20) | Corn steep liquor (20 mM) YE (3) | KH\(_3\)PO\(_4\) (0.8), Na\(_2\)HPO\(_4\) (0.4), MgSO\(_4\)\(_7\) H\(_2\)O (0.5) | Shaking (150) | 25 | 6.0 | 5 | 3.00 | Elisashvili et al. (2009) |
| **HAI 796**                         |                                                          |                                |                          |                                          |
| **Lactarius sulphureus**             | PDB (24) | ME (10), peptone (1) | – | Shaking (150) | 25 | – | 7 | – | Demir and Yamac (2008) |
| **Lenzites betulina**                | Beer wort (7% of Balling’s scale) | – | – | Batch fermentation (150) | 25 | – | 4 | 4.00 | Lobunok et al. (2003) |
| **Lentinus edodes**                 | PDB (24) | ME (10), peptone (1) | – | Shaking (150) | 25 | – | 7 | – | Demir and Yamac (2008) |
| **Lentinus strigosus**               | PDB (24) | ME (10), peptone (1) | – | Shaking (150) | 25 | – | 7 | – | Demir and Yamac (2008) |
Table 1 continued

| EPS producer            | Main parameters of growth medium, type of medium and concentration of components (g l\(^{-1}\)) | Culture conditions | EPS yield (g l\(^{-1}\)) | References                      |
|-------------------------|--------------------------------------------------------------------------------------------------|--------------------|--------------------------|----------------------------------|
|                         | Carbon source | Nitrogen source | Salts | Type of culture (rev. min\(^{-1}\)) | Temperature (\(^\circ\)C) | Initial pH | Optimal time (day) |                                   |
| *Phellinus gilvus*      | Glucose (30)  | Corn steep powder (5) | KH\(_2\)PO\(_4\) (0.68), K\(_2\)HPO\(_4\) (0.87), MgSO\(_4\)\(_7\)H\(_2\)O (1.23) | Batch fermentation | 30      | 4.0      | 11                | 5.30 Hwang et al. (2003)           |
| *Phellinus igniarius*   | Maltose (20)  | YE (3), (NH\(_4\))\(_2\)SO\(_4\) (2) | KH\(_2\)PO\(_4\) (0.8), Na\(_2\)HPO\(_4\) (0.4), MgSO\(_4\)\(_7\)H\(_2\)O (0.5) | Shaking (150) | 25      | 6.0      | 5                 | 1.80 Elisashvili et al. (2009)     |
| *Phellinus sp.*         | Glucose (50)  | Glutamic acid (4), (NH\(_4\))\(_2\)SO\(_4\) (4) | KH\(_2\)PO\(_4\) (1), MgSO\(_4\) (1) | Batch fermentation | 28 | 6.5 | 5 | 10.90 Ma et al. (2014) |
| *Pleurotus dryinus*     | Glucose (20)  | YE (3), (NH\(_4\))\(_2\)SO\(_4\) (2) | KH\(_2\)PO\(_4\) (0.8), Na\(_2\)HPO\(_4\) (0.4), MgSO\(_4\)\(_7\)H\(_2\)O (0.5) | Shaking (150) | 25 | 6.0 | 5 | 1.10 Elisashvili et al. (2009) |
| *Pleurotus pulmonarius* | Galactose (30) | YE (4) | KH\(_2\)PO\(_4\) (1), MgSO\(_4\)\(_7\)H\(_2\)O (0.2) | Shaking (120) | 22 | – | 18 | 0.42 Smiderle et al. (2012) |
| *Pleurotus sajorcaju*   | Glucose (20)  | (NH\(_4\))\(_2\)SO\(_4\) (5), YE (2), Peptone (1) | KH\(_2\)PO\(_4\) (1), MgSO\(_4\)\(_7\)H\(_2\)O (0.2) | Shaking (120) | 30 | 6.5-7.0 | – | – Telles et al. (2011) |
| *Pleurotus sajorcaju*   | Glucose (20)  | (NH\(_4\))\(_2\)SO\(_4\) (2.5), peptone (1) | KH\(_2\)PO\(_4\) (1), MgSO\(_4\)\(_7\)H\(_2\)O (0.2), CaCO\(_3\) (1) | Batch fermentation (300) | 30 | 4.0 | 20 | 0.94 Silveira et al. (2015) |
| *Polyporus arcularius*  | PDB (24)      | ME (10), peptone (1) | – | Shaking (150) | 25 | – | 7 | – Demir and Yamaç (2008) |
| *Trametes versicolor*   | Fructose (20) | YE (2), peptone (1), (NH\(_4\))\(_2\)SO\(_4\) (5) | KH\(_2\)PO\(_4\) (1), MgSO\(_4\)\(_7\)H\(_2\)O (0.2) | Shaking (–) | 27 | 6.0 | 7 | 8.00 Bolla et al. (2010) |
| *Trametes versicolor*   | Maltose (20)  | YE (3), (NH\(_4\))\(_2\)SO\(_4\) (2) | KH\(_2\)PO\(_4\) (0.8), Na\(_2\)HPO\(_4\) (0.4), MgSO\(_4\)\(_7\)H\(_2\)O (0.5) | Shaking (150) | 25 | 6.0 | 5 | 1.40 Elisashvili et al. (2009) |

ME malt extract, MG monosodium glutamate, YE yeast extract, PDB potato dextrose broth, PE potato extract, VMSM Vogel minimum salt medium, MSS mineral salt solution

VMSM* composition: Na\(_3\) citrate—2.5 g l\(^{-1}\), KH\(_2\)PO\(_4\)—5.0 g l\(^{-1}\), NH\(_4\)NO\(_3\)—2.0 g l\(^{-1}\), MgSO\(_4\)\(_7\)H\(_2\)O—0.2 g l\(^{-1}\), CaCl\(_2\)•2H\(_2\)O—0.1 g l\(^{-1}\), biotin—25 mg l\(^{-1}\), citric acid—5.0 mg l\(^{-1}\), ZnSO\(_4\)•7H\(_2\)O—5.0 mg l\(^{-1}\), Fe(NH\(_4\))\(_2\)(SO\(_4\))\(_2\)•6H\(_2\)O—1.0 mg l\(^{-1}\), CuSO\(_4\)•5H\(_2\)O—0.25 mg l\(^{-1}\), MnSO\(_4\)•H\(_2\)O—0.05 mg l\(^{-1}\), H\(_3\)BO\(_3\)—0.05 mg l\(^{-1}\), NaMoO\(_4\)•2H\(_2\)O—0.05 mg l\(^{-1}\); MSS* composition: MgSO\(_4\)•7H\(_2\)O—1.20 g l\(^{-1}\), NaCl—0.06 g l\(^{-1}\), KH\(_2\)PO\(_4\)—0.20 g l\(^{-1}\), CaCl\(_2\)—0.20 g l\(^{-1}\), FeSO\(_4\)•7H\(_2\)O—0.10 g l\(^{-1}\), ZnCl\(_2\)—0.018 g l\(^{-1}\)
For example, Chen et al. (2015) obtained only 0.59 g l\(^{-1}\) of EPS after 40 days of *Fusarium oxysporum* JN604549 cultivation. Ascomycota and Basidiomycota strains produced amounts of maximum EPS at a temperature range between 20 and 30 °C. In turn, the pH values of the culture medium are usually acidic or neutral and oscillate around a range of 4.0–7.0 for maximum EPS production. It was observed that the pH values varied during the cultivation days. For example, *B. rhodina* DABAC-P82 strains changed the initially acidic pH value from 3.7 to 5.9–6.5 after 1 day of cultivation (Selbmann et al. 2003). The EPS production efficiency depends on the type of the C source. The main source of C in most Ascomycota and Basidiomycota cultures was glucose, but potato dextrose broth (PDB) and sugars such as sucrose, maltose, mannitol, lactose, and fructose were used as well. The concentration of the compound serving as a C source is an additional important factor in the synthesis of EPSs. It has been shown that the most optimal concentration of the C source usually varies between 30 and 50 g l\(^{-1}\) (Table 1). Another important factor in the production of EPS in fungi is the presence of organic N sources (e.g. corn steep liquor, yeast extract, peptone, malt extract, soybean powder) and inorganic N sources comprising ammonium sulfate, sodium nitrate, and ammonium chloride, as well as mineral salts in the growth medium (Table 1). The highest yield (10.9 g l\(^{-1}\)) of Basidiomycota EPS was obtained in the *Phellinus* sp. P0988 culture with glutamic acid and ammonium sulfate (4 g l\(^{-1}\)) used as the main N sources and glucose (50 g l\(^{-1}\)) as the main C source (Ma et al. 2014). The highest yield (42.2 g l\(^{-1}\)) of Ascomycota EPS was described for *Aureobasidium pullulans* culture with yeast extract (1 g l\(^{-1}\)) and sucrose (50 g l\(^{-1}\)) as the main N and C sources, respectively (Yadav et al. 2014).

In cultures of Ascomycota *B. rhodina* strain, a Vogel Minimum Salt Medium (VMSM) with ammonium nitrate as the main N source but also with biotin and traces (1.0 mg l\(^{-1}\)) of ammonium ferrous sulfate was used (Barbosa et al. 2003; Vasconcelos et al. 2008). This VMSM and mineral salt solution (MSS) used in Basidiomycota *Grifola frondosa* strain culture was a very rich source of different salts (Table 1). Phosphorous and magnesium are components of almost all cultures used for derivation of fungal EPSs and seem a most important mineral additives stimulating EPS production. Such compounds as potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)) and dipotassium hydrogen phosphate (K\(_2\)HPO\(_4\)) are most effectively used by fungi as a phosphorus source. In addition, magnesium sulfate (MgSO\(_4\)·7H\(_2\)O), sodium phosphate (Na\(_2\)HPO\(_4\)), sodium chloride, and potassium chloride are usually used for supplementation of media for cultivation of EPS producers.

### EPSs isolation, extraction, and purification methods

In contrast to cell-wall or cytosolic Ps, EPSs do not require drastic methods of extraction with hot water or organic solvents (Madla et al. 2005; Chen et al. 2008a, b; Mahapatra and Banerjee 2013a). Usually, to obtain crude EPS, the culture fluid is precipitated by alcohols such as ethanol (absolute or 95 %) or methanol and only in some cases by acetone or isopropanol at a temperature of 4 °C for a period of 12–24 h (Table 2). The main extraction method of EPS obtaining from Ascomycota and Basidiomycota strains cultures is the precipitation with 95 % ethanol used in a proportion 1/4 (v/v) of supernatant and ethanol. Particularly in Ascomycota strains, primary purification of supernatant with 5 % trichloroacetic acid (TCA) is sometimes applied (Yadava et al. 2012). After dialysis against water, crude EPSs are generally stored as vacuum-dried or lyophilized powder. The next step of EPS purification is deproteinization thereof using Sevage reagent (Chen et al. 2011, 2013a, b, c). The main methods used for purification of Ascomycota and Basidiomycota EPSs are Ion Exchange Chromatography (IEC) and a type of Size Exclusion Chromatography (SEC)–Gel Permeation Chromatography (GPC) (Table 2) (Shu and Lung 2004; Chen et al. 2011; Ma et al. 2015).

### EPS characteristics

Ascomycota and Basidiomycota EPSs are usually soluble in water or in a NaCl solution but data concerning the degree of EPS solubility in water are limited. In some cases, solubility of EPSs in alkali solutions (mainly 1 M NaOH) was determined (Table 2). The chemical structures and properties of EPS such as the monosaccharide composition, the linkage type, the molecular weight, or the conformation chain were usually evaluated by different experimental analyses including chromatography technology such as TLC, HPLC, and GLC as well as spectrum analysis such as FTIR and 1D and 2D NMR spectroscopy or GLC-MS (Kozarski et al. 2012; Osińska-Jaroszuk et al. 2014; Silveira et al. 2015). Ascomycota and Basidiomycota EPSs are mainly heteropolysaccharides but in the case of homopolysaccharides glucose is their only monomer (Table 2). The sugar content of various EPSs is varied but glucose, mannose, galactose, xylose, fucose, and rhamnose monomers have frequently been found in fungal EPSs. Moreover, individual monosaccharides synthesized by different groups of Ascomycota and Basidiomycota fungi had different molecular weight (Table 2). EPSs isolated from the Ascomycota and Basidiomycota strains have a different linkage pattern but a 1 → 6 linkage type is dominant, and presence of different types of linkage has been described for Ascomycota EPSs. Guo et al. (2013)
| EPS producer | The main steps during the procedure of EPS characteristics | EPS characteristics |
|--------------|-------------------------------------------------|---------------------|
| **Isolation** | Precipitation method (supernatants/alcohol proportion v/v) | Solubility |
| **Purification** | Period of dialysis against water (h) | Compositions |
| **Chromatographic methods** | EPS solution | Linkage type | $M_w$ (kDa) |
| **References** | | | |
| **Ascomycota** | | | |
| *Alternaria alternate* | 95 % ethanol (1/5), 16 h, 4 °C | – | – | – | – | Nehad and El-Shamy (2010) |
| *Aspergillus sp. Y16* | 95 % ethanol (1/3) | 48 | Water | IEC | Glc | EPS1-Man, Gal, EPS2-Man, Glc | 1 → 2; 1 → 6, 1 → 6 | EPS1-15.0 EPS2-6.0 | Chen et al. (2011) |
| *Aspergillus sp. RYLF17* | 95 % ethanol (1/4), 24 h, 4 °C | – | – | – | – | – | – | – | Yadava et al. (2012) |
| *Aspergillus versicolor* | 95 % ethanol (1/3) | 48 | Water | IEC | Glc, Man | α-1 → 6 | 500.0 | Chen et al. (2013a) |
| *Aureobasidium pullulans RYLF-10* | 95 % ethanol (1/4), 24 h, 4 °C | – | Water | – | – | – | – | – | Yadav et al. (2014) |
| *Botryosphaeria rhodina MMLR* | Absolute ethanol (1/3) | 48 | GPC | Water | NaOH | Glc | β-1 → 6 | – | Barbosa et al. (2003) and Vasconcelos et al. (2008) |
| *Botryosphaeria rhodina MMGR* | Absolute ethanol (1/3) | 48 | GPC | Water | NaOH | Glc | β-1 → 6 | – | – |
| *Botryosphaeria rhodina MMPI* | Absolute ethanol (1/3) | 48 | GPC | Water | NaOH | Glc | β-1 → 6 | – | – |
| *Botryosphaeria rhodina MMMFR* | Absolute ethanol (1/3) | 48 | GPC | Water, 1 M NaOH | Glc | β-1 → 6 | – | – |
| *Botryosphaeria rhodina DABAC-P82* | Ethanol (1/2), 16 h, 4 °C, repeated twice | 24 | Water | GPC | Glc | β-1 → 6; β-1 → 3 | 4.9 | Selbmann et al. (2003) |
| *Cordyceps sinensis Cs-HK1* | 95 % ethanol (5 ratios: 1/0.2, 1/0.4, 1/1, 1/2, 1/5) | – | – | – | Glc | – | EPS0.2-47,400.0 EPS0.4-47,400.0 EPS1-253,000.0 EPS2-630.0 EPS3-16.0 | Leung et al. (2009) and Huang et al. (2013) |
| EPS producer | The main steps during the procedure of | EPS characteristics | References |
|-------------|--------------------------------------|---------------------|------------|
|             |  | EPS solution | Chromatographic methods |  |  |  |  |  |  |  |
|             |  | Precipitation method | (supernatants/alcohol proportion v/v) | Period of dialysis against water (h) | Solubility | Compositions | Linkage type | *M*<sub>w</sub> (kDa) |
| Fusarium coccophilum BCC2415 | 95 % ethanol (1/4), 12 h, –20 °C | Water | GPC | Water | – | – | 2.8 | Madla et al. (2005) |
| Fusarium oxysporum JN604549 | 95 % ethanol (1/3), 72 | Water | IEC | Water | Gal, Glc, | β-1 → 6 | 61.2 | Chen et al. (2015) |
| Fusarium oxysporum Dzf17 | 95 % ethanol (1/3), 48 h, 4 °C | – | Water | Water | – | – | – | Li et al. (2014) |
| Fusarium oxysporum Y24-2 | 95 % ethanol (1/4) | 72 | Water | IEC | Water | Gal, | β-1 → 2 | 36.0 | Guo et al. (2010, 2013) |
| Fusarium solani SD5 | Absolute ethanol (1/5), 24 h, 4 °C | 24 | Water | GPC | Water | Gal, Rha | α-1 → 2 | 187.0 | Mahapatra and Banerjee (2012) |
| Hypocreales sp. NCHU01 | 95 % ethanol (1/4) | – | – | 1 M NaOH | 60 °C, 1 h | – | – | – | Yeh et al. (2014) |
| Morchella crassipes | 95 % ethanol (1/4), 16 h, 4 °C | 0.2 M NaCl | GPC | 0.2 M NaCl | – | – | 19.6 | He et al. (2012) |
| Penicillium commune | 95 % ethanol (1/3) | 48 | Water | IEC | Water | Glc, Man, Gal | α-1 → 2 | 18.3 | Chen et al. (2013c) |
| Penicillium griseofulvum | 95 % ethanol (1/3) | 48 | Water | IEC | Water | Man, Gal | α-1 → 2 | 20.0 | Chen et al. (2013b) |
| Penicillium vermiculatum CCM F-276 | Ethanol (1/4) repeated three times | – | Water | GPC | Water | Glc, Gal, Man | β-1 → 6 | 77.0 | Kogan et al. (2002) |
| Phoma herbarum CCFEE5080 | Absolute ethanol (1/2), 16 h, 4 °C, repeated twice | 24 | Water | GPC | Water | Glc | β-1 → 3 | 7.4 | Selbmann et al. (2002) |
| Trichoderma pseudokoningii | 95 % ethanol (1/3), 16 h, 4 °C | – | Water | IEC | Water | Rha, Xyl, Fuc, Man, Glc, Gal | – | 31.9 | Huang et al. (2012) |
| EPS producer | Isolation | Purification | EPS characteristics | References |
|--------------|-----------|--------------|---------------------|------------|
| *Agaricus nevoi* HAI 610 | 95% ethanol (1/3), 12 h, 20 °C | – | – | – | Elisashvili et al. (2009) |
| *Antrodia camphorata* | 95% ethanol (1/3), 12 h, 4 °C, repeated twice | – | – | – | Shu and Lung (2004) |
| *Antrodia cinnamomea* BCRC 35396 | 95% ethanol, 12 h, 4 °C | – | – | – | Wu et al. (2006) |
| *Clavariadelphus truncatus* | 95% ethanol (1/4), 12 h, 4 °C | – | – | – | Lin and Sung (2006) |
| *Cerrena unicolor* IBB 681 | 95% ethanol (1/3), 12 h, 4 °C | – | – | – | Demir and Yamaç (2008) |
| *Cerrena maxima* | 95% ethanol (1/3), 12 h, 4 °C | – | – | – | Demir and Yamaç (2008) |
| *Corpinus comatus* | 95% ethanol (1/4), 12 h, 4 °C | – | – | – | Demir and Yamaç (2008) |
| *Cryptococcus laurentii* DSMZ 70766 | Absolute isopropanol (1/3) | – | – | – Man, GlcA, Glc, Gal, Xyl | Smirnou et al. (2014) |
| *Cryptococcus neoformans* ATCC 24067 | 95% ethanol (1/3), CTAB-PS | 24 | Water | Water Xyl, GlcA, Man, Gal, Glc | Frases et al. (2008) |
| *Flammulina velutipes* SF-06 | 95% ethanol (1/3), 24 h, 4 °C | 10 | Water | ICE | EPS1-Rha, Glc EPS2-Rha, Gal | Ma et al. (2015) |
| *Fomes fomentarius* | 95% ethanol (1/4), 12 h, 4 °C | – | – | – | Chen et al. (2008a, b) |
| *Ganoderma applanatum* KFRI 646 | 95% ethanol (1/4), 12 h, 4 °C | 72 | Water | Glc, Gal, Man, Xyl | Lee et al. (2007) |
Table 2 continued

| EPS producer          | The main steps during the procedure of EPS characteristics | References           |
|-----------------------|------------------------------------------------------------|-----------------------|
| Isolation             | Purification                                              |                       |
|                       | Precipitation method                                      |                       |
|                       | (supernatants/alcohol proportion v/v)                     |                       |
|                       | Period of dialysis against water (h)                      |                       |
|                       | EPS solution                                              |                       |
|                       | Chromatographic methods                                  |                       |
|                       | Solubility                                                |                       |
|                       | Compositions                                              |                       |
|                       | Linkage type                                              |                       |
|                       | Mw (kDa)                                                  |                       |
|                       | EPS solution                                              |                       |
|                       | Chromatographic methods                                  |                       |
|                       | EPS characteristics                                       |                       |
|                       | References                                                |                       |

**Ganoderma carnosum**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: HPAEC
- Chromatographic methods: Fuc, Gal, Glc, Man
- Linkage type: 1 → 3; 1 → 4
- Solubility: Water, NaOH (60 °C)
- Compositions: EPS1-390.840.0, EPS2-309.70
- Mw (kDa): 1
- References: Demir and Yamac (2008)

**Ganoderma lucidum UF20706**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Water (poor soluble)
- Chromatographic methods: 0.5% NaOH
- Linkage type: –
- Solubility: –
- Compositions: –
- Mw (kDa): –
- References: Fraga et al. (2014)

**Ganoderma lucidum CCGMC 5.616**
- 95% ethanol (–), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/3), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Water, NaOH (60 °C)
- Chromatographic methods: 1 M NaOH
- Linkage type: 1 → 3; 1 → 4
- Solubility: –
- Compositions: –
- Mw (kDa): –
- References: Tang and Zhang (2002)

**Grifola frondosa**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: GPC
- Chromatographic methods: 1 M NaOH (60 °C)
- Linkage type: –
- Solubility: –
- Compositions: –
- Mw (kDa): –
- References: Shih et al. (2008)

**Inonotus levii HAI 796**
- 95% ethanol (1/3), 12 h, 20 °C
- Precipitation method: 95% ethanol (1/3), 12 h, 20 °C
- Period of dialysis against water: 20°C
- EPS solution: Water
- Chromatographic methods: –
- Linkage type: –
- Solubility: –
- Compositions: –
- Mw (kDa): –
- References: Elisashvili et al. (2009)

**Laetiporus sulphurous**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Water (poor soluble)
- Chromatographic methods: 0.5% NaOH
- Linkage type: –
- Solubility: –
- Compositions: –
- Mw (kDa): –
- References: Lobanok et al. (2003)

**Lentinus edodes**
- 95% ethanol (1/1), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/1), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Water (poor soluble)
- Chromatographic methods: 0.5% NaOH
- Linkage type: –
- Solubility: –
- Compositions: –
- Mw (kDa): –
- References: Demir and Yamac (2008)

**Lentinus strigosus**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Buffer, pH 6.8
- Chromatographic methods: SEC/MALS
- Linkage type: –
- Solubility: Water
- Compositions: Mal, Arab, Xyl, Man, Glc
- Mw (kDa): EPS1–8 628.0, EPS2–1 045.0, EPS3–6 109.0, EPS4–3 355.5
- References: Hwang et al. (2003)

**Lenzites betulina**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Buffer, pH 6.8
- Chromatographic methods: SEC/MALS
- Linkage type: –
- Solubility: Water
- Compositions: –
- Mw (kDa): –
- References: –

**Phellinus gilvus**
- 95% ethanol (1/4), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/4), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Buffer, pH 6.8
- Chromatographic methods: SEC/MALS
- Linkage type: –
- Solubility: Water
- Compositions: Mal, Arab, Xyl, Man, Glc
- Mw (kDa): EPS1–8 628.0, EPS2–1 045.0, EPS3–6 109.0, EPS4–3 355.5
- References: –

**Phellinus igniarius HAI 795**
- 95% ethanol (1/3), 12 h, 20 °C
- Precipitation method: 95% ethanol (1/3), 12 h, 20 °C
- Period of dialysis against water: 20°C
- EPS solution: Buffer, pH 6.8
- Chromatographic methods: SEC/MALS
- Linkage type: –
- Solubility: Water
- Compositions: –
- Mw (kDa): –
- References: Elisashvili et al. (2009)

**Phellinus sp. P0988**
- 95% ethanol (1/3), 12 h, 4 °C
- Precipitation method: 95% ethanol (1/3), 12 h, 4 °C
- Period of dialysis against water: 4°C
- EPS solution: Buffer, pH 6.8
- Chromatographic methods: SEC/MALS
- Linkage type: –
- Solubility: Water
- Compositions: –
- Mw (kDa): –
- References: Ma et al. (2014)
| EPS producer | The main steps during the procedure of EPS characteristics | References |
|--------------|----------------------------------------------------------|------------|
|              | EPS solubility | Compositions | Linkage type | M_w (kDa) |            |
| Isolation    | Purification   | Chromatographic methods |            |           |            |
| Precipitation method (supernatants/alcohol proportion v/v) | Period of dialysis against water (h) |            |            |           |            |
| Pleurotus dryinus IBB 903 | 95% ethanol (1/3), 12 h, 20 °C | – | – | – | – | – | Elisashvili et al. (2009) |
| Pleurotus pulmonarius | 95% ethanol (1/3) | + | – | IEC | – | Man, Gal, Glc 3-O-methyl-Gal 1 → 3; 1 → 6 | Smiderle et al. (2012) |
| Pleurotus sajor-caju CCB 019 | Acetone (1/3), 24 h, 4 °C | – | – | – | – | Man, Gal, Glc 3-O-methyl-Gal | Telles et al. (2011) |
| Pleurotus sajor-caju | 95% ethanol | – | – | HPAEC | – | Man, Gal 3-O-methyl-Gal 1 → 6 | 64.0 | Silveira et al. (2015) |
| Polyporus arcularius | 95% ethanol (1/4), 12 h, 4 °C | – | – | – | – | – | – | Demir and Yamac (2008) |
| Trametes versicolor | Isopropanol (1/1), 24 h, 4 °C | – | – | – | – | – | – | Bolla et al. (2010) |
| Trametes versicolor IBB 897 | Absolute ethanol (1/3), 12 h | – | – | – | – | – | – | Elisashvili et al. (2009) |

Arab, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; GlcA, glucuronic acid; Mal, maltose; Man, mannose; Rha, rhamnose; Xyl, xylose; GPC, Gel Permeation Chromatography type of Size Exclusion Chromatography (SEC); HPAEC, High Performance Anion Exchange Chromatography; IEC, Ion Exchange Chromatography; SEC/MALS, the combination of SEC with Multi-Angle Light Scattering Analysis (MALS); CTAB-PS, isolation of EPS by cetyltrimethylammonium bromide precipitation
reported that EPS from *Fusarium oxysporum* Y24-2 consists of a disaccharide repeating units with the following structure (n \( \approx \) 111): \( \text{[1\rightarrow2]-}\beta-D\text{-Galf(1\rightarrow6)-}\alpha-D\text{-}\text{Glc}(1\rightarrow)n \). A homogenous EPS from *Aspergillus versicolor* is mainly composed of (1→6)-linked \( \alpha\text{-d-}\text{gluco} \)copyranose residues, slightly branched by single \( \alpha\text{-d-} \)mannopyranose units attached to the main chain at C-3 positions of the glucan backbone (Chen et al. 2013a). *B. rhodina* MMGR produces EPS containing \( \beta\text{-1 \rightarrow6} \) branched glucose residues (Barbosa et al. 2003; Vasconcelos et al. 2008). *Penicillium griseofulvum* produces an EPS composed of a long chain of galactofuranan and a mannose core. The galactofuranan chain consists of (1→5)-linked \( \beta\text{-galactofuranose} \), with additional branches at C-6 consisting of (1→4)-linked \( \beta\text{-galactofuranose} \) residues and phosphate esters. The mannan core is composed of (1→6)-linked \( \alpha\text{-mannopyranose} \) substituted at C-2 by (1→3)-linked \( \alpha\text{-mannopyranose} \) residues, disaccharide, and trisaccharide units of (1→2)-linked \( \alpha\text{-mannopyranose} \) (Chen et al. 2013b). There are only few studies on the linkage type of EPS isolated from Basidiomycota. Silva et al. (2015) reported that the EPS produced by edible mushrooms *Pleurotus sajor-caju* was a mannogalactan composed of mannose (37.0 %), galactose (39.7 %), and 3-O-methyl-galactose (23.3 %) and comprised a main chain of (1→6)-linked \( \alpha\text{-d-Galp} \) and 3-O-methyl-\( \alpha\text{-d-Galp} \) units. *Ganoderma lucidum* produces EPSs that are mainly \( \beta\text{-1 \rightarrow4} \)-linked branched glucans containing (1→3) linkages as well (Fraga et al. 2014).

**Potential medical applications**

Given their diversity, fungal EPSs could be used in medicine, for example as antioxidant and antimicrobial agents applied for acceleration of wound healing and fighting bacterial and viral infections as well as antitumor agents activating immune response in the host and supporting chemotherapy treatment (Zhang et al. 2002, 2007; Chen and Seviour 2007; Liu et al. 2009).

**Antioxidative and antimicrobial activities**

Antioxidative properties have been described for fungal compounds, mainly represented by phenolic compounds, \( \beta\text{-tocopherol} \) and \( \beta\text{-carotene} \), PS-protein complexes, and EPSs (Palacios et al. 2011). Until now, it has been demonstrated that ethanol extracts of nearly 150 species of fungi exhibit antioxidative activity (Chang and Miles 2004). These antioxidative properties have been recognized for several EPSs of both Ascomycota and Basidiomycota strains (Table 3). Antioxidant activity assigned to PSs consists in chelation of ferrous ions Fe\(^{2+}\) or prevention of Fenton’s reaction and inhibition of lipid peroxidation as well as enhancement of the enzymatic activity of the antioxidant system such as superoxide dismutase, catalase, or glutathione peroxidase (Kozarski et al. 2014). Antioxidants may react directly with reactive oxygen species or indirectly by reduced oxidation reaction metabolites, not allowing formation of oxygen free radicals. Therefore, PSs show greater antioxidant properties than monosaccharides because the most important factor that has an influence on the ability of EPSs to scavenge free radicals is the size of the carbohydrate molecule. The choice of a proper method of extraction of PS and PS-protein complexes, especially the type of applied solvents, also affects the antioxidant activity of purified preparations. For example, water extracts of fungal PSs contain proteins and phenol compounds with antioxidant activity (Zhou and Chen 2011; Kozarski et al. 2014).

There are many reports available describing the antibacterial properties of fungal EPSs, especially those from Basidiomycota strains from the genus *Cerrena, Ganoderma, Lenzites* and *Pleurotus*, in relation to both gram-positive and gram-negative bacteria (Demir and Yamaç 2008). EPS from *Ganoderma applanatum* showed antibacterial properties against *Staphylococcus aureus* and a toxic effect against *Vibrio fischeri* cells (Osińska-Jaroszuk et al. 2014). For Ascomycota (*Hirsutella* strain) EPS, also antimicrobial activity against *Bacillus subtilis* and *Micrococcus tetragenus* was studied (Li et al. 2010).

**Bioactive properties and potential biotechnological (medical, environmental, and agricultural) applications of EPS**

Many bioactive properties of Ascomycota and Basidiomycota EPSs such as antioxidative, antimicrobial, immunomodulatory, antitumor, hypolipidemic, hypoglycemic, and hepatoprotective activity can find medical applications. The activities of fungal EPSs in such processes as mineral solubilization, heavy metal sorption and hydrocarbon removal as well as eliciting plant resistance created a possibility of potential environmental and agricultural applications of these EPSs in biofertilization, soil/water bioremediation, and plant bioprotection (Table 3). The bioactive properties of PSs are known to depend on many factors, including the structure, monosaccharide components, molecular mass, conformation, configuration of glycosidic bonds, and extraction and isolation methods (Zhou and Chen 2011).
Table 3  Examples of bioactive properties and potential medical, environmental, and agricultural applications of EPSs obtained from selected Ascomycota and Basidiomycota strains

| Bioactivities               | Ascomycota                                      | Basidiomycota                                  |
|-----------------------------|-------------------------------------------------|------------------------------------------------|
|                             | EPS producer References                         | EPS producer References                         |
|                             | References                                      | References                                      |
| **Potential medical applications** |                                                 |                                                 |
| Antioxidative               | Aspergillus sp. Y16                             | Agaricus brasiliensis                          |
|                             | Chen et al. (2011)                              | Kozarski et al. (2011)                          |
|                             | Cordyceps sinensis                             | Ganoderma applanatum                           |
|                             | Leung et al. (2009)                             | Kozarski et al. (2012)                          |
|                             | *Fusarium oxysporum*                           | *Ganoderma lucidium*                           |
|                             | Chen et al. (2015)                              | Jia et al. (2009), Liu et al. (2010) and Kozarski et al. (2012) |
|                             | *Morchella crassipes*                          |                                                 |
|                             | He et al. (2012)                                |                                                 |
|                             | *Lentinus edodes*                              |                                                 |
|                             | *Trametes versicolor*                           |                                                 |
|                             | *Phellinus sp. P0988*                          | Ma et al. (2014)                                |
|                             | *Flammulina velutipes SF-06*                    | Ma et al. (2015)                                |
| **Antimicrobial**            | *Hirsutella sp.*                               | Cerrena unicolor                                |
|                             | Li et al. (2010)                                | Demir and Yamaç (2008)                         |
|                             | *Ganoderma applanatum*                         | *Ganoderma applanatum*                         |
|                             | *Ganoderma carnosum*                           | Demir and Yamaç (2008)                         |
|                             | *Lenzites betulina*                             | Demir and Yamaç (2008)                         |
|                             | *Polyporus arcularius*                         | Demir and Yamaç (2008)                         |
| **Immunomodulatory**        | *Fusarium coccophillum BCC2415*                 | Ganoderma applanatum                           |
|                             | Madla et al. (2005)                             | Osinski-Jaroszuk et al. (2014)                 |
|                             | *Cordyceps sinensis*                           | Pleurotus sajor-caju                           |
|                             | Zhang et al. (2010)                             | Silveira et al. (2015)                         |
| **Antitumor**               | *Trichoderma pseudokoningii*                    | Agaricus blazei                                 |
|                             | Huanga et al. (2012)                            | Yu et al. (2009)                                |
|                             | *Cordyceps militaris*                          | Fomes fomentarius                               |
|                             | Kim et al. (2010)                               | Chen et al. (2008a, b)                          |
|                             | *Cordyceps sinensis*                           | Osinski-Jaroszuk et al. (2014)                 |
|                             | Zhang et al. (2010)                             | *Ganoderma applanatum*                         |
|                             |                                                 | *Ganoderma tsugae*                             |
|                             |                                                 | Pleurotus sajor-caju                            |
|                             |                                                 | CCB 019                                        |
| **Wound management**        | *Cryptococcus laurentii*                        |                                                 |
|                             | Smirnou et al. (2014)                           |                                                 |
| **Hypoglycemic**            | *Cordyceps militaris*                          | Tremella fuciformis                             |
|                             | Kim et al. (2001)                               | Cho et al. (2007)                               |
|                             | *Lentinus edodes*                               | Phellinus baumii                                |
|                             | Yang et al. (2002)                              | Cho et al. (2007)                               |
|                             | *Botryosphaeria rhodina*                        |                                                 |
|                             | Miranda-Nantes et al. (2011)                    |                                                 |
| **Hypolipidemic**           | *Cordyceps militaris*                          | Phellinus linteus                               |
|                             | Yang et al. (2000)                              | Kim et al. (2001)                               |
|                             | *Botryosphaeria rhodina*                        | Cerrena unicolor                                |
|                             | Miranda-Nantes et al. (2011)                    | Kim et al. (2001)                               |
|                             |                                                 | Coprinus comatus                                |
|                             |                                                 | Yamaç et al. (2009)                             |
|                             |                                                 | *Lenzites betulina*                             |
|                             |                                                 | Yamaç et al. (2009)                             |
| **Hepatoprotective**        | *Antrodia cinnamomea*                           |                                                 |
|                             | Ho et al. (2008)                                |                                                 |
Immunomodulatory and antitumor activities and wound management

Antitumor activity of PSs is closely related to their immunomodulatory and immunostimulant activity, which is affected by many physical and chemical properties. Particles of β-(1 → 3) glucans with the molecular weight less than 20 kDa have either no or low immunomodulatory activity (Bohn and BeMiller 1995). There is a correlation between the conformation and weight of the molecule because only a molecule with molecular weight of at least 90 kDa may take a triple helix structure. There are a number of papers on the immunomodulatory and antitumor action of EPSs from both Ascomycota and Basidiomycota strains (Table 3). Lee et al. (2007) has demonstrated that the effect of EPS from G. applanatum on TNF-α was dependent on its molecular size. Within the range of fungal PS, the most active antitumor properties are exhibited by β-glucans binding by β-(1 → 3)-glycosidic bonds. Not without significance is also the additional share of side chains linked by β-(1 → 6) glycosidic bonds affecting the degree of branching molecules, which is reflected in increased antitumor activity of β-(1 → 3) glucan (Chen and Seviour 2007). The action of PS mainly involves stimulation of macrophages and dendritic cells (Kim et al. 2010) for production of various kinds of cytokines, including TNF-α, IFN-γ, and IL-1β, and stimulation of NK cells, T and B, which is connected with inhibition of cancer cell growth (Lindequist et al. 2005; Lee et al. 2010). After recognition and attachment of the PS molecule, immune processes such as production of free radicals, phagocytosis, or production of cytokines involved in inflammation are activated (Brown and Gordon 2005; Chan et al. 2009). Chen et al. (2008a, b) showed that EPS from Fomes fomentarius has a direct antiproliferative effect in vitro on SGC-7901 human gastric cancer cell. Crude EPS obtained by Osin´ska-Jaroszuk et al. (2014) from G. applanatum exhibited antitumor activity against carcinoma cells (lines SiHa) and stimulated the production of IL-6 and TNF-α by the macrophage line THP-1. EPSs produced by Pleurotus sajor-caju act as an anti-inflammatory agent reducing nociception and edema (Silveira et al. 2015). Kim et al. (2010) demonstrated that the cordlan PS isolated from Cordyceps militaris induced phenotypic maturation of dendritic cells demonstrated. Zhang et al. (2010) reported immunomodulatory function and antitumor activity of an EPS fraction EPSF prepared from Cordyceps sinensis, which significantly enhanced the Neutral Red uptake capacity of peritoneal macrophages and splenic lymphocyte proliferation in B16-bearing mice and inhibited metastasis of B16 melanoma cells to lungs and livers.

EPS recovered from the culture supernatant of the human pathogenic fungus Cryptococcus neoformans can elicit the immunological response providing convenient source of EPS preparations suitable for immunological studies and of EPS-based vaccines for prevention of cryptococcosis (Datta and Pirofski 2006; Frases et al. 2008; Table 3).

Furthermore, EPS obtained from culture of Cryptococcus laurentii belonging to the same genus seems to be a very promising biotechnological product in wound management, as it significantly improved excisional wound

| Bioactivities                                      | Ascomycota         | References | Basidiomycota       | References |
|---------------------------------------------------|---------------------|------------|---------------------|------------|
| Potential environmental and agricultural application |                     |            |                     |            |
| Mineral solubilization (biofertilization)          | *Penicillium* sp.   | Wakelin et al. (2004) | *Pleurotus ostreatus* | Seneviratne et al. (2008) |
| Heavy metal sorption (bioremediation)              | *Aspergillus fumigatus* | Lian et al. (2008) |                     |            |
|                                                    | *Hansenula anomala*  | Breierová et al. (2002) |                     |            |
|                                                    | CCY 38-1-22         | Da Silva et al. (2014) |                     |            |
|                                                    | *Colletotrichum* sp. | Moon et al. (2006) |                     |            |
|                                                    | *Pestalotiopsis* sp. | Radulović et al. (2008) |                     |            |
|                                                    | KCTC 8673           |                     |                     |            |
|                                                    | *Aureobasidium pullulans* |                     |                     |            |
|                                                    | CH1                 |                     |                     |            |
| Hydrocarbon removal (bioremediation)               | *Fusarium oxysporum*| Li et al. (2011) |                     |            |
| Eliciting plant resistance (bioprotection)         | Dzf17               |                     |                     |            |
|                                                   |                     |                     |                     |            |

Table 3 continued
healing in rats in in vitro experiments (Smirnou et al. 2014).

**Hypoglycemic, hypolipidemic, and hepatoprotective activities**

Several PSs from edible fungi have a proven hypoglycemic effect via a decrease in the glucose level in blood or modulation of glucose/insulin metabolism. A majority of publications on this topic are related to studies of intracellular PSs (Mao et al. 2009). For example, intracellular PS obtained from *Ganoderma lucidum* decreased fasting serum glucose and insulin levels, and the epididymal fat/BW ratio in type-2 diabetic mice (Xiao et al. 2012) and delayed progression of diabetic renal complications (He et al. 2006). There are also a growing number of reports concerning the ability of some EPSs to reduce glucose levels (Table 3). Cho et al. (2007) reported that EPSs from two different Basidiomycota strains, *Tremella fuciformis* and *Phellinus baumii*, exhibited a considerable hypoglycemic effect and improved insulin sensitivity through the regulation of peroxisome proliferator-activated receptor (PPAR)-γ-mediated lipid metabolism. Oral administration of EPS produced by *Cerrena unicolor*, *Coprinus comatus*, and *Lenzites betulina* significantly decreased glucose levels in the serum in streptozotocin-induced diabetic rats (Yamac et al. 2009).

One of the pharmacological properties of fungal EPS is the ability to lower the level of cholesterol and the content of lipids in blood (Table 3). After application of glucan, increased excretion of bile acids and short chain fatty acids was observed. This process inhibits the incorporation of acetate to serum lipids, a substrate required for the synthesis of sterols and fatty acids. This is probably related to the gelling properties of glucans, which also inhibit absorption of cholesterol and triglycerols (Guillamon et al. 2010). Significant reduction of total cholesterol and triglyceride levels in plasma was observed in rats fed with *C. militaris* and *Lentinus edodes* EPSs (Yang et al. 2000, 2002). Botryosphaeran, a water-soluble EPS of the β-(1 → 3; 1 → 6)-D glucan type isolated from the culture medium of *Botryosphaeria rhodina*, administered by gavage, also reduced the plasma levels of total cholesterol and low density lipoprotein-cholesterol in hyperlipidemic rats (Miranda-Nantes et al. 2011). Hypolipidemic and hypoglycemic activities have also been described for EPSs obtained from Ascomycota strains from species *C. militaris* and *B. rhodina* cultures (Table 3).

The possible role of EPS in hepatoprotection has also been discussed. For example, the hepatoprotective activity of water extracts containing EPS from *Antrodia cinnamo-meza* on ethanol-induced cytotoxicity in AML12 hepatocytes was determined (Ho et al. 2008).

**Potential environmental and agricultural applications**

The mechanisms used by microorganisms for mineral solubilization have been attributed mainly to acidification, exchange reactions, and chelation (Yadav and Tarafdar 2003). It was found that the ability to release nutrients from minerals (weathering) by fungi resulted from the action of fungal EPS in cooperation with organic acids synthesized by fungi, causing precipitation and dissolution of soil minerals through acidification of surrounding hyphae and chelation of different ions (Welch et al. 1999; Rogers and Bennett 2004; Sheng 2005; Rosling et al. 2009; Xiao et al. 2012). Acidic metabolites adsorbed on the EPS surface bind SiO$_2$ ions from silicate minerals, thereby contributing to K$^+$ release in the soil solution (Liu et al. 2006). Fungal EPSs also contain their own acidic constituents, e.g. carboxylic acid, uronic acids, or functional groups enhancing mineral dissolution (Welch et al. 1999). Many saprotrophic fungi producing EPS, including strains from the genera *Penicillium* (*P. simplicissimus*, *P. giseofulvum*, *P. radicium*) and *Aspergillus* (*A. fumigatus*, *A. niger*), effectively release phosphorus and potassium through degradation of minerals e.g. Ca phosphates, Fe/Al phosphates, mica, montmorillonite, and K-feldspar and belong to a group of phosphate- or potassium-solubilizing microorganisms (PSM, KSM) (Welch et al. 1999; Wakelin et al. 2004; Barroso and Nahas 2005; Sheng 2005; Yi et al. 2008; Smits 2009; Mahapatra and Banerjee 2013b). *Penicillium* and *Aspergillus* strains are very often a fungal component of a special kind of tested (lab-stage) biofertilizers like fungal–bacterial and fungal–rhizobial biofilm biofertilizers (FBBs and FRBs, respectively) (Wakelin et al. 2004; Lian et al. 2008). There are commercial biofertilizers with *Penicillium* strains e.g. Provide, TagTeam (produced by Philom BIOS; Canada).

EPSs play a key role in formation of biofilm facilitating colonization of soil particles, seeds, and roots systems. The degree of phosphate solubilization increased up to 230 % after the use of biofilm of *Penicillium* spp., *Pleurotus ostreatus*, and *Xanthoparmelia mexicana* in comparison to fungal monocultures (Seneviratne et al. 2008).

**EPS as a potential component of bioremediation preparations removing heavy metals and hydrocarbons**

Metal biosorption by EPSs involves ionic interactions (e.g. ion exchange, electrostatic interaction) and physical entrapments (Breierová et al. 2002; Da Silva et al. 2014).
EPSs possess a substantial quantity of functional groups (amine, phosphate, hydroxyl, carboxyl, and urinate), which increase the negative charge of EPSs and their ion exchange properties and flocculation activities, and can coordinate with metal ions (complexation) and form organic precipitation (Breierová et al. 2002; Moon et al. 2006; Abdel-Aziz et al. 2012). Under the presence of heavy metals, EPS is more heterogenic and contains higher amounts of different components and reactive groups than under absence of metal (Breierová et al. 2002). After purification of crude pullulan obtained from *Aureobasidium pullulans* CH1 strain, when glucose was its only component, no metal (Cu, Fe, Zn, Mn, Pb, Cd, Ni and Cr) biosorption was reported (Radulovic et al. 2008). Exopolymers of *Hansenula anomala* CCY 38-1-22 bound 90 % of the total amount of Cd ions sorbed by this resistant strain, while the sensitive strain of *Saccharomyces cerevisiae* CCY 21-4-100 accumulated this metal predominantly in the cellular compartments (94 %) (Breierová et al. 2002). The biosorption of Cd and Pb ions on EPS produced by the fungus *Colletotrichum* sp. contributed to removal of 79 and 98 % of cadmium and lead, respectively, from a solution with the initial concentration of these metals of 100 mg l\(^{-1}\) (Da Silva et al. 2014). Moon et al. (2006) found that each gram of pestan (a specific EPS produced by *Pestalotiopsis* sp. KCTC 8637) absorbed 120 mg of lead or 60 mg of zinc. Fungal EPSs can be as effective in biosorption as fungal biomass (Moon et al. 2006), and can be used as potential biosorbents for removal of heavy metals from wastewaters. The interaction of a marine isolate of the white-rot fungus *Flavodon flavus* NIOCC#312 EPS with specific lignin-degrading enzymes (e.g. peroxidases and laccase) appears to help in degradation of toxic organic compounds by breaking down polycyclic aromatic hydrocarbons before their contact with mycelium (Raghukumar et al. 2006).

**EPSs as potential biocontrol preparations eliciting plant resistance**

In the literature, there are many reports concerning the ability of bacterial EPSs and fungal cell wall PSs to induce plant resistance (Tamm et al. 2011) but the ability of fungal EPSs to induce resistance is still poorly studied. To our knowledge, there are only two reports about fungal EPS acting as an elicitor of systemic plant resistance (Table 3). Li et al. (2014) have shown that an oligosaccharide obtained from the EPS of the endophytic fungus *Fusarium oxysporum* Dz17 contributed to an increase in the activity of defense-related enzymes such as phenylalanine ammonia lyase, polyphenoloxidase, and peroxidase in *Dioscorea zingiberensis* suspension cells and seedling cultures. El Oirdi et al. (2011) have shown that EPS of *Botrytis cinerea* acts as a suppressor of the jasmonic acid (JA) signaling pathway, induces accumulation of salicylic acid (SA) in tomato, and enhances resistance against the hemibiotrophic pathogen *Pseudomonas syringae*. The phenomenon of induced systemic plant resistance—systemic acquired resistance (SAR) mediated by a SA-dependent process and induced systemic resistance (ISR) mediated by JA and ethylene (ET) involves elevation of the level of phenylpropanoid pathway metabolites not only at the site of introduction of the elicitor but also in the entire plant, even in tissues distant from the infection site (Walters et al. 2013).

**Conclusion**

Many research groups conduct studies on the EPSs from Ascomycota and Basidiomycota fungi, considering their potential use in various fields of science and industry. The data described above imply that the current work on fungal EPSs is the tip of the iceberg, and many exciting scientific findings are still to come. While knowledge about the structure of EPSs is becoming increasingly clear, details of the mechanisms of EPS action in various systems are only beginning to be understood. The multifarious properties of EPSs create a possibility of their wide future application in both biotechnology and medicine.

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