G.J.B.A.H.S., Vol.6(3):5-12 (July-September, 2017) ISSN: 2319 – 5584

GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(Published By: Global Institute for Research & Education)

www.gifre.org

MICROBIAL XYLANASES AND THEIR INDUSTRIAL APPLICATIONS AS WELL AS FUTURE PERSPECTIVES: A REVIEW

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Abstract

Xylan is the most abundant and principal type of hemicellulose. It is a linear polymer of β-D xylopyranosyl units linked by (1–4) glycosidic bonds. Xylanases are most predominantly present in plant cell walls and are produced by different kinds of microorganisms like bacteria, fungi, protozoans and some yeast. Cellulase-free xylanases are important in pulp biobleaching as alternatives to the use of toxic chlorinated compounds because of the environmental hazards and diseases caused by the release of the adsorbable organic halogens. Xylanases has a wide range of applications in pulp and paper, food, animal feed, textiles and pharmaceuticals. In this review, we have focused on the studies of structural composition of xylan, sources of xylanases, production of xylanases and their potential industrial applications.

Keywords: *Xylan, xylanases, microorganisms*

1. Introduction

Enzymes are distinct biological polymers that catalyze the chemical reactions and convert substrates to particular products. They are specific in function and speed up reactions by providing alternative pathways of lower activation energy without being consumed. These are the fundamental elements for biochemical processes and utilized in a number of food processing industries (Haq et al., 2006). The plant cell wall is composed of cellulose (35–50%), hemicellulose (20–30%, mainly xylan) and lignin (20–30%). Cellulose and hemicellulose binds with lignin by covalent and non-covalent interactions. Xylan is a heteropolysaccharide containing O-acetyl, arabinosyl and 4-O-methyl-D-glucuronic acid substituents. It is substituted with L-arabinose, D-galactose, D-mannoses, and glucouronic acid through glysosidic bonds with acetic acid and ferulic acid by ester bonds (Collins et al., 2005; Ahmed et al., 2011). Xylanases (E.C.3.2.1.8) are the key enzymes, which play an important role in the breakdown of xylan (Sharma and Sharma, 2016c). The depolymerisation action of endo-1,4-xylanases (1,4- β -xylan xylanohydrolase; EC 3.2.1.8) and β -D- xylosidase (1,4-b-xylan xylohydrolase; EC 3.2.1.37) results in the change of the polymeric substance into xylooligosaccharides and xylose (Gomez et al., 2008; Juturu and Wu, 2014). Thus, the action of a xylanase enzyme helps to break down plant cell walls. This activity has applications in the food and paper-making industries, along with uses in agriculture and for human health. It is produced by bacteria (Kiddinamoorthy et al., 2008; Sanghi et al., 2007), fungi (Nair et al., 2008; Sharma and Sharma, 2013), actinomycetes (Ninawe et al., 2007) and yeast (Liu et al., 1998). Recently, interest in xylanase has markedly increased due to its wide variety of biotechnological applications such as pre-bleaching of pulp, improving the digestibility of animal feed stocks, modification of cereal-based stuffs, bioconversion of lignocellulosic material and agro-wastes to fermentable products, clarification of fruit juices and degumming of plant fibers (Kapoor et al., 2001; Virupakshi et al., 2005) etc. A large variety of xylanases produced by microorganisms become a major group of industrial enzymes that are capable to degrade xylan to renewable fuels and chemicals (Hatanaka, 2012), in addition to their use in food, paper and pulp industries (Golugiri et al., 2012; Singh et al., 2013). In recent years, there has been growing awareness in applying green biotechnology to bleaching processes to decrease pollution as well as improve the quality of pulp produced. Biobleaching and biopulping processes have been explored frequently over the past 15 years (Singh et al., 2013). Cellulase-free xylanases active at high temperature and pH are gaining importance in pulp and paper industry as they reduce the need for toxic chlorinated compounds making the bleaching process environment-friendly (Srinivasan, et al., 1999; Viikari et al., 1994). Thus, biotechnologies developed to convert biomass into saleable products that progressively substitute raw materials resulting from non-renewable resources are becoming commercially worthy.

2. Structure of Xylan

Plant cell wall polysaccharides are the most abundant organic compounds found in nature. Hemicellulose are polysaccharides more heterogeneous than cellulose and are second most abundant organic in the plant cell wall. The major hemicelluloses polymer in cereals and hardwood is xylan, representing up to 30-35 per cent of the total dry mass (Joseleau et al., 1992). Hemicelluloses are structurally unrelated to celluloses and are so named as these were incorrectly believed to be the precursor of celluloses (Singla and Chauhan, 1995). Among hemicelluloses, xylan is a heterogenous polysaccharide in which β-1, 4-linked D-xylopyranose residues are the main constituents depending on their origin, xylan may also contain variable amounts of arabinosyl and 4-0-methylglucoronic acid residues and acetyl groups. The most important enzyme in the xylan biodegradation is the endo-1, 4- β-xylanase (EC3.2.1.8) that releases xylopyranose units (Madlala et al., 2001). It comprises up to 30 percent of cell wall material of annual plant, 15-30 per cent of hardwoods and 7-10 per cent of softwood (Jeya et al., 2005). Xylan and glucomannan form the basic backbone polymer of hemicelluloses in wood. In hard wood there are twice as many 4-0 methyl glcuronic acid side groups; on an average two out of ten xylan units are substituted with uronic acid. The ratio of arabinose side groups to xylose residues in softwood xylan is 1:8. Xylan in hardwood and softwood differ in their degree of polymerization which in hardwood xylan is about 15-200 and in softwood about 7-130 (Fihlo et al., 1994).

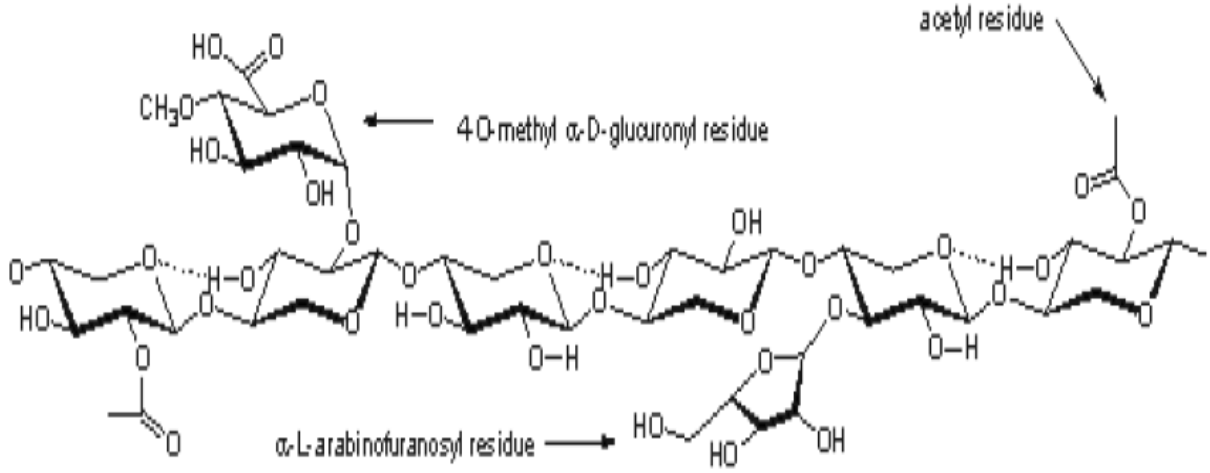

Fig1. Structure of xylan

3. Xylanolytic Enzymes

Xylanases catalyze the hydrolysis of xylans. These enzymes are produced mainly by microorganisms and take part in the breakdown of plant cell walls, along with other enzymes that hydrolyze polysaccharides, and also digest xylan during the germination of some seeds (e.g. in the malting of barley grain). Xylanases also can be found in marine algae, protozoans, crustaceans, insects, snails and seeds of land plants (Sunna and Antranikian 1997). Xylan being a heterogeneous polymer requires the concentrated action of complex enzyme system, degrading the backbone as well as side chains of xylans and converting it into constituent sugars (Amani et al., 2007). Several hydrolytic enzymes are involved in complete breakdown of branched acetyl xylan (Dodd and Cann, 2010).

3.1 Endo -1-4-β-xylanases

Endo-1, 4-β-xylanase (1, 4-β-D-xylan xylanohydrolase; EC 3.2.1.8) cleaves the glycosidic bonds in the xylan backbone, bringing about a reduction in the degree of polymerization of the substrate. Xylan is not attacked randomly, but the bonds selected for hydrolysis depend on the nature of the substrate molecule, i.e. on the chain length, the degree of branching, and the presence of substituents (Puls and Poutanen 1989; Li et al., 2000). Endoxylanases have been differentiated according to the end products they release from the hydrolysis of xylan (e.g. xylose, xylobiose and xylotriose and arabinose).Thus, xylanases may be classified as non-debranching (arabinose non-liberating) or debranching (arabinose liberating) enzymes. Fungal and bacterial endoxylanases are almost exclusively single subunit proteins with molecular weight values ranging from 8.5 to 85 kDa and isoelectric point (pI) values between 4.0 and 10.3, most of them are glycosylated (Coughlan et al., 1993; Polizeli et al., 2005).

3.2 β- Xylosidases

β-D- Xylosidases (1, 4-β-D-xylan xylohydrolase; EC 3.2.1.37) can be classified according to their relative affinities for xylobiose and larger xylooligosaccharides. It may be monomeric, dimeric or tetrameric with molecular weight ranging from 26 to 360 kDa (Octavio et al., 2006). Purified β-xylosidases usually do not hydrolyze xylan, their best substrate is xylobiose and their affinity for xylooligosaccharides is inversely proportional to its degree of polymerization. They are able to cleave artificial substrates such as p-nitrophenyl- and o-nitrophenyl-β-D-xylopyranoside (Polizeli et al., 2005). An important role attributed to β- xylosidases comes into play after the xylan has suffered a number of successive hydrolyzes by xylanase.

3.3 α – Glucuronidases

α- Glucuronidase (EC 3.2.1.131) hydrolyzes the α-1, 2 bonds between the glucuronic acid residues and β-D-xylopyranosyl backbone units found in glucuronoxylan (Kaneko et al., 1993).

3.4 α -Arabinofuranosidases

Arabinofuranosidases removes L-arabinose residues substituted at positions 2 and 3 of the β-D-xylopyranosyl. There are two types with distinct modes of action, exo-α-L-arabinofuranosidase (EC 3.2.1.55) which degrades p-nitrophenyl-α- L-arabinofuranosides and branched arabinans and endo-1, 5-α-L-arabinase (EC 3.2.1.99) which only hydrolyzes linear arabinans (De Vries et al., 2000).

3.5 Acetylxylan esterase

Acetylxylan esterase (EC 3.1.1.6) removes the O-acetyl substituents at the 2 and 3 positions of xylose residues in acetylated xylans. Acetylxylan plays an important role in the hydrolysis of xylan, since the acetyl side-groups can interfere with the approach of enzymes that cleaves the backbone by steric hindrance and their elimination thus facilitates the action of endoxylanases (Octavio et al., 2006).

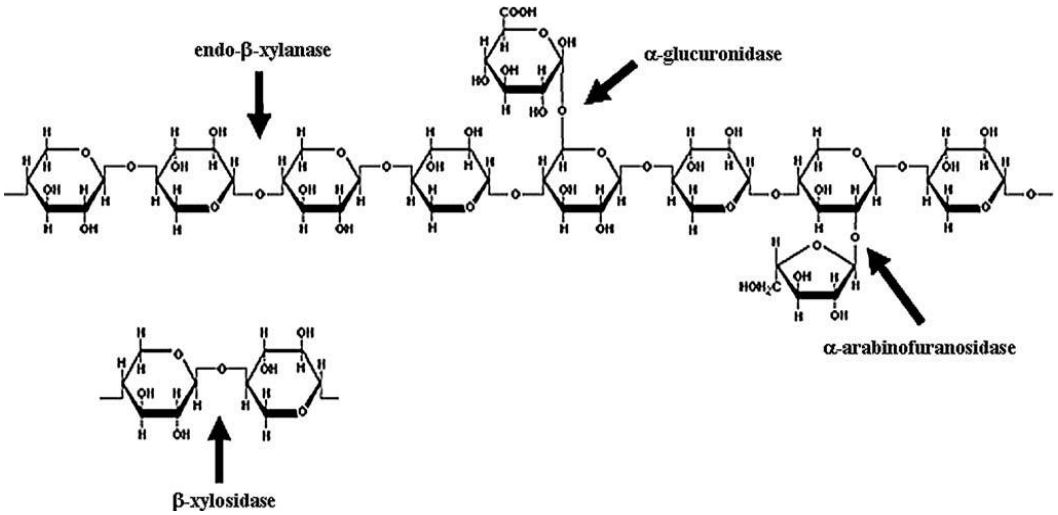

Fig2. Mechanism of xylanases

4. Sources of Xylanases

Xylanases are produced by a wide range of bacteria and fungi, including aerobes, anaerobes, mesophiles, thermophiles and extremophiles. Both fungi and bacteria have been heavily exploited for their abilities to produce a wide variety of hydrolytic enzymes (Sharma and Sharma, 2016a). Amongst the prokaryotes, bacteria and cyanobacteria from marine environments produce xylanase (Anna-malai et al., 2009). There is information about xylanase from plants, which is endoxylanase from Japanese pear fruits during over maturing period and higher animals such as mollusc, are also able to produce xylanase (Yamaura et al., 1997). There are reports related to isolation and purification of xylanase from various other sources such as anaerobic bacterium *Clostridium acetobutylicum*, immature cucumber seeds and germinating barley (Sizova et al., 2011). Xylanase production from *Myceliophthora thermophila* SH1, a thermophlillic fungus under solidstate fermentation has been reported (Sharma and Sharma, 2013).

4.1 Bacterial xylanases

Xylanases produced by bacteria and actinomycetes (*Bacillus* sp., *Pseudomonas* sp., *Streptomyces* sp.) are effective in a broader pH range of 5-9, with the optimum temperature for xylanase activity between 35ºC to 60ºC (Beg et al., 2001; Motta et al., 2013). Bacterial strains studied for their xylanase activity (Amore et al., 2014; Dhiman et. al, 2008). Studies on *Bacillus* spp. showed higher xylanase activity at alkaline pH and high temperature. Therefore, bacterial xylanases are used in industrial application due to their alkali tolerance and thermostability (Mandal, 2015).

4.2 Fungal xylanases

Fungi (*Aspergillus* spp., *Fusarium* spp., *Penicillium* spp.) are important producers of xylanase due to high yields and extracellular release of the enzymes (Nair and Shashidhar, 2008). Also, fungal xylanases have higher activity, compared with bacteria or yeast. However, xylanases derived from fungal sources have some characteristics that make them unavailable for some industrial applications (Mandal, 2015). Most of these

xylanases are efficient at temperature below 50ºC and a pH range of 4-6 (Beg et al., 2000). For example, fungal xylanases can't be used in the pulp and paper industry that needs an alkaline pH and temperature more than 60 º C (Mandal, 2015). Another problem with fungal xylanases is the presence of a cellulase, few studies reporting fungal xylanase without cellulase activity (Subramaniyan and Prema, 2002).

5. Xylanase Production

The carbon source plays another major role in the economics of xylanase production. In order to replace the cost of the xylan, cost effective natural lignocellulosic substrates like wheat bran, sugarcane bagasse, rice straw, corn cobs etc. are used for the production of xylanase. Xylanase production can be performed on a variety of cheaper lignocellulosic materials, such as wheat bran, wheat straw, rice husk, rice bran, rice straw, corncob, corn stalk, sorghum straw, apple pomace and sugarcane bagasse have been found to be most suitable substrates for solid state fermentation in certain microbes (Yang et al., 2006; Heck et al., 2006). Wheat bran was found to be the best substrate for xylanase production by alkalophilic *Paenibacillus polymyxa* CKWX1 and alkalophilic Streptomyces T-7 (Keskar et al., 1992). From the industrial point of view, xylanases are important enzyme in the bioconversion of hemicellulose, which is a significant component of lignocellulosic material. Filamentous fungi are particularly interesting producers of xylanases from an industrial point of view, due to the fact that they excrete xylan degrading enzymes into the medium, eliminating the need for cell disruption prior to purification (Sunna and Antranikian, 1997; Polizeli et al., 2005). Xylanase production by wild-type *Aspergillus niger* ANL301, newly isolated from wood-waste, was monitored at 24 h intervals for a period 168 h in media containing different carbon sources by (Okafor et al., 2007). The production of xylanase by a local isolate *Trichoderma* spp. FETL C32 via solid state fermentation system using sugar cane baggase: palm kernel cake as substrates was investigated by Pang and co workers (Pang et al., 2006)**.** The various biotechnological techniques like submerged and solid state fermentation are employed for xylanase biosynthesis (Gawande and Kamat, 1999; Kansoh and Gammel, 2001). The submerged fermentation is most beneficial as compared to other techniques due to more nutrients availability, sufficient oxygen supply and less time required for the fermentation (Bim and Franco, 2000 and Gouda, 2000). The production of microbial xylanases is preferred over plant and animal sources because of their availability, structural stability and easy genetic manipulation. Most xylanase manufacturers produce these enzymes using submerged fermentation.

5.0 Industrial applications of xylanases

In recent years, the biotechnological use of xylans and xylanases has grown remarkably (Subramaniyan and Prema, 2002; Beg et al., 2001; Techapun et al., 2003). Xylanase began to be used in the 1980s, initially in the preparation of animal feed and later in the food, textile and paper industries. Most commercial xylanases are produced by *Trichoderma, Bacillus, Aspergillus, Penicillium, Aureobasidium, and Talaromyces sp* (Godfrey et al., 1996).

5.1 Baking and brewing industry

The application of xylanolytic enzymes has increased for the last few decades owing to their potential effectiveness in bread making (Butt et al., 2008). Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of Rheological properties of dough, bread specific volume and crumb firmness (Martinez-Anaya et al., 1997). The xylanases, like the other hemicellulases, break down the hemicellulose in wheat-flour, helping in the redistribution of water and leaving the dough softer and easier to knead. During the bread- baking process, they delay crumb formation, allowing the dough to grow (Polizeli et al., 2005). With the use of xylanases, there has been an increase in bread volumes, greater absorption of water and improved resistance to fermentation (Harbak and Thygesen 2002; Camacho and Aguilar 2003). Also, a larger amount of arabinoxylo oligosaccharides in bread would be beneficial to health (Polizeli M.L et al., 2005). In biscuit-making, xylanase is recommended for making cream crackers lighter and improving the texture, palatability and uniformity of the wafers (Polizeli et al., 2005). Recently, recombinant yeast of wine was constructed with the gene for xylanase of *Aspergillus nidulans*, xlnA, resulting in a wine with a more pronounced aroma than is conventional (Ganga et al., 1999). During the manufacture of beer, the cellular wall of the barley is hydrolyzed releasing long chains of arabinoxylans which increase the beer's viscosity rendering it "muddy" in appearance. Thus, xylanases are used to hydrolyze arabinoxylans to lower oligosaccharides diminishing the beer's viscosity and consequently eliminating its muddy aspect (Debyser et al., 1997; Dervilly et al., 2002).

5.2 Pulp and paper industries

The pulp and paper industry has been scanning for novel biotechnology methods utilized for the replacement for a portion of the chemicals utilized as a part of the paper making process. Biopulping is the pretreatment of wood or non- wood by lignin-degrading fungi prior to routine pulping process. Notwithstanding, the downsides are the time used in the pretreatment (around 2–4 weeks) and yield loss, as the organisms will be at the same time attacked by the polysaccharides and lignin. To defeat these disadvantages, xylanase pretreatment expanded the dissemination of sodium hydroxide in both hardwoods and softwoods and enhanced the traditional

pulping process (Woldesenbet et al., 2012). Other than the utilization of xylanase in bleaching through lignin removal, the utilization of xylanases additionally help in expanding pulp fibrillation, decrease of beating times in unique pulp and expanded freeness in reused fibers (Savitha et al., 2009). It has been demonstrated from a few studies that xylanase prebleaching is an environment friendly, economically cheap innovation and can diminish the amount of bleached chemicals required to achieve a given brightness in the resulting chemical bleaching stage. Pretreatment with xylanases enhances the effectiveness of chemical extraction of lignin from pulp and minimizes the necessity of chlorine dioxide (ClO_2) (Khonzue et al., 2011). On the contrary, enzymatic treatments of pulp using xylanase have been useful in terms of both lower costs and improved fiber qualities. To obtain white and bright pulp suitable for manufacturing good quality papers, it is necessary to remove the constituents such as lignin by using bleaching process and its degradation products, resins and metal ions**.** The effectiveness of xylanase treatment before chemical bleaching application may be due to cleavage of linkage of residual lignin to hemicellulose, prominent to increased accessibility of the pulp to bleaching chemicals and thereby enhanced the extraction of lignin during sub- sequent bleaching stages (Azeri et al., 2010). Overall, major advantages of biobleaching are: reduced consumption of bleaching chemical, reduced absorbable organic halogen compounds, improved pulp and paper quality, improved brightness, reduced effluent toxicity and pollution load.

5.3 Animal feed

Xylanases used as pretreatment of forage crops, improve the nutritional properties of agricultural silage and grain feed (Subramaniyan and Prema, 2002; Kuhad and Singh, 1993; Bedford and Classen, 1992), thus improving the digestibility of ruminant feeds and facilitating composting (Gilbert and Hazlewood, 1993). However, the complete removal of xylan is not wanted, because hemicelluloses are important components of diet and their removal may increase bowel diseases (Mandal, 2015).

5.4 Biofuels

Production of biofuels is gaining great importance as the energy resources are shrinking. The combined action of xylanase with several enzymes such as mannanase, lignase, xylosidase, glucanase, glucosidase etc can be applied for the generation of biofuels (ethanol and xylitol), from lignocellulosic biomass (Dominguez, 1998). Ethanol from renewable resources has been of interest in recent decades as an alternative fuel or oxygenate additive to the current fossil fuels (Sharma and Sharma, 2016b). The production of bioethanol requires the delignification of lignocellulose to liberate cellulose and hemicellulose. The next steps include the depolymerization of the carbohydrate polymers to produce free sugars and the fermentation of mixed pentose and hexose to produce bioethanol (Lee, 2009).

5.5 Treatment of plant cells

Treatment of tobacco suspension cells (*Nicotiana tabacum* CV.KY 14) with a purified endoxylanase from *Trichoderma viride* increased the levels of acylated sterol glycosides and induces the synthesis of phytoalexins (Moreau et al., 1994). Additionally, a truncated bacterial xylanase gene from *Clostridium thermocellum* has been demonstrated in rhizosecretion in transgenic tobacco plants (Borisjuk et al., 1999). Some xylanases improve cell wall maceration for the production of plant protoplasts (Beg et al., 2001).

5.6 Surfactants

Alkyl glycosides are surfactants widely used in industrial applications, being produced commercially from monomeric sugars. Using polysaccharide is more feasible for their industrial production, because several steps in the process can be omitted (Matsumara et al., 1999). Therefore, xylanase presents a challenging opportunity. Other application of xylanase is in the detergent industry, as it improves the cleaning ability of detergents that are more efficient in cleaning fruit, vegetable, soils and grass stains (Kumar et al., 2004; Dhiman et al., 2008).

5.7 Retting of Flax fibers

A combined xylanase-pectinase system is used in the debarking process, which the first step in wood is processing, the addition of xylanases enhancing the retting process. Other applications of this combined system are used in the degumming of bast fibers such as flax, hemp, jute and the fiber liberation from plant instead of retting (Beg et al., 2001).

6. Future Perspectives

In future, emphasis will be placed on the proper and economical utilization of abundantly present xylan and lignin compounds. Paper industries and agricultural activities release significant quantity of xylan, which gets deposited in rivers and ponds. Therefore in order to maintain the ecological balance and to fullfill the ever increasing demand of fuels and energy attention must be paid to the proper conversion of these waste hemicellulosic compounds to renewable sources of energy and biofuels. Knowledge of molecular aspects of xylanase and cloning in suitable expression vectors will be the second major target. This is so because new

industrial uses of xylanases have been explored and such kind of xylanases is required that is stable and active over a broad range of pH and temperature. A principal hurdle in the commercialization of enzymatic processes is the bulk production of enzymes at a cost effective rate. In order to meet this goal, such strategies should be explored by which cost effective bulk production can be achieved. In the coming new time, new methods will be developed for an easy and cheap production of xylanases to fullfill the demands of various industries.

7. Acknowledgment

The authors gratefully acknowledge the financial support given by Department of Science and Technology (DST), New Delhi- India in the form of "INSPIRE FELLOWSHIP", JRF [IF-110299].

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