higher concentration of KOH increased the saponification reactions. As a conclusion, the highest yield of transesterification reaction was obtained from 1:5 molar ratio of huzelnut and methanol with 2% of KOH with the chemical composition of methyl oleate (82.34%), and methyl linoleate (11.28%), methyl palmitate (5.71%) and methyl stearate (2.37%). These transesterification reaction conditions will be tested for bio- diesel production from agricultural wastes.

SW06.W33–29
Cloning and expression of carbon cycle relevant enzymes of *Ralstonia eutropha* H16

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Ralstonia eutropha is a Gram-negative, strictly respiratory facultative chemolithoautotrophic bacterium which can use hydrogen and carbon dioxide as sole sources of energy and carbon in the absence of organic substrates. It has attracted great interest for biotechnology for its ability to degrade a large list of chloroaromatic compounds and chemically related pollutants. Moreover the production of biodegradable polymer polyhydroxyalkanoates on an industrial scale has already been applied [1]. *R. eutropha* serves as a model organism for the mechanisms involved in the control of autotrophic carbon dioxide fixation, hydrogen oxidation and denitrification.

In our project the main objective is the cloning of different enzymes, like carbonic anhydrases and carboxylases, which allow the organism to fixate carbon dioxide. Important enzymes for the carbon dioxide fixation under lithoautotrophic growth conditions are Rubisco as well as PEP-carboxylase and pyruvate-carboxylase. These enzymes are not able to directly use carbon dioxide as substrate. Carbonic anhydrases are responsible for the conversion of CO_2 to HCO_3^-, which can be used by these carboxylases. Four different carbonic anhydrases as well as the PEP-carboxylase and the Pyruvate carboxylase of *R. eutropha* H16 were cloned and expressed in *Escherichia coli* BL21. The specific enzyme activities of the carbonic anhydrases were measured by Infrared Spectroscopy. The enzyme activities of the carbolxlases were determined spectophotometrically in an assay linked to the NADH-dependent reduction of oxaloacetic acid. The overall aim of the study is the overexpression of carbonic anhydrases and carboxylases to provide a basis for efficient carboxylation reactions performed by *R. eutropha* H16 under lithoautotrophic conditions.

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SW06.W33–30
Cloning and characterization of a new dye degrading laccase from *Bacillus amyloliquefaciens* 12B1

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Industrial dyeing of textile consumes large amounts of water and energy, while 5–40% of the dyestuffs used are released in the effluent. Some dyes are recalcitrant to direct microbial degradation and there is an ongoing search for the introduction of cleaner and more efficient technologies that will enable degradation of these compounds [1]. Promising new technology is the use of oxidative enzymes [2]. Laccases (EC1.10.3.2), a family of blue multicopper oxidases, are capable of oxidizing a wide range of aromatic compounds, with concomitant reduction of molecular oxygen to water [3]. Identifying and characterizing new laccases from bacteria and evaluating their application potential will greatly help us to better use them in industrial processes.

To obtain new laccases with ideal characteristics, one hundred soil isolated *Bacillus* sp. across Serbia have been screened for spore laccase activity. Ten percent of screened strains demonstrated laccase activity on their spores. A new laccase gene (cotA) was cloned from newly isolated *Bacillus amyloliquefaciens* strain and expressed in *Escherichia coli*. Temperature optimum, pH optimum and temperature stability were deteremined for both wt spore-bound laccase and recombinant laccase. The recombinant protein CotA demonstrated activity towards canonical laccase substrates ABTS, syringaldazine and 2.6-dimetoxyphenol. Highest oxidizing activity towards ABTS was obtained at 80°C. pH optimum of recombinant laccase is 5.0. Higher thermostability at 80°C was observed for recombinant enzyme. Oxidation of azo and aminochlorotriazine dyes was demonstrated and thus potential for industrial application confirmed. Due to high temperature optimum of this enzyme it is expected to use it in the treatment of hot effluents from textile industry.

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Escherichia coli F_OF_1-ATPase activity under glycerol fermentation at different pH and role of hydrogenases

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Escherichia coli is able to ferment glycerol and produce molecular hydrogen (H_2) by using different hydrogenases (Hyd) [1]. The important aspect in regulation of H_2 production by *E. coli* formate hydrogen lyase (FHL) and its energetics is the requirement of the F_oF_1-ATPase, which has been shown to be *N,N'*-dicyclohexylcarbodiimide (DCCD) sensitive during glycerol fermentation [2].

In this study overall and DCCD-sensitive ATPase activity of membrane vesicles was investigated with glycerol-fermented *E. coli* wild type BW25113 and mutant DHP-F2 (MC4100 *ΔhypF*) (with deficiency of Hyd-1; 2; 3 and 4) at different pH.

ATPase activity of wild type strain was ~3-fold higher ($p \leq 0.05$) at pH 7.5 compared with that at pH 6.5. Membrane vesicles ATPase activity was higher in wild type glucose-fermented cells ~1.5-fold at pH 7.5 compared with that in glycerol-fermented cells. DCCD inhibited markedly ATPase activity ~11-fold ($p \leq 0.05$) at pH 7.5 and at pH 6.5– ~1.3-fold ($p \leq 0.025$). Compared with wild type cells, ATPase activity at pH 7.5 was decreased in ~2.2-fold ($p \leq 0.02$) with *ΔhypF* mutant. DCCD inhibited ATPase activity of *ΔhypF* was lowered ~1.4-fold ($p \leq 0.025$) at pH 7.5 and but not at pH 6.5.