In vivo characterization of 3-ketoacyl-acyl-carrier protein (ACP) synthase III (KASIII)

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Introduction:
3-ketoacyl-acyl-carrier protein (ACP) synthase III (KASIII) is an enzyme that catalyzes the chemical reaction acetyl-CoA + malonyl-ACP → acetoacetyl-ACP + CoA + CO₂. This enzyme participates in fatty acid biosynthesis. Fatty acid synthesis is the creation of fatty acids from acetyl-CoA and malonyl-CoA precursors. Bacillus subtilis (B. subtilis) is the organism used for fatty acid analysis.

The B. subtilis was first grown in a petri dish with solid LB over night. A colony was picked and placed in a LB liquid culture tube. These cultures grew up at various rates from 8 to 21 hours. The optimal density was determined with a spectrometer to inoculate the minimal medium cultures. The growth in the minimal medium was monitored in a spectrometer to inoculate the minimal medium cultures. Over night. A colony was picked and placed in a LB liquid carrier protein (ACP): Cells are collected as A600nm for cell collection.

Methylation is the addition of a methyl group to a substrate or the substitution of an atom or group by a methyl group (R'). It is a form of alkylation with a methyl group, rather than a large carbon chain, replacing a hydrogen atom.

Figure 2: Cells are freeze dried using a lyophilizer (A). Cells are collected as dried pellets (B).

Figure 3: Fatty acid extraction begins with methylation. A mixture of methanol, concentrated HCL, and chloroform are added to the cells and heated at 90 Celsius for 30 minutes.

Figure 4: Water, hexane, and chloroform is added to the samples. The samples are placed in a vortex for 1 minute. Samples are then placed in a centrifuge for 5 minutes at 3000 X g.

Figure 5: The top layer, which contains the organic fatty acids, is extracted and collected into clean analytical tubes. A hexane/chloroform mixture is added to the cells and heated at 90 Celsius for 30 minutes.

Figure 6: The extracted fatty acid mixture concentrated by using nitrogen gas to evaporated the liquid composition (hexane and chloroform).

Figure 7: The concentrated samples are run through a gas chromatography-mass spectrometer (GC-MS) for fatty acid analysis.

Figure 8: Phylogenetic analysis of 11 KASII genes.

Figure 9: The two blue profiles are closely related on the tree. The two green profiles are also closely related. The genes highlighted in blue appear to have similar in function, whereas, the genes highlighted in green appear to be functionally different.

Results and Discussion
Eleven of the twelve genes were expressed in B. subtilis and generated fatty acid profiles capable of recovering B. subtilis growth to varying extent (fig 1C). The phylogenetic tree (fig. 9) shows the genetic relationship between the 11 genes tested. The closer two genes are on the tree the more closely they are related at the amino acid level. In comparing some of the samples, KAS BalL2 and KAS BS have similar fatty acid profiles. In contrast KAS BV and KAS CG1 have very different fatty acid profiles (fig. 9). Both of these examples are closely related on the tree of KAS III genes. The difference in the green highlighted fatty acid profiles could lead to further research and understanding of similarities and differences in their structures. This understanding could then lead to the development and engineering of novel enzymes.

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